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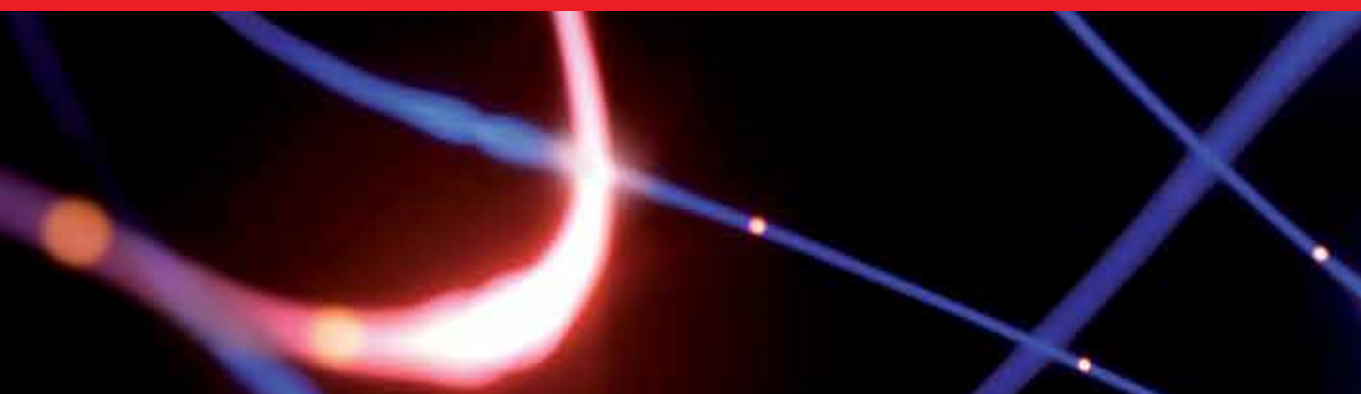
The Amygdala

Where Emotions Shape Perception, Learning and Memories

Edited by Barbara Ferry



THE AMYGDALA - WHERE EMOTIONS SHAPE PERCEPTION, LEARNING AND MEMORIES

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http://dx.doi.org/10.5772/63124
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Contributors

Aline Desmedt, Diego Andolina, Antonella Borreca, Haim Ovadia, Joseph Weidenfeld, Mark Packard, Jarid Goodman, Christa McIntyre, Yukihiko Yasui, Tal Dotan Ben-Soussan, Joseph Glicksohn, Patrizio Paoletti, Patricia Bauer, Maria Garcia-Amado, Lucia Prensa, Gina Forster, Raluca Simons, Lee Baugh, Guoshi Li, Diana Peterson, Laura Corbit, Rose Chesworth, Marco Tamietto, Matteo Diano, Alessia Celeghin, Arianna Bagnis, Jimmy Stehberg, Rodrigo Moraga-Amaro, Sergio Linsambarth

Notice

First published in Croatia, 2017 by INTECH d.o.o.
eBook (PDF) Published by IN TECH d.o.o.
Place and year of publication of eBook (PDF): Rijeka, 2019.
IntechOpen is the global imprint of IN TECH d.o.o.
Printed in Croatia

Legal deposit, Croatia: National and University Library in Zagreb

Additional hard and PDF copies can be obtained from orders@intechopen.com

The Amygdala - Where Emotions Shape Perception, Learning and Memories
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p. cm.

Print ISBN 978-953-51-3249-3

Online ISBN 978-953-51-3250-9

eBook (PDF) ISBN 978-953-51-4757-2

Meet the editor

Dr. Barbara Ferry is a senior researcher at the National Center for Scientific Research (CNRS), France. After obtaining her PhD degree in Neuroscience at Louis Pasteur University, Strasbourg, France, in 1997, she joined the Center for the Neurobiology of Learning and Memory, in Irvine, California, for her postdoctoral studies, from 1997 to 2000. During this time, she studied the role of the α- and β-noradrenergic systems in the basolateral amygdala in the consolidation processes of inhibitory avoidance learning in the rat. In 2000, she obtained a post in the CNRS (France) and joined the UMR 7521 unit, where she studied the role of the lateral entorhinal cortex and basolateral amygdala in the modulation of the olfactory memory trace during conditioned aversion learning in the rat, from 2000 to 2006. Then, she joined the UMR 5292 (Lyon, France) and focused her work on identifying the behavioral, pharmacological, and molecular mechanisms that control olfactory memory formation during associative learning in the rat, with a particular emphasis on the basolateral amygdala. Recently, the expertise in the olfactory area she acquired during her career enabled her to develop a new research project to determine the processes underlying human scent identification by police dogs, through a worldwide collaboration.

Contents

Preface

Among the components of the limbic system, the amygdala is a fascinating structure that is involved in the processes of liking and disliking and in the ways our emotions drive our actions and affect the strength of our memories.

Over these last 20 years, advances in techniques for examining brain activity have led to new insights into the functional contribution of the amygdala to emotions, learning, and related memories.

Combined with new conceptual breakthroughs, the research data obtained in animals and humans reviewed in this book have helped uncover the functional contribution of the amygdala to these processes.

In addition, and consistent with the relations between amygdala malfunction and the occurrence of a number of disorders that affect the personality and learning abilities, this book presents recent advances in various research areas that provide insights into the contribution of the amygdala to some neurological and neuropsychiatric pathologies, including Alzheimer's disease, schizophrenia, and anxiety and stress disorders.

In order to address these topics, results from several research fields have been used, and the very latest data obtained by leading world experts in different aspects of amygdala function are presented. Of course, due to the rate of research advancement, all the chapters presented here correspond to reviews or original articles responding to precise questions addressed by experts using highly specific techniques.

All the data presented in each chapter should be viewed as pieces of a puzzle that represent all the different research areas that have to be taken into consideration in discussing the role of the amygdala in emotion, learning, and memory.

More than 20 years ago, when I started my research on the amygdala, it was already a hot topic, and our knowledge concerning its role in emotion, learning, and memory was growing very fast. Even though publication of research data increased exponentially since, the mystery shrouding the amygdala is still in the spotlight. The fascination and excitation aroused by the functional complexity of this structure never seem to vanish, and there is ample reason to believe that studies relating learning and memory to their neural substrates will continue as a result of the many advances in the use of new investigation tools. In this context, the discovery and the use of new techniques will certainly contribute to progress in amygdala research.

Although the primary goal of this book is to inform experts and newcomers of some of the latest data in the field of brain structures involved in mechanisms underlying emotional learning and memory, I hope it will also help stimulate discussion on the functional role of the amygdala and connected brain structures in these mechanisms.

Dr. Barbara Ferry
Centre of Research in Neuroscience Lyon
Université Claude Bernard Lyon 1
France

Section 1

The Role of the Amygdala in Emotion and Memory - Human Studies

Chapter 1

Human Amygdala in Sensory and Attentional Unawareness: Neural Pathways and Behavioural Outcomes

Matteo Diano, Alessia Celeghin, Arianna Bagnis and Marco Tamietto

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.69345

Abstract

One of the neural structures more often implicated in the processing of emotional signals in the absence of visual awareness is the amygdala. In this chapter, we review current evidence from human neuroscience in healthy and brain-damaged patients on the role of amygdala during non-conscious (visual) perception of emotional stimuli. Nevertheless, there is as of yet no consensus on the limits and conditions that affect the extent of amygdala's response without focused attention or awareness. We propose to distinguish between attentional unawareness, a condition wherein the stimulus is potentially accessible to enter visual awareness but fails to do so because attention is diverted, and sensory unawareness, in which the stimulus fails to enter awareness because its normal processing in the visual cortex is suppressed. Within this conceptual framework, some of the apparently contradictory findings seem to gain new coherence and converge on the role of the amygdala in supporting different types of non-conscious emotion processing. Amygdala responses in the absence of awareness are linked to different functional mechanisms and are driven by more complex neural networks than commonly assumed. Acknowledging this complexity can be helpful to foster new studies on amygdala functions without awareness and their impact on human behaviour.

Keywords: blindsight, hemispatial neglect, subcortical, superior colliculus, pulvinar, attention, consciousness

1. Introduction

The amygdala (Amg) is a composite subcortical structure that comprises more than 12 subnuclei having distinctive patterns of input-output connections with the rest of the brain [1, 2]. This histological and connectional heterogeneity reflects its multifaceted functions. In fact, the Amg has long been known to have a central role in the processing of emotions, but it also serves as an interface between emotion and cognitive functions, including decision-making, learning and attention [3, 4]. Over the past two decades, evidence has accumulated which shows that Amg exerts some of its functions also when the subject is not aware of the nature, content or even presence of the triggering emotional stimulus [5]. The present chapter will discuss findings related to Amg functions in humans during conditions in which the subject is not aware of the presence of an emotional visual stimulus. We will cover Amg main functional/anatomical afferent and efferent pathways that seem particularly relevant during emotion perception in the absence of awareness, and the consequences of such unconscious perception along several dimensions, such as expressive or instrumental actions, psychophysiological and neuroendocrine alterations or modulation of motivated behaviour. Before addressing each specific issue, there are several preliminary considerations, both theoretical and methodological, about the relevance of studying Amg's contribution to emotion processing without awareness [5, 6].

First, Amg functions and circuitry have been well preserved across evolution and appeared early during phylogenetic as well as ontogenetic development. For example, the Amg is present in reptiles, birds and mammals [1], and its neurogenesis in humans and other primates is complete at birth [7], and its connections lay down by the second week of age [8]. Therefore, studying Amg's role in the perception of emotional stimuli when visual awareness is lacking enables us to focus on processes related to basic or core aspects of emotion perception and responses. These primitive aspects of emotion processing likely evolved before more sophisticated functions like perceptual awareness and core feelings. These primordial Amg functions have been implicated in the specialization of more recent cortical functions across the primate lineage as well as during development and maturation [9], including present-day organization of the cortical visual system [10, 11]. Hence, this also provides a valuable testing ground for gauging cross-species continuity of functions and comparison. Second, by examining stimulus properties and categories that evoke Amg activity without awareness, or that by comparison fail to do so, we may be able to abstract from common taxonomies, such as those distinguishing animate from inanimate objects, faces from bodies and so on, to reveal cross-category commonalities between stimulus types and attributes that could not be anticipated by looking at cortical segregation of stimulus categories [12, 13]. Lastly, Amg clearly rests at the intersection between conscious as well as non-conscious emotional processing [14]. To the extent that these two different modes of processing incoming sensory information co-exist in the brain, assessing which operations the Amg undertakes without awareness helps to unravel functions that may be overridden, modulated or even actively blocked during conscious perception and cortical top-down regulation. This can add valuable insights to the longstanding debate on whether perceptions with and without awareness are qualitative or quantitatively different phenomena, whether and how they interact and interfere to shape

the ultimately conscious representation of the external world, and which are, if anything, the specific evolutionary benefits that determined conservation of emotion processing without awareness across evolution [6].

The rest of the chapter proceeds as follows. We will first introduce a conceptual and terminological distinction between different types of emotion perceptions without awareness, as they entail profoundly different mechanisms and are sampled through distinctive experimental designs. Second, we will review neuroimaging evidences demonstrating Amg activity during emotion perception without awareness, how they have been interpreted, and current controversies and limitations. Third, we will discuss the neural timing of the Amg response during presence/absence of visual awareness for the triggering emotional stimulus, and how data acquired with high temporal resolution techniques can elucidate and accommodate apparent inconsistencies originating from fMRI results. Fourth, we will consider functional and anatomical evidence about the neural networks that seem crucial in conveying sensory information to the Amg in the absence of awareness. Fifth, we will concentrate on stimulus categories and properties that can be processed non-consciously by the Amg and finally, we will summarize the behavioural and psychophysiological consequence of emotion perception without awareness. Throughout the chapter, we will concentrate on vision because this is the best-known system in terms of connections with the Amg in human and non-human primates, and because the majority of human studies investigating Amg's role in processing emotions without awareness used visual stimuli.

2. Different types of unawareness for emotions and how they are studied

A host of techniques and experimental manipulations have been used to render emotional stimuli not consciously perceivable. For example, during backward masking an emotional stimulus (e.g. a facial expression) is briefly presented and then immediately followed by a masking stimulus (e.g. a neutral or scrambled face). If the stimulus onset asynchrony between the first and masking stimulus is sufficiently brief, then the observer cannot consciously report the presence or the emotional content of the first stimulus [15, 16]. Binocular rivalry or continuous flash suppression exploits the mutual inhibition between monocular channels in the primary visual cortex (V1) by presenting different images to the corresponding regions of the two eyes [17–19]. In such condition, only one image enters visual awareness, whereas the other image is suppressed from awareness. Other popular paradigms include dual-task designs where the subject's attention is engaged in an attention-absorbing task, such as matching judgment between neutral stimuli, while an emotional stimulus is presented at task-irrelevant and unattended locations [20, 21]. In the attentional blink, a rapid stream of stimuli is presented and subjects are asked to detect the presence of a target stimulus. However, if a second target appears in rapid succession after a first successfully detected target (typically within 500 ms), the latter fails to be reported [22]. Many other paradigms such as priming, Stroop-task, dot-probe designs or the redundant target paradigm have been used to sample emotion perception without awareness, each with its own advantages and limitations [23–27].

Although detailed coverage of these different methods goes beyond the purposes of this chapter, they can be conveniently grouped in two broad categories that entail different functional mechanisms [6]. Dual-task, attentional blink, visual search or Stroop paradigms render the emotional stimulus not consciously visible by interfering with attentional mechanisms. Psychophysical evidence indicates indeed that visual stimuli outside the focus of attention are not, or are only partially, seen consciously [28]. Accordingly, when attentional resources are engaged in a task, cortical activity that is evoked in visual areas by unattended (i.e. task-irrelevant) stimuli is suppressed or significantly reduced by top-down influences from fronto-parietal regions that control voluntary attention [29]. We refer to these phenomena as *attentional unawareness*. Emotional stimuli seem to constitute an exception, as the processing of emotional information seems less dependent on attentional resources than neutral information. As we will discuss later, this mechanism seems to depend on Amg [30].

In contrast, failure to become aware of a stimulus may also result from sensory reasons, although attentional selection mechanisms can operate normally [31]. For example, if the stimulus intensity is too weak (i.e. below the detection threshold) or the presentation time is too brief (i.e. subliminal), the stimulus often does not generate a conscious sensation notwithstanding we pay attention to it [32, 33]. Backward masking, binocular rivalry or flash suppression do not modulate attention, but temporarily interfere with normal functioning in the ventral occipito-temporal cortex, which is known to be crucial for visual awareness [18, 34, 35]. In this latter case, we refer to this type of non-conscious processing as *sensory unawareness*.

Attentional and sensory unawareness are thus qualitatively different phenomena that can be investigated to explore different Amg functions, while still remaining within the domain of non-conscious processes. For example, studying emotion perception during attentional unawareness is well-suited to examine the role of Amg in biasing orientation towards affective stimuli, and investigate what mechanism enables Amg to eventually promote privileged access of emotional signals to awareness. Sensory unawareness can instead reveal alternative pathways by which visual stimuli can reach the Amg, or their impact towards on-going activities, behaviour or judgments, while still remaining unseen. Lastly, patients with brain damage represent an invaluable additional source of information to broaden our knowledge of Amg functions without awareness. Patients with hemispatial neglect due to right temporo-parietal lesions typically fail to pay attention to the contralesional (left) space, and stimuli appearing on that side often go undetected [36]. Therefore, the study of Amg response to emotional stimuli in such patients can add insights into the mechanism governing attentional unawareness. On the other hand, patients with cortical blindness following destruction of the visual cortex offer a case study to investigate the distinction between conscious and non-conscious emotion processing due to sensory causes, as opposed to attentional, and the role of Amg therein [37]. Indeed, such patients are permanently blind to stimuli presented inside the scotoma (the visual field region affected by the cortical lesion), including supra threshold and long-lasting stimuli [38–41]. Lastly, patients with focal damage to the Amg offer the ultimate ground-truth to translate correlational evidence typical of fMRI into causal evidence on Amg functions, by observing whether and how the influence of emotional stimuli during attentional or sensory unawareness is modified or abolished following Amg lesion [42].

3. Amygdala response during sensory and attentional unawareness: evidence and limits

Neuroimaging studies on healthy participants in which attention was manipulated have shown that stimulus-evoked activity in the Amg, along with that of other cortical and subcortical structures, is not suppressed when emotional stimuli are unattended [21, 43–45]. Although this has been sometimes interpreted as evidence of strict automaticity in Amg response to emotion, the current evidence is mixed on this issue. For example, Vuilleumier et al. [21] showed that Amg activation in response to fearful facial expressions is independent of attention, whereas Pessoa et al. [20] reported that when attention is engaged elsewhere by a demanding task, Amg response is suppressed. These apparently contradictory results may be partly explained by differences in the tasks and experimental design, which prevent simple or straightforward comparisons. In fact, in the original study by Pessoa and collaborators [20], the subjects had to evaluate the gender during trials in which attention was focused on the faces, whereas they were asked to judge the same/different orientation of peripheral bars when faces were unattended. In addition to the focus of attention on faces versus bars, therefore, the cognitive load, type of judgment and task requirements also varied between the two conditions, whereas in the study by Vuilleumier et al. [21] only the focus of attention changed. Also, Pessoa et al. [20] used a block design, which samples Amg activity across various repetitions of the same condition and is thus more liable to habituation and less sensitive to physiological responses induced by single events, whereas Vuilleumier et al. [21] used an event-related design where attention varied between single trials. Another major confounding factor concerns the different response the Amg displays to various emotion categories. For instance, Williams et al. [45] found that Amg activity in response to happy facial expressions was greater when faces were attended, whereas for fearful expressions activity was greater when the faces were unattended. Findings collected in neuroimaging studies on patients with hemispatial neglect seem more convergent towards the automaticity of Amg's activity in response to unattended stimuli. Indeed, stimuli presented in the contralateral and pathologically unattended left side of such patients can activate the Amg as well as cortical areas directly connected to it, such as the orbitofrontal cortex or the insula [46–48]. The advantage of addressing the issue of Amg automaticity in neglect patients is based on the fact that no explicit or intentional manipulation of attention is required from the subject, thereby discounting issues related to task differences and attentional load between conditions.

Investigations on sensory unawareness have consistently shown that unseen emotional stimuli elicit activity in the Amg, often along with activity in the superior colliculus and pulvinar [11, 16, 17, 19, 49–58]. But how robust is Amg's response to unseen as opposed to seen stimuli? Some reports found indeed that Amg activity during unawareness and awareness is the same, others described that in several cases, unseen emotional stimuli yield responses higher than those reported during conscious perception of the same stimuli [43, 59], whereas still others reported significantly greater activity in Amg when participants were aware of emotional expressions [56, 57, 60]. Also in this case, methodological differences seem at least partly responsible for the inconsistencies. In fact, assessing the neural bases of emotion perception during sensory unawareness ideally involves a direct comparison between perceived and

unperceived, albeit physically identical, stimuli. Evidence of this type is, however, difficult to observe in healthy individuals, because many manipulations that render a stimulus invisible for the subject inevitably also render the stimulus spatially and temporally different from its consciously visible counterpart. At present, studies on patients with cortical blindness following destruction of the visual cortex possibly provide the best opportunity to clarify the neural basis and properties of non-conscious perception of emotional stimuli. These patients are able to discriminate emotional stimuli that they report not to have seen, for example by 'guessing' whether the stimulus expresses happiness or fear [61]—a phenomenon known as affective blindsight—and their proficiency is associated with activity in the Amg [61–68]. As it often happens when mixed results are reported, interpretations and theoretical views on the role of Amg tend to group along two extremes: those endorsing a strict notion on Amg automaticity and independency from awareness, and those supporting that awareness is a necessary condition for Amg response to occur. We and others have proposed that neural networks for conscious and non-conscious perception of emotions are not entirely different or segregated [5, 30, 69–71]. In this context, Amg not only contributes to both modes of processing, but its initial response without awareness actually helps to determine whether the stimulus will reach awareness and how it will modulate behavioural and bodily reactions. Therefore, the temporal dimension of Amg response becomes critical to interpret its role in emotion perception without awareness, while also offering an additional framework to understand more coherently the seemingly abstracted and different findings summarized above [6].

4. Timing of Amg response: fast signals for slow measures

The speed of processing has always been regarded as one hallmark of non-conscious emotion perception [72]. However, human studies on Amg engagement in emotion processing without awareness typically used fMRI, which has high spatial but poor temporal resolution. In fact, fMRI studies usually average together events occurring during a temporal window of about 2 s, due to the sluggishness of blood oxygen level-dependent (BOLD) response. On the other hand, non-invasive methods with higher temporal resolution in the order of milliseconds, such as EEG and MEG, have traditionally had limitations in sampling neural activity in deep structures such as the Amg [73]. Nevertheless, recent technical advancements in sources analysis, such as the synthetic aperture magnetometry (SAM) and sliding windows analysis, increased precision and sensitivity in detecting MEG signals from deep brain structures.

One early study combining MEG and MRI methods reported early event-related synchronization in the Amg at 20–30 ms after stimulus onset, whereas synchronization in the primary visual cortex occurred later at about 40–50 ms after stimulus onset [74]. A more recent MEG study revealed a dissociation between rapid Amg response to automatic fearful face processing and a later response that interacted with voluntary attention. On each trial, participants had to discriminate the orientation of peripheral bars while task-irrelevant neutral or fearful faces were presented centrally. Rapid increase in gamma band activity in response to threatening faces (30–60 ms) was shown to be independent of task load and under attentional unawareness, while a significant interaction of emotion with attention manipulation was seen at later

latencies (280–340 ms), subsequent to fronto-parietal activity [75]. Coherently, two other MEG studies used dynamic causal modelling (DCM) to test the explanatory power of the automatic Amg response mediated via the subcortical route versus a model predicting only cortical mediation associated with stimulus awareness over Amg activity. Early brain activity was better explained by a model including an automatic Amg response via the subcortical pathway, whereas at longer latencies both models had comparable explanatory power [76, 77]. Therefore, MEG data offer new clues to resolve the longstanding controversy concerning automaticity of Amg response based on fMRI results, as described above [78]. On such bases, it seems that Amg automaticity is a function of time, and these findings have been interpreted according to a two-stage model of emotion-attention interaction. Early Amg responses afford early discrimination between threat and neutral stimuli. These responses occur independently of awareness and attention, possibly because the influence of the fronto-parietal cortex in reducing the representation strength of task-irrelevant and unattended emotional information during attentional competition requires more time to be effective. Conversely, later Amg responses are modulated by attention because the same top-down fronto-parietal mechanisms have had sufficient time to enhance the representation of task-relevant and attended information. Notably, both the early automatic and later attention-modulated Amg responses lie within the time window of one volume acquisition of fMRI studies, likely resulting in the contamination of the rapid effects [6].

Admittedly, intracranial electrophysiological recordings offer the most reliable source of evidence concerning both automaticity of Amg response and its dependency on attention and visual awareness. Three recent studies addressed this issue by recording signals directly from electrodes implanted in the Amg of patients undergoing pre-surgical assessment of pharmacologically intractable epilepsy. Pourtois and colleagues [79] employed the same dual-task paradigm previously used by Vuilleumier et al. [21] to gauge Amg automaticity with fMRI measures. Recordings from lateral Amg in patients with temporal lobe epilepsy showed an early neural response, in the 140–290 ms post-stimulus onset that differed between fearful and neutral faces. Notably, this early response occurred independently of, and prior to, attentional effect starting at 700 ms post-stimulus onset. Likewise, Sato et al. [80] showed greater gamma-bend activity in response to fear compared to neutral faces between 50 and 150 ms. Even though this study confirmed early responses to emotional stimuli, sensory or attentional unawareness was not manipulated and stimuli were projected centrally for 1 s. Lastly, a recent study by Ménzed-Bértolo at al. [81] found fast Amg responses at 74 ms post-stimulus onset, which were specific for fearful faces compared to neutral or happy facial expressions. Moreover, fast Amg responses were selective to the low spatial frequencies' components of fearful faces. This sensitivity to low spatial frequencies is important because it is in keeping with the properties of the magnocellular pathway, which is supposed to relay visual signals to the Amg via a subcortical pathway devoted to fast and non-conscious emotion perception.

The present findings raise two interrelated issues of the utmost relevance. The first one concerns how visual information exploitable for non-conscious emotion perception reaches the Amg. The second relates to the encoding properties of the pathway(s) that channel visual information to the Amg without awareness, thereby defining which visual properties, stimulus attributes and categories can undergo emotion processing and trigger appropriate responses. In the next two sections we will deal separately with each of these issues.

5. Pathways to the Amg relevant for non-conscious emotion perception

The canonical pathway for the transmission of visual information from the retina to the Amg passes through the occipito-temporal cortex along the ventral stream, with the main projection originating from the anterior part of the inferior temporal cortex (TE) [82]. However, earlier studies in rats underlined the role of midbrain structures in providing a rapid but coarse analysis of the affective value of auditory as well as visual stimuli and in relaying such information to the Amg, hence bypassing the primary sensory cortices [72, 83–87]. Neuroimaging data on healthy subjects in which sensory unawareness for emotional stimuli had been induced by experimental manipulations have revealed that the superior colliculus, pulvinar and Amg constitute a functional network that shows increased positive covariation of activity in response to non-consciously perceived emotional signals [11, 16, 54, 57, 88, 89]. By contrast, the major cortical pathway relaying visual input to the Amg does not show substantial activity and functional connectivity under the same conditions of sensory unawareness but does so during conscious perception of emotional stimuli [17, 56, 57]. Similar findings have been reported in patients with affective blindsight presented with unseen facial and bodily expressions. This indicates that a functional subcortical pathway to the Amg is engaged in emotion perception during sensory unawareness [5, 65, 66, 68, 90–94]. The involvement of the superior colliculus and pulvinar is in keeping with their connectional pattern and physiological properties. Notably, the superficial layers of the superior colliculus (SC) receive direct retinal input only from the magnocellular and koniocellular channels originating from the parasol and bistratified retinal ganglion cells, respectively [95–97]. Also the medial subdivision of the inferior pulvinar receives direct projections from the retina, in addition to input originating from the superior colliculus and targeting the centromedial and posterior subdivisions of the inferior pulvinar [6]. Hence, these subcortical structures are ideally positioned to convey visual input to the Amg and bypass transient or permanent inactivation of the visual cortices. The functional role of the superior colliculus and pulvinar in processing emotional expressions has also received independent support from recent single cell recordings in monkeys [98]. In fact, a subpopulation of neurons in the superior colliculus responds to face and face-like visual stimuli, and its response properties are not influenced by low spatial frequency filtering of the images. Moreover, neural response magnitude and latency to face stimuli in the superior colliculus significantly correlate with those in the pulvinar. Another cell recording study from the same group showed that monkey pulvinar neurons display differential activity to specific emotional expressions [99].

Granted the role of a subcortical functional pathway to the Amg devoted to processing emotion under sensory unawareness, are these structures also anatomically connected, thereby forming a structural pathway? While tracer studies have demonstrated the existence in birds and rodents of anatomical connections between the superior colliculus, pulvinar and Amg, similar evidence in primates was lacking until recently [14, 100]. Yet Day-Brown et al. [101] have shown in tree shrews that projections to the lateral Amg originate also from the dorsal pulvinar. This latter part of the pulvinar receives visual input from the superior colliculus, thereby forming a disynaptic pathway to the Amg. The authors suggested that this pathway potentially relays non-topographic visual information from the SC to the Amg, its functional role being that of alerting the animal to potentially dangerous signals [101]. In an attempt to verify whether such

anatomical connections also exist in the human brain, we used diffusion tensor imaging (DTI) and tractography techniques to characterize *in vivo* the connectivity between the superior colliculus, pulvinar and Amg in normal observers and its changes in blindsight GY are the initials of the patient's name that it has been tested in the paper. This way of report the name is to protect the privacy of the patient [102]. We found fibre connections between pulvinar and Amg and also between superior colliculus and Amg via the pulvinar in the healthy observer as well as in the patient GY. The destruction of the visual cortex led to qualitative and quantitative modifications along the pathways connecting these three structures, and the changes were confined to the patient's damaged hemisphere, thereby strongly supporting the notion that the subcortical route conveys visual information critical for sustaining affective blindsight and non-conscious emotion perception. A recent tractography study by Rafal and collaborators [103] used a different tractography method in 20 healthy subjects, as well as in eight monkeys, to trace possible direct connections between colliculus, pulvinar and Amg. The results in humans were closely comparable to our previous findings, and the study also provided the first anatomical evidence of direct connections between Amg, pulvinar and colliculus in the monkey brain.

Clearly, the existence of such a subcortical pathway does not exclude the possibility that the Amg receives visual input also from other structures, nor the role of cortical areas in different forms of conscious or non-conscious emotion perception [104]. For example, both the lateral geniculate nucleus and the pulvinar send collateral projections that bypass V1 and target extrastriate visual areas, including areas along the ventral stream that can then relay visual information back to the Amg. Also, two other disynaptic subcortical pathways to the Amg have been recently demonstrated in mice, along with their functional role in triggering innate defensive responses to threatening visual stimuli. Both these pathways originate from the superior colliculus, but one includes the parabigeminal nucleus as intermediate station leading to the Amg [105], whereas the other involves the lateral posterior nucleus of the thalamus [106]. Whether these and other potential pathways beyond the well-documented colliculus-pulvinar-Amg one play a crucial role for emotion perception without awareness in humans remains to be established. Lastly, these two-route perspective involving cortical versus subcortical input to the Amg has been often conceived or presented as alternative to the two-stages account discussed above, as emerging from analyses of the temporal profile of Amg responses. However, there is no necessary contradiction between these two views nor must they be seen as mutually exclusive. Conversely, empirical evidence seems to indicate they co-exist in the intact brain, and they gain new coherence when considered under the light of the distinction between sensory and attentional unawareness introduced above [6]. In fact, when V1 is not able to process visual information normally, because of either experimental manipulation inducing sensory unawareness or permanent damage to V1, the subcortical route seems the primary non-canonical pathway to convey rapidly visual information to Amg and sustain non-conscious emotion processing. During attentional unawareness in healthy subjects or in patients with neglect, however, the visual cortex is normally functioning and coarse magnocellular input can also reach the Amg from cortical areas in the ventral stream through an initial forward sweep [30, 71]. This can afford rapid processing of unattended stimuli prior to voluntary attentional control [79, 107] or fine-grained and conscious stimulus perception.

6. Stimulus categories and properties triggering amygdala response without awareness

Facial expressions effectively communicate other's emotions during social interactions and, until recently, most investigations of human emotions predominately concentrated on processes associated with viewing faces (e.g. Ref. [108]). It is therefore not surprising that research on emotion perception without awareness primarily used facial expressions [16, 53, 54, 109]. This has contributed to the prevailing assumption that Amg activity during non-conscious emotion perception is selective for facial expressions [10, 110]. However, recent investigation seems to challenge this view from two parallel lines of findings. On the one hand, Amg activity contingent upon sensory and attentional awareness in healthy as well as brain damaged patients emerged from non-facial stimuli, thereby extending evidence of non-conscious emotion processing to other stimulus categories. Bodily expressions of emotions, both static and dynamic, have been the most extensively studied non-facial stimuli [46, 47, 62, 67, 68, 92–94, 110, 111]. Stimuli that represent evolution-determined threats, such as spiders and snakes, have also been tested under conditions of sensory and attentional unawareness. These stimuli induced enhanced physiological arousal and amygdala activity [112–115] particularly in individuals who were phobic to these types of stimuli, and activated Amg also when unattended because they were presented in the affected side of patients with hemispatial neglect [47]. On the other hand, the alleged special status of faces in triggering non-conscious perception and Amg activity is at odds with negative evidence when non-emotional facial attributes are tested, such as personal identity or gender [116]. Furthermore, facial expressions of complex social emotions, such as arrogance or guilt, also fail to undergo non-conscious emotion processing in patient with affective blindsight (Celeghin, Adenzato, et al., in preparation).

A certain degree of functional similarity between these different stimulus categories, resulting in their similar role in sustaining non-conscious emotion processing and Amg response, challenges theories exclusively concerned with analysis of the specific visual features. In fact, evidence suggests an approach that cuts across gross physical stimulus differences, as there exist between facial and bodily expressions, or between these latter and snakes, to focus more on the functional properties of visual signals. Under the assumption that the special role of faces is not fixed by their physical properties but by their functional ones, the findings reported above converge with the idea that non-conscious emotion processing is not specific for faces, but rather for biologically primitive emotional signals that can be encoded from low spatial frequencies, that are clearly associated with action tendencies and to which we are evolutionary prepared to respond [5]. Accordingly, complex affective scenes derived from the international affective picture system (IAPS) cannot be processed non-consciously in patients with affective blindsight [117] and do not activate Amg under attentional unawareness tested in patients with neglect [118].

Evidence therefore suggests that the analysis of the emotional content of complex scenes, facial identity or expressions of social emotions may depend critically on conscious visual perception and on the detailed processing of the high spatial frequency information that is typically performed by the cortical visual system [119]. We have already discussed findings

about fast Amg responses for low but not high spatial frequency fearful expressions [81, 120]. In an attempt to determine the causal role and behavioural consequences of Amg activity during non-conscious perception of low spatial frequencies expressions, we have recently tested two patients with affective blindsight in a combined behavioural/fMRI experiment. Fearful and neutral faces were filtered so as to contain only low or only high spatial frequency information. We reasoned that, if non-conscious emotion perception during sensory unawareness relies on a subcortical pathway to Amg and magnocellular channels, then the patients should be able to correctly guess the emotional expressions of faces filtered for displaying only low spatial frequency information and this behavioural effect should be associated with Amg activity. Conversely, the same expressions filtered in high spatial frequency should knock out the behavioural effect and Amg response should drop significantly. Preliminary evidence indeed confirms our hypothesis and provides direct support for the role of subcortical structures in mediating affective blindsight.

7. Consequences of Amg activity during non-conscious emotion perception

What are the consequences of Amg activity without stimulus awareness? Do they alter on-going behaviour, psychophysiological reactions or expressive responses towards normally seen environmental stimuli? And, lastly, are these responses felt consciously, even though they cannot be linked to the external triggering event?

Non-conscious perception of emotional stimuli associated with Amg activity often induce behavioural consequences that are accompanied by characteristic psychophysiological correlates of changes in the emotional state of the (unaware) observer. These behavioural and psychophysiological outcomes are often different from those associated with conscious perception, as they tend to be stronger and faster in the former case [35, 47, 67]. This suggests that non-conscious perception of emotional stimuli is not simply a degraded counterpart of conscious perception, but a different mode of processing visual signals.

For example, emotional stimuli that are unattended nevertheless interfere with on-going tasks [25, 121], and behavioural consequences include delayed disengagement of attention [122], faster and easier detection than neutral stimuli, as shown in visual search [123, 124], attentional blink paradigms [22] or in patients with neglect [35, 125–128]. Notably, damage to the Amg abolishes some of these behavioural effects [42]. Likewise, attitudes and preferences towards neutral stimuli may be shifted towards more positive or more negative evaluations depending on whether the neutral stimuli are preceded by, or paired with, unperceived emotional stimuli [129, 130]. For example, consumption behaviours or preference judgments can be influenced by exposure to masked facial expressions, despite subjective feelings remain unaltered [131, 132]. Notably, however, when subjects are aware of the presence and nature of the emotional stimuli these effects sometimes disappear [130, 133].

Psychophysiological changes that are associated with non-conscious perception of emotional stimuli include enhanced skin conductance [15, 134] increased frequency of eye blink

(indicating startle reactions or avoidance) [64], changes in stress hormone levels [135], increased pupil dilation [47, 67] and heart rate changes [136]. These changes index arousal and their function is to prepare the organism for reacting to impeding and salient events. Similarly, undetected emotional stimuli also induce spontaneous facial reactions that reflect the affective valence of the stimuli, as recorded using electromyography (EMG) [67, 137]. This spontaneous tendency to synchronize our facial expressions with the emotional meaning of other individuals' expressions is likely to play a part in social interactions [138].

A different source of evidence on the impact of stimulus processing without awareness comes from studies that used indirect manipulations. For example, studies on patients with affective blindsight have used indirect methods to investigate possible online interactions between consciously and non-consciously perceived emotions, as well as the influence exerted by the former over on-going recognition of seen stimuli [63, 139–141]. A classic example of such indirect methods is the redundant target paradigm, in which stimuli are presented either singly to the intact field or paired simultaneously with another stimulus in the blind field. Typically, reaction times (RTs) to the seen stimulus are faster during redundant stimulation than during single presentation to the intact field. With such method, unimodal (visual/visual) and cross-modal interactions (visual/auditory) between consciously and non-consciously perceived emotional stimuli have been observed in such patients. For example, presenting a facial expression to the blind field of patients with blindsight biases their judgment of the emotional prosody of a sentence fragment [90, 117]. For example, a fearful prosody in the voice is perceived as more fearful when it is presented synchronously with a fearful facial expression in the blind visual field, and this effect is associated with enhanced Amg activity. These findings converge with the notion that emotion processing with and without stimulus awareness co-exist and interact in the intact brain, though they can be dissociated because of focal brain damage or experimental manipulation.

But can the bodily changes and responses triggered by unseen emotional stimuli be themselves experienced consciously as feelings? The classical view is that we become aware of such bodily responses when linking them to conscious representations of their external (e.g. an angry expression or a sudden noise) or internal causes (e.g. our thoughts). In fact, some evidence indicates that we are unable to report a conscious feeling despite the fact that, at the same time, our behaviour reveals the presence of an affective reaction triggered by the exposure to an external stimulus of which we are unaware. Despite this, however, it is conceivable that we can become aware of our physiological changes without any conscious representation of their underlying causes. This seems to be a common situation in clinical conditions such as alexithymia, pathological anxiety or depression. Also, one study on patients with affective blindsight has shown that the presentation of an unseen stimulus previously paired with an aversive event enhances eye-blink startle reflex, and this enhancement corresponded to the reported level of negative emotional feelings [142].

8. Concluding considerations

If emotional stimuli can be processed without awareness, activate the Amg, and still induce coherent responses, what role is left for consciousness in emotions? Some clues come from

the observation that the responses observed when emotion processing is accompanied by awareness are often quantitatively or qualitatively different from those induced by unconscious processing. Enhanced influence of non-consciously perceived emotional signals on physiological or expressive responses is in line with evidence that cortical activity and awareness may exert an inhibitory modulation over subcortical areas or automatic responses [143–145]. The fact that such inhibition is absent during non-conscious perception of emotional stimuli could also explain the apparently paradoxical finding that subcortical activity can be enhanced during non-conscious compared to conscious perception of emotional stimuli in healthy subjects [43, 58]. Likewise, conscious perception of the eliciting stimulus can overrule subjective affective experience in response to an aversely conditioned stimulus, and the decoupling between phenomenal affective experience and actual physiological changes is associated with increased activity in the ventrolateral prefrontal cortex [129, 142]. These findings contradict the common assumption that emotional feelings merely reflect cortical readouts of peripheral and autonomic arousal. Therefore, the added value of the conscious perception of emotional stimuli seems primarily that of integrating representations of the external and internal world in order to achieve context-dependent and higher-order decoupling and flexibility between sensory input and behavioural output. Consciousness also allows control and planning, as well as anticipation of desirable or functional responses.

From the opposite vantage point, emotions seem to play a prominent role in the generation and development of state consciousness. The basic physiological reactions triggered by emotional stimuli involve the moment-to-moment mapping of our bodily states and interoceptive information crucial for homeostatic regulation. Because homeostatic processes provide the sense of invariance that accompanies every subjective experience, they constitute a neurobiological mechanism for the invariance of the sense of self and the continuity of our first-person experience of the world [146–148]. On this picture, basic aspects of the physiological reactions to emotional stimuli overlap with physiological responses related to corrections of homeostatic imbalance and thought to be necessary for the general level of consciousness [146, 149, 150]. It is not a coincidence that these emotional responses are controlled by neural structures in the brainstem that also control the level of consciousness. Accordingly, several scholars consider raw emotional feelings as the precursors or basic forms of consciousness, and have rooted it in subcortical processes rather than (only) in full-blown subjective cognitions implemented in higher-order cortical structure [145, 149, 151–153]. In keeping with this perspective, children with total congenital absence of the cerebral cortex can nevertheless exhibit appropriate affective responses and feelings can be even strengthened [154]. Moreover, direct electrical brain stimulation in subcortical and brainstem structures that evoke observable behavioural and physiological reactions associated with reward and punishment in animals, also induce conscious affective feelings when stimulated in humans [145, 153]. Therefore, even when we remain unaware of the external determinants of an emotional response, such that the eliciting stimulus does not become a content of our conscious visual experience, the chain of physiological reactions it triggers nevertheless contributes to modulate our state of vigilance and behaviour, which are constitutive components of our state of consciousness.

Acknowledgements

Alessia Celeghin, Matteo Diano and Marco Tamietto are supported by a 'Vidi' grant from the Netherlands Organization for Scientific Research (NWO) (grant 452-11-015), by a FIRB—*Futuro in Ricerca 2012*—grant (RBFR12F0BD), and by a PRIN 2015—*Programmi di Ricerca Scientifica di Rilevante Interesse Nazionale*—grant (2015NA4S55_003) from the Italian Ministry of Education University and Research (MIUR). Parts of this chapter are reproduced from the authors' previous publications [5].

Author details

Matteo Diano[1,2], Alessia Celeghin[1,2], Arianna Bagnis[2] and Marco Tamietto[1,2,3]*

*Address all correspondence to: m.tamietto@uvt.nl

1 Center of Research on Psychology and Somatic diseases (CoRPS), Tilburg University, The Netherlands

2 Department of Psychology, University of Torino, Italy

3 Department of Experimental Psychology, University of Oxford, UK

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Chapter 2

Inner Design Technology: Improved Affect by Quadrato Motor Training

Patrizio Paoletti, Joseph Glicksohn and
Tal Dotan Ben-Soussan

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67586

Abstract

The relation between positive affect and negative affect is a predictor of emotional well-being. In addition, healthy neuronal synchronization is associated with higher emotional well-being and positive affect. Related to this, recent studies have consistently reported that Quadrato Motor Training (QMT), a sensorimotor-cognitive training, increases alpha synchronization and emotional well-being in healthy participants. QMT was further found to improve creativity, reflectivity, and mindfulness-related experiences in healthy participants. In the current research, we have examined the effect of QMT on emotional well-being using the Affect Balance Scale (ABS), comparing two 1-week training programs: (1) breathing meditation retreat with QMT training (QMT, $n = 42$) and (2) breathing meditation retreat without QMT (BM, $n = 42$). While both groups reported improved affect and self-efficacy following the training, the QMT group reported significantly higher ABS scores following the retreat. QMT can thus improve well-being and emotional regulation as measured by the ABS. The current results strengthen previous claims that different practices, such as BM and QMT, may improve emotional well-being. These results are discussed in the context of the possible mechanisms mediating training-induced improved affect, focusing on the amygdala and neuronal synchronization. In conclusion, incorporating specifically structured motor and mindful practices may serve as important tools to facilitate greater emotional well-being.

Keywords: affect, self-efficacy, Quadrato Motor Training, synchronization

1. Introduction

Accumulating behavioral and physiological evidence suggests that emotions are grounded in *core affect*, namely, the fluctuating level of pleasant or unpleasant arousal. Neuroimaging

studies reveal that participants' subjective ratings of valence (i.e., pleasure/displeasure) evoked by positive and negative experiences are correlated with neural activity in specific brain regions (orbitofrontal cortex and amygdala, respectively) [1]. Initial processing of emotional content occurs in various structures of the limbic system, such as the amygdala [2]. The amygdala has been especially linked to fear processing, ranging from fear conditioning, the modulation of attention and memory for fear-related stimuli, the induction of fear-related behaviors [3–5], stress response, and memory consolidation of newly acquired information through its projections to other brain structures [6].

The problem is that as we age, brain structures mediating the experience of negative affect become more easily activated [7, 8]. Such heightened sensitivity leads to a vicious cycle of increased manifestation of negative affect under stress, which in turn is related to additional aversive emotional and physical health outcomes [9]. Luckily, this mostly automatic process of enhanced emotional sensitivity [10, 11] can be influenced through training, such as meditation. For example, expert meditators showed less activation in the amygdala during focused attention meditation compared to novice meditators [12]. Decreased activation in the amygdala during a computerized emotion visualization task was also reported following an eight-week (2 h of class time per week) mindful attention training [13].

Along with decreased activation of the amygdala, meditation is known to enhance alpha synchronization, which has been suggested to support emotional regulation [14–18]. In fact, different practices, such as breathing meditation (BM), improve emotional and physical well-being as well as cognitive functions (e.g., information processing and attention) [17–22]. For example, intensive meditation/emotion regulation interventions have been found to reduce negative affect, rumination, depression, and anxiety and to increase positive affect and *Mindfulness* [23].

Mindfulness, as an attribute of consciousness, can be defined as the clear minded awareness of the present moment [20]. Mindfulness can be achieved by many ways, and is important for disengaging from automatic thoughts (such as "no one likes me," "I can't stand feeling this way"), habits and unhealthy behavioral patterns. It plays a key role in fostering and orienting attention, thus adding clarity to the experience, which in turn can significantly contribute to well-being and happiness [20, 24, 25]. Mindfulness-based training is associated with reduced anxiety [26]; adaptive response to stress [27]; decreased ego-defensive responsivity under threat [24]; decreased difficulties regulating emotions [28]; and reduced emotional interference resulting from unpleasant stimuli and less prolonged physiological reactivity to negative emotional stimuli [29].

Similar to sitting meditation techniques, a novel movement meditation, Quadrato Motor Training (QMT), has also been found to improve different cognitive functions, such as creativity and spatial cognition (for review see [18]). QMT was further found to increase alpha synchronization [30, 31], mindfulness, and emotional well-being [17]. In a recent pilot study, we further found improved emotional well-being following a month of daily QMT in contrast to Simple Motor Training (SMT) in university students [32], as assessed using the Affect Balance Scale (ABS) [33].

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Bradburn's [33] classic work on the structure of psychological well-being provided the initial distinction between positive and negative affect. Thus, Bradburn's Affect Balance Scale [33] is based on the definition of "happiness" as a preponderance of positive over negative affect (PA and NA, respectively). Affect balance can be defined as the difference between positive affect and negative affect, with a high score meaning that positive feelings outbalance negative feelings [33], which can, in turn, promote resilience and coping with stress [34–36].

High General Self-Efficacy (GSE) is also considered a resource that buffers against stressful experiences, as high self-efficacious individuals perceive demands as challenging, not as threatening [37]. Physical exercise, as well as different meditation practices, such as Tai Chi, has been found to enhance self-efficacy (for reviews, see Refs. [38, 39]). Nevertheless, to the best of our knowledge, no studies have compared the effects of sitting versus movement meditation on self-efficacy.

Consequently, in the current study, we chose to examine the effects of QMT and of breathing meditation (BM) on emotional well-being using the Affect Balance Scale (ABS) and on General Self-Efficacy (GSE) [40]. To this aim, we compared two one-week intense training programs: (1) breathing meditation retreat with QMT training (QMT) and (2) breathing meditation retreat without QMT (BM). Since both physical exercise and meditation have previously been linked to increased synchronization and emotional well-being [15, 16, 18] and based on our previous results demonstrating QMT-induced enhanced local alpha synchronization in the frontal and parietal lobes as well as alpha parietal-limbic and occipito-limbic connectivity and emotional well-being, we hypothesized that the unique combination of mindfulness and specifically structured movement will have a greater effect on emotional well-being, resulting in greater improvement in affect balance and self-efficacy.

2. Methods

2.1. Participants and design

A total of 84 participants volunteered in the study, which took place in the Research Institute for Neuroscience, Education and Didactics of the Patrizio Paoletti Foundation. The participants signed an informed consent, and the study was approved by the ethics committee of Bar-Ilan University. At each time point, participants also reported their emotional and psychological condition using two questionnaires, the Affect Balance Scale (ABS) [33] and General Self-Efficacy (GSE) Scale [40]. The ABS and GSE were completed at the beginning of the week and at the end of the retreat.

Volunteers were recruited from participants who were registered for two different one-week intense retreats organized by the Ideas—Knowledge of Excellence, International School of Self-Awareness: (1) breathing meditation retreat with QMT training (QMT, n = 42) and (2) breathing meditation retreat without QMT (BM, n = 42). As specific brain activation patterns are linked to different kinds of exercise and participants' physical exercise preferences, the volunteers could decide for themselves in which intense training to participate. The retreats

consisted of lectures, discussions, and meditations. Participants arrived on day 1, which included an evening introduction and guided meditation. Days 2–5 consisted of lectures, discussion, and 3–4 h of meditation that was done in a group setting. On the 6th day, participants met for the morning session and the retreat ended at noon. Participants were given explicit instructions both before and during guided meditations at the beginning of the retreat, though the frequency of instruction during the guided meditations decreased as the retreat continued. Across the retreats, the sitting meditation sessions consisted of breathing meditation, emphasizing physical and mental relaxation and maintaining attention on the breath [41].

The QMT group included additional two sessions of daily QMT sessions, which were conducted once in the morning and the afternoon of days 2–5 in group setting. During QMT, each participant stood at one corner of a 0.5 m × 0.5 m square and was asked to make a step in one of three directions in response to verbal instructions given by the instructor. Participants were instructed to keep the eyes focused straight ahead and their hands loose at the side of the body. They were also told to immediately continue with the next instruction and not to stop due to mistakes. At each corner, there are three possible directions to move. The training thus consists of 12 possible movements (3 directions × 4 corners): 2 forward, 2 backward, 2 left, 2 right, and 4 diagonals. For example, if the sequence required is 1, 2, 1, 2, 1, 2, 3, 2, 4, 3, 1…. this means moving to the first corner, then to the second, then back to the first, and so on. See **Figure 1** for a graphical illustration.

2.2. Measures

2.2.1. Affect Balance Scale (ABS)

The purpose of the Affect Balance Scale is to assess positive and negative affect as indicators of life satisfaction or well-being [33]. It comprises a 10-item scale, with five items that measure positive affect and five that measure negative affect, asking the participants to answer if they have felt certain emotions in the past week on a four-point scale ranging from never, rarely, sometimes, and frequently. Final scoring was conducted by subtracting the average of the negative affect (NA) items from the average of the positive affect (PA) items [33–35].

The ABS has been extensively used in empirical research with a broad range of populations since its development. In a study of 2735 adults, Bradburn [33] reported the reliability coefficients for each component (PA, NA) of the ABS score. These were 0.83 for PA and 0.81 for NA, reflecting the consistency of the subscales [33]. Reliabilities reported in other studies in the literature have ranged from 0.55 to 0.73 for PA and from 0.61 to 0.73 for NA [42]. In the current study, we found the reliability coefficients for pre- and posttraining to be 0.66 and 0.68 for PA and 0.55 and 0.65 for NA.

2.2.2. The General Self-Efficacy Scale (GSE)

The General Self-Efficacy Scale has the aim to assess a general sense of perceived self-efficacy, through a 14-item questionnaire that reflects the respondent's beliefs regarding his or her

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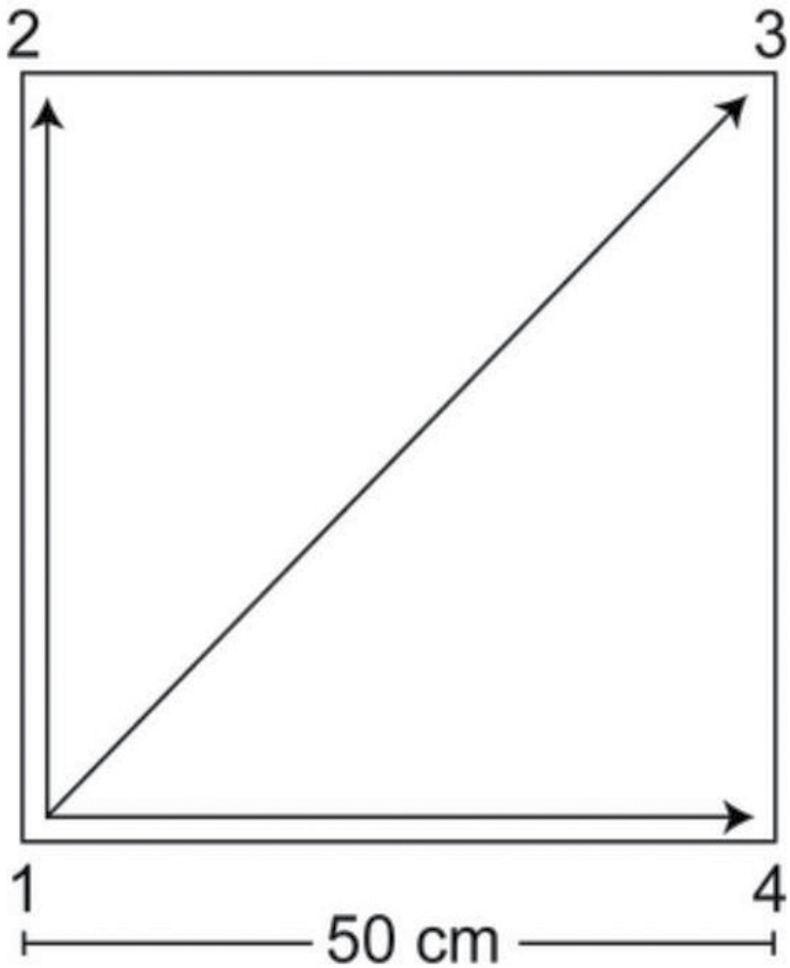

Figure 1. Graphical illustration of the Quadrato Motor Training.

capacities [40]. The participant was asked to state the degree to which he or she agrees with each of the statements in the questionnaire on a scale ranging from "1" (strongly disagree) to "5" (strongly agree). The total scores on the questionnaire range from 14 to 70, with the highest score reflecting higher self-efficacy. The reliability of the GSE is generally high ($\alpha = 0.90$). The scale's reliability coefficient obtained in this study was 0.90 and 0.89, pre- and posttraining, respectively.

2.3. Statistical analysis

To answer the question regarding the effects of QMT on well-being, we ran a Group (QMT, BM) × Training (pre-, post-) analysis of variance (ANOVA) on the ABS score, and on the GSE

score, separately, adopting the Greenhouse-Geisser criterion. Whenever needed, we added *post hoc paired sampled t* tests.

3. Results

There were no baseline (pre-) difference between groups for the ABS [$t(82)$=1.16, ns]. Mean and STD for QMT and BM were 0.8 (±0.9) and 1.1 (±0.7). A significant Group × Training interaction was found for ABS [$F(1,82) = 4.83$, $p < 0.05$] with significantly higher increase in ABS score for QMT compared to the BM intense training [$t(82) = -2.2$, $p < 0.05$]. See **Figure 2**, presenting the data for the change in ABS.

No base line differences nor a Group × Training interaction were found for GSE [$F(1,82) = 0.99$, ns]. The main effect for training was significant for both ABS and GSE [$F(1,82) = 60.16$ and 38.65, respectively, $p < 0.01$], indicating an increase in score for both measures following training. For the GSE, the increase was from 50.39 to 54.64 posttraining.

In addition, while there was no correlation between ABS and GSE before training ($r = 0.14$, ns), we found a significant correlation between these measures following the retreat ($r = 0.23$, $p < 0.05$, $n = 84$), as can be seen in **Figure 3**.

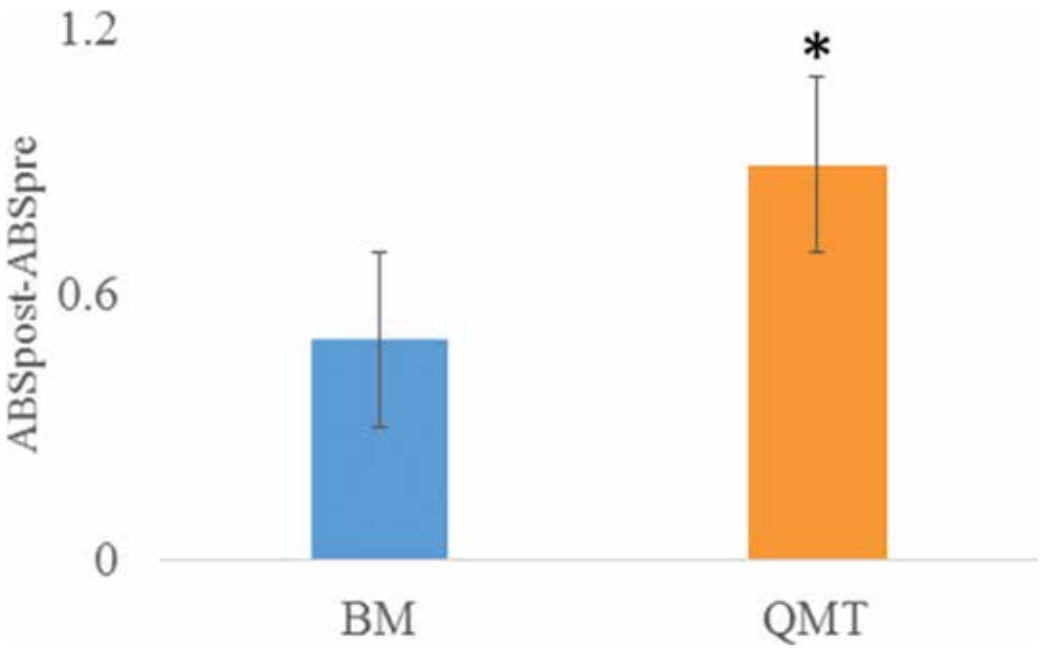

Figure 2. Change in Affect Balance Scale score as a function of Group and Training. Change in Affect Balance Scale score was calculated by subtracting ABS score before the training (ABSpre) from the ABS score following the training (ABSpost).

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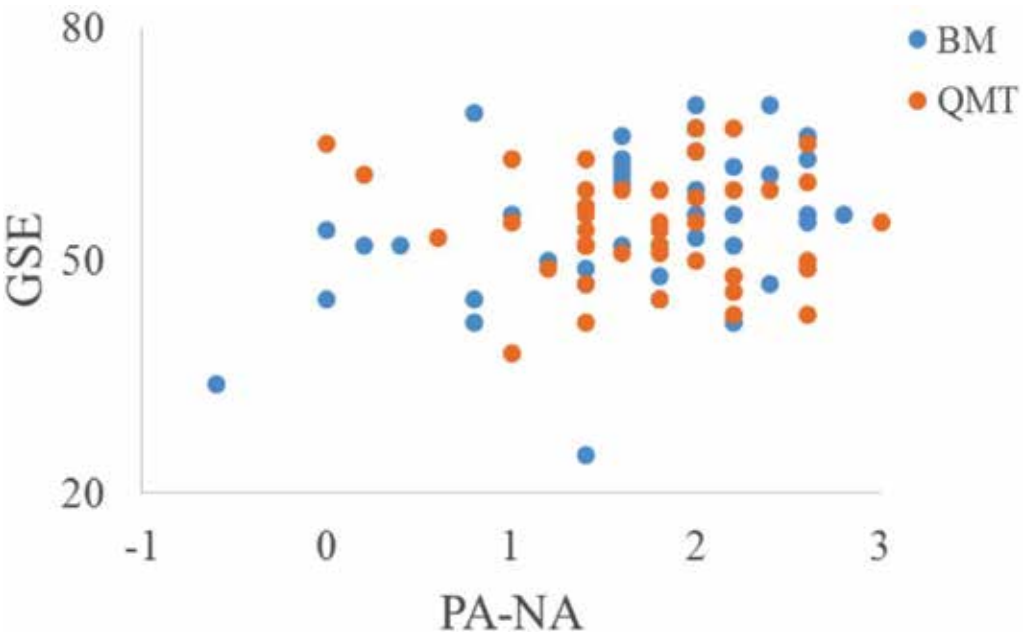

Figure 3. Correlation between Affect Balance Score (PA-NA) and General Self-Efficacy (GSE) following training.

4. Discussion

In the current study, we have found that 1 week of intense training combining breathing meditation and QMT can significantly increase the affect balance. The current results are consistent with previous findings that mindfulness-based interventions were associated with lowered intensity and frequency of negative affect [20, 43]. They are further in line with previous findings regarding QMT-induced affect balance in contrast to simple motor training (SMT) in university students [32], emphasizing the specific effects of QMT, as opposed to simple motor training without the mindfulness aspect. Here we further demonstrate that the combination of BM and QMT has an added value to affect balance compared to intense BM training.

4.1. Possible underlying mechanisms mediating QMT-induced increased affect balance

We build on the previous QMT-induced electrophysiological and cognitive changes and the meditation literature to posit the possible underlying mechanisms of QMT-induced changes in affect. We suggest three interrelated possible underlying mechanisms that might mediate this change: (1) neuronal synchronization and connectivity with limbic areas, especially within the alpha band (8–12 Hz); (2) activity of the default mode network (DMN); and (3) improved allocation of attention following training.

First, based on our previous results and model of an interrelationship between alpha synchronization, improved cognitive, emotional, and bodily function [18, 44–46], we hypothesize that the effects of QMT on affect balance may have resulted from increased alpha activity. Alpha activity has consistently been found to increase following QMT, especially within frontal and parietal areas, as well as between occipito-limbic areas [30, 31, 46, 47]. The occipito-limbic circuit links the occipital cortex and amygdala and is usually activated in response to emotional processing [48–50]. Interestingly, this network is dysfunctional in affective disorders and impulsivity [51].

In fact, alpha activity has been linked to emotional well-being [52, 53]. Supportive evidence of this hypothesis comes also from the fact that both physical exercise and meditation increase frontal alpha activity [53, 54], and that this effect is thought to be independent of meditation technique and degree of experience [14].

Second, neuronal synchronization in the alpha range is negatively correlated with default mode network (DMN) activity [55]. The DMN, which includes regions such as the amygdala, hippocampus, and ventromedial prefrontal cortex, is thought to mediate negative emotions. While DMN activity is related to negative emotion, meditation is known to decrease DMN activity [56, 57].

Third, the DMN is further related to mind-wandering and autobiographic self-processing (for review see [58]). An antagonistic network to the DMN is the *attentional extrinsic system* related to self-processing, defining the self as the momentary agent of experience [57]. Mental states that explicitly involve the control of attention allocation, such as mindfulness training, require the participant to bring greater awareness to the present moment and to the body [56, 59, 60]. This allocation of attention may permit the modulation of negative emotions (e.g., [61, 62]). QMT, similar to other mindfulness training paradigms, increases attention to the body and to the present moment but perhaps in a greater frequency, as it requires constant attention to the incoming instruction and consequently responding to it [17]. This in turn can be mediated by increased synchronization [18], thus enabling a state of increased affect balance.

4.2. Self-efficacy, the DMN, and the importance of aims

In addition, we found that self-efficacy significantly increased following a week of intense training. The construct of self-efficacy reflects an optimistic self-belief that one can cope with adversity and difficulty in various domains of human functioning [37]. Self-efficacy facilitates goal-setting and can be regarded as a source of resilience and, therefore, is relevant for behavior change. The current result is especially important as reactivity to stress increases as we grow older due to a lifetime of repeated activations of the neural systems that mediate negative affect, including the limbic system and the amygdala [44, 63].

Automatic responses to affective stimuli and stress can disrupt performance during demanding goal-directed behavior as compared to neutral stimuli [10]. Noteworthy in this context is the fact that DMN activity decreases during goal-directed tasks compared to baseline rest conditions; while the sensorimotor and attention-related cortices become activated during goal-directed tasks [64, 65]. These important observations have practical implications for

health and well-being, through the orientation of attention and can be modulated by training mindfulness. For example, neuroimaging studies have shown that even simply verbally labeling affective stimuli activates right ventrolateral prefrontal cortex and attenuates responses in the amygdala through the ventromedial prefrontal cortex [66–68]. DMN activation can be reduced with attention focused on breath-related sensations [69].

This goal-directed behavior occurs both in breathing meditation, in which the participant is instructed to focus on his breath, as well as during the QMT, emphasizing the importance of training. In addition, we found that while there was no correlation between ABS and GSE scores prior to training, there was a significant correlation between them following training. The relationship between affect balance and self-efficacy in the literature is not strong [70] and can perhaps be strengthened by training.

In conclusion, the current results suggest that QMT can be advantageous for improving affect, and that training can improve perceived self-efficacy. The current findings are in line with recent results demonstrating QMT-induced increased connectivity in the limbic system and strengthen previous claims that mindfulness practices and sensorimotor training may improve well-being. The examination of emotional changes resulting from mindful movement trainings should be conducted in parallel to the examination of changes in neuronal synchronization and functional and structural connectivity. Although we have not conducted this in the current study, future studies should be conducted in order to verify whether local alpha oscillations and limbic connectivity as well as the DMN comprise the underlying electrophysiological mechanism mediating the change in affect. We are currently working in this direction.

At this historic moment, which is characterized by exponential external technological development, which increasingly influences our lives, we need tools to help us cope better with induced stress. What may be required in order to keep pace with this rapid development is a work on the *Inner Design Technology*, in which people, through different training paradigms such as mindfulness and QMT, can manage their emotions. Inner Design Technology and neuroplasticity though training may aid in advancing a state of improved affect and cognition.

Author details

Patrizio Paoletti[1], Joseph Glicksohn[2,3] and Tal Dotan Ben-Soussan[1]*

*Address all correspondence to: research@fondazionepatriziopaoletti.org

1 Research Institute for Neuroscience, Education and Didactics, Patrizio Paoletti Foundation, Assisi, Italy

2 The Leslie and Susan Gonda (Goldschmied) Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat Gan, Israel

3 Department of Criminology, Bar-Ilan University, Ramat Gan, Israel

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Chapter 3

Amygdala and Jaw Movements: A Hodological Review

Yukihiko Yasui

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67581

Abstract

The organization of emotional motor behavior including jaw movements is governed by neural circuits of the limbic system, such as the amygdala and hypothalamus. GABAergic neurons in the central amygdaloid nucleus (CeA) exert an inhibitory influence on premotor neurons for the trigeminal motor nucleus (Vm) in the parvicellular reticular formation (RFp) of the medulla oblongata. The CeA also influences glutamatergic posterior lateral hypothalamic neurons and non-dopaminergic neurons in the retrorubral field of the midbrain, both of which send their axons to Vm-premotor neurons in the RFp. In addition, the CeA may modulate the activity of Vm motoneurons via projections to the mesencephalic trigeminal nucleus whose neurons convey inputs from the masticatory muscle spindles and periodontal ligament receptors to jaw-closing motoneurons in the Vm. These pathways from the amygdala to the trigeminal motor system in the lower brainstem may underlie the regulation of emotional jaw movement.

Keywords: amygdala, motor trigeminal nucleus, jaw movement, neural pathway, emotion

1. Introduction

The amygdala is an almond-shaped set of neurons located deep in the medial temporal lobe of the cerebral hemisphere. Although the amygdala is ontogenetically a part of the basal ganglia, it is a major component of the limbic system. The limbic system is composed of the phylogenetically old limbic lobe, such as the cingulate gyrus and hippocampal formation, and subcortical structures including the amygdala, hypothalamus, anterior thalamic nucleus, and connecting parts (for review, see Ref. [1]). The amygdala is functionally involved in the regulation of instinctive behavior (including sexual and feeding behavior), and in autonomic function as well. The amygdala has also attracted recent attention as a center of emotional expression.

According to Ledoux [2] and Ledoux et al. [3], emotional expression consists of a three-step evaluative process: (1) the acceptance of sensory stimuli, (2) the evaluation of the biological value of the sensory stimuli, and (3) the expression of emotion and subjective experience based on such evaluation. As shown in **Figure 1**, the amygdala receives not only a variety of sensory information from the thalamus and association cortices but also visceral sensory information from brainstem nuclei, such as the nucleus of the solitary tract and parabrachial nucleus. This information serves as emotional stimuli. Within the context of the bases of information of these emotional stimuli sent via the hippocampal formation, the amygdala evaluates the biological value of the emotional stimuli, subsequently inducing a subjective experience of emotion and expression of emotion. Such subjective experiences of emotion (e.g., feelings of rage or pleasure) are processes, which occur within our minds, with the expressions of emotion manifesting as emotional behavior, as well as autonomic and endocrine responses. Emotional behaviors are visible physical changes, of which the orofacial (i.e., jaw) movements being perhaps the more notable.

In this chapter, I would like to describe possible neuronal pathways from the amygdala to the trigeminal motor system that are responsible for emotional jaw movement.

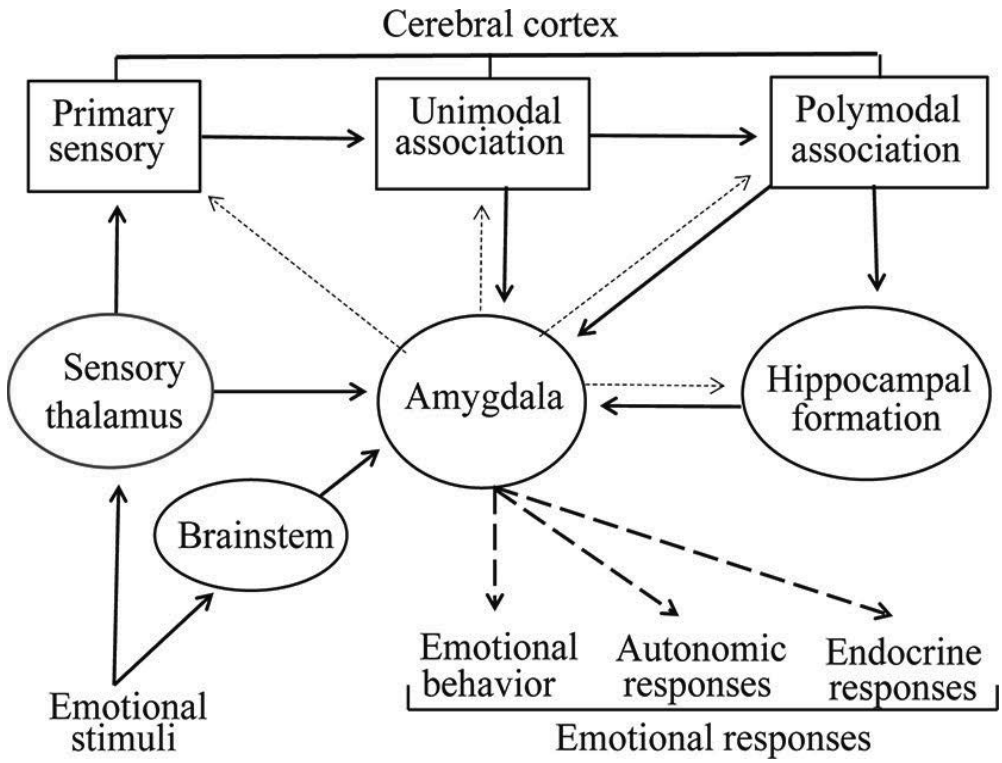

Figure 1. Schematic overview of emotional processing neural circuits (modified from LeDoux [3]). Various forms of sensory inputs that become emotional stimuli are sent to the amygdala via the thalamus and association cortices as well as via the brainstem nuclei. The context of these emotional stimuli is sent to the amygdala via the hippocampal formation. The amygdala integrates this information and evaluates the biological value of emotional stimuli. Subsequently, subjective experience of emotion and expression of emotion are induced. The expression of emotion consists of emotional behaviors, autonomic responses, and endocrine responses.

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2. Amygdala and emotional motor system

The limbic region, known to be involved in emotional functions, has descending motor pathways and influences the responses of both skeletal muscles and autonomic functions. These descending motor pathways are called the "emotional motor system," while the voluntary motor pathways from the motor and premotor cerebral cortex to the brainstem and spinal cord are called the "somatic motor system" [4].

Both the somatic motor and emotional motor systems have medial and lateral components. In the somatic motor system, the medial component consists of pathways, which directly and indirectly control motoneurons innervating axial and proximal body musculature as well as neck muscles. These pathways originate not only in the motor and premotor cortices but also in the brainstem structures, such as the reticular formation, superior colliculus, and red and vestibular nuclei.

The lateral component of the somatic motor system consists of descending pathways originating mainly in the motor cortex and controlling motoneurons innervating distal body muscles, that is, arm, hand, leg, and feet muscles. In the brainstem, the medial and lateral components of the somatic motor system control motoneurons innervating external eye muscles and orofacial muscles, respectively.

The emotional motor system originates from the amygdala, bed nucleus of the stria terminalis, and hypothalamus. Its medial component projects to all parts of the spinal cord, and to all sensory and motor nuclei in the brainstem via, among others, serotonergic neurons in the raphe nuclei and noradrenergic neurons in the locus coeruleus. Due to the diffuse nature of these pathways, the medial component is not involved in specific motor actions but is implicated in setting the general level of activation of all neurons in the brainstem and spinal cord.

The lateral component of the emotional motor system consists of pathways, which control not only motoneurons involved in forming specific emotional behaviors but also neurons involved in regulating autonomic functions accompanying such behaviors. This projection system regulates motoneurons in the brainstem and spinal cord controlling respiration, blood circulation, vocalization, and mating behavior mainly via the periaqueductal gray [5]. Other projections of the lateral component are the descending pathways from the central amygdaloid nucleus (CeA), the bed nucleus of the stria terminalis, and the lateral hypothalamic area (LHA), terminating in the caudal pontine and medullary lateral tegmentum (for review, see Ref. [6]). The lateral tegmentum of the pons contains the parabrachial nucleus and reticular formation around the motor trigeminal nucleus (Vm), while that of the medulla oblongata contains the parvicellular reticular formation (RFp) and ventrolateral medulla. The parabrachial nucleus and ventrolateral medulla contain many neurons involved in autonomic functions, while the reticular formation around the Vm and RFp contains numerous premotor neurons projecting directly to the orofacial motor nuclei including the Vm, facial nucleus (VII), and hypoglossal nucleus (XII).

In light of the above, it is highly likely that projection fibers from the limbic region, such as from the amygdala, to the lateral tegmentum are involved in the regulation of autonomic

functions as well as the control of orofacial muscle movements during aggression, freezing, and other emotional behaviors.

3. Somatotopic arrangement of Vm motoneurons

Jaw movement occurs mainly through the masticatory and suprahyoid muscles attached to the mandibular bone. The masticatory muscles, mylohyoid muscle, and the anterior belly of the digastric muscle are innervated by motoneurons in the Vm located in the dorsolateral tegmentum of the pons. Within the Vm, neurons innervating each muscle are assembled and constitute subgroups. The motoneurons innervating the jaw-closing muscles, such as the masseteric and temporalis muscles, are assembled in the dorsolateral part of the Vm (Vm-dl), while those innervating the jaw-opening muscles, such as the mylohyoid and the anterior belly of the digastric muscle, are assembled in the ventromedial part of the Vm (Vm-vm) [7, 8].

4. Premotor neuron pools for Vm

Jaw movement is triggered by Vm motoneurons, which, in turn, are activated by input from periphery and/or upper motor centers in the brain transmitted to the Vm via interneurons called premotor neurons, primarily located in the brainstem. The Vm-premotor neurons send their axons directly to the Vm, with many distributed in the lower brainstem, such as the pons and medulla oblongata [9–11] (**Figure 2**). A large number of Vm-premotor neurons are found in the sensory trigeminal nuclei including the mesencephalic trigeminal nucleus (Vmes), and principal and spinal trigeminal nuclei. A greater number of them are distributed in the lateral reticular formation, including the reticular formation around the Vm, RFp, and intermediate reticular nucleus (IRt) of the pontomedullary brainstem.

The reticular formation around the Vm, considered an extension of the medullary RFp, consists of the supratrigeminal area dorsal to the Vm, the intertrigeminal area between the principal sensory trigeminal nucleus and the Vm, the juxtatrigeminal area just medial to the Vm, and the reticular formation just ventral to the Vm. The Vm-premotor neurons (excepting Vmes neurons) are generally distributed bilaterally and play a major role in the initiation and regulation of jaw movement, serving as interneurons in brainstem reflexes and transmitting information from upper motor centers to Vm motoneurons. By contrast, Vmes neurons, which innervate jaw-closing muscle spindles and periodontal mechanoreceptors [12–14], send their axons ipsilaterally to Vm motoneurons for controlling jaw movement [13, 15].

The RFp of the medulla oblongata also contains numerous premotor neurons for the facial (VII) and hypoglossal (XII) nuclei. With the aid of a fluorescent retrograde double-labeling technique, Li et al. [16] demonstrated that the RFp contains many premotor neurons projecting bilaterally to one of the orofacial motor nuclei (the Vm, VII, and XII) by branching axons. Their data also indicated that single neurons in the RFp project simultaneously to two

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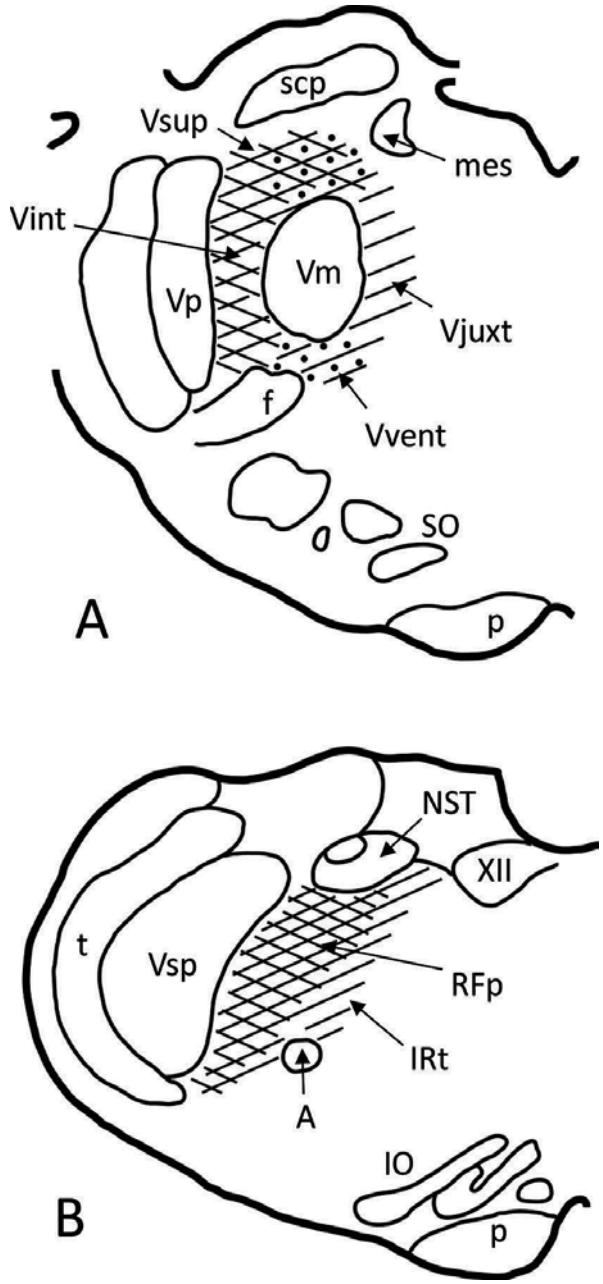

Figure 2. Schematic diagrams of the pons (A) and medulla oblongata (B) depicting the Vm-premotor neuron pools of the rat. A, ambiguus nucleus; f, facial nerve; IO, inferior olivary complex; IRt, intermediate reticular nucleus; mes, mesencephalic trigeminal tract; NST, nucleus of the solitary tract; p, pyramis; RFp, parvocellular reticular formation; scp, superior cerebellar peduncle; SO, superior olivary complex; t, spinal trigeminal tract; Vint, intertrigeminal area; Vjuxt, juxtatrigeminal area; Vm, motor trigeminal nucleus; Vsp, spinal trigeminal nucleus; Vsup, supratrigeminal area; Vvent, reticular formation ventral to the Vm; Vp, principal trigeminal nucleus; XII, hypoglossal nucleus.

orofacial motor nuclei (Vm and VII, Vm and XII, or VII and XII) [17]. These data suggest that RFp neurons may serve to synchronize not only the activity of Vm, VII, or XII motoneurons on both sides but also the activity of Vm and VII, Vm and XII, or VII and XII motoneurons. However, it remains unknown whether or not single premotor neurons project by way of axon collaterals simultaneously to all orofacial motor nuclei.

5. Descending pathways from the amygdala to the Vm

Stimulation of the amygdala results in changes of autonomic functions and emotional behaviors, and is known to have an effect on orofacial movements, particularly jaw movement [18]. As noted above, jaw movement is controlled by Vm motoneurons. Control pathways from the amygdala to the Vm have been examined using anterograde and/or retrograde axonal tracing in anatomical studies. Such axonal tracing combined with immunohistochemistry or *in situ* hybridization has provided the means of further investigation of neurotransmitters, their receptors and transporters in the neural circuits.

5.1. Neuroanatomical organization

5.1.1. CeA-RFp-Vm pathway

The direct CeA-Vm pathway was suggested by Mascaro et al. [19], who observed retrograde-labeled neurons in the CeA after Fluoro-gold (FG) injection into the Vm. On the other hand, anterograde-tracing studies have shown that biotinylated dextran amine (BDA)-labeled fibers with bouton-like varicosities are distributed around the Vm but not within the Vm after BDA injection into the CeA, suggesting a low probability of the existence of a direct CeA-Vm pathway [20, 21].

However, the existence of a disynaptic pathway from the CeA to the Vm via the supratrigeminal area [22] or via the lateral reticular formation of the pons [23] has been confirmed by anatomical studies using a combined degeneration and horseradish peroxidase method. A physiological study [24] also produced data supporting the existence of disynaptic inputs from the amygdala to the Vm mediated by the supratrigeminal area. It has also been established that descending projection fibers from the CeA are distributed in the RFp of the lower brainstem [25–27]. Furthermore, the RFp of the medulla oblongata contains many premotor neurons that project directly to the Vm [9–11, 28, 29]. Taken together, these data make it likely that the CeA also exerts its influences on the regulation of jaw movement through the disynaptic pathway from the CeA to the Vm via the RFp of the medulla oblongata.

We confirmed the existence of a CeA-RFp-Vm pathway by using a combination of anterograde- and retrograde-tracing techniques [20]. When ipsilateral injections of BDA into the CeA and of cholera toxin B subunit (CTb) into the Vm were made in a rat, a significant overlap of BDA-labeled axons and CTb-labeled neurons was found in the RFp region just ventral to the nucleus of the solitary tract and medial to the spinal trigeminal nucleus throughout the caudal-most part of the pons and the rostral half of the medulla oblongata (**Figure 3**).

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When the neuropil of the RFp was viewed under an electron microscope, BDA-labeled axons showed symmetrical synaptic contacts predominantly with dendrites and additionally with somata of the RFp neurons, some of which were labeled with CTb.

5.1.2. CeA-PLH-RFp-Vm pathway

Anterograde-tracing studies with *Phaseolus vulgaris*-leucoagglutinin [30, 31] and BDA [32] indicate that the posterior lateral hypothalamus (PLH) receives a dense projection from the CeA; the PLH is just medial to the subthalamic nucleus and has been identified as the parasubthalamic nucleus by Wang and Zang [33].

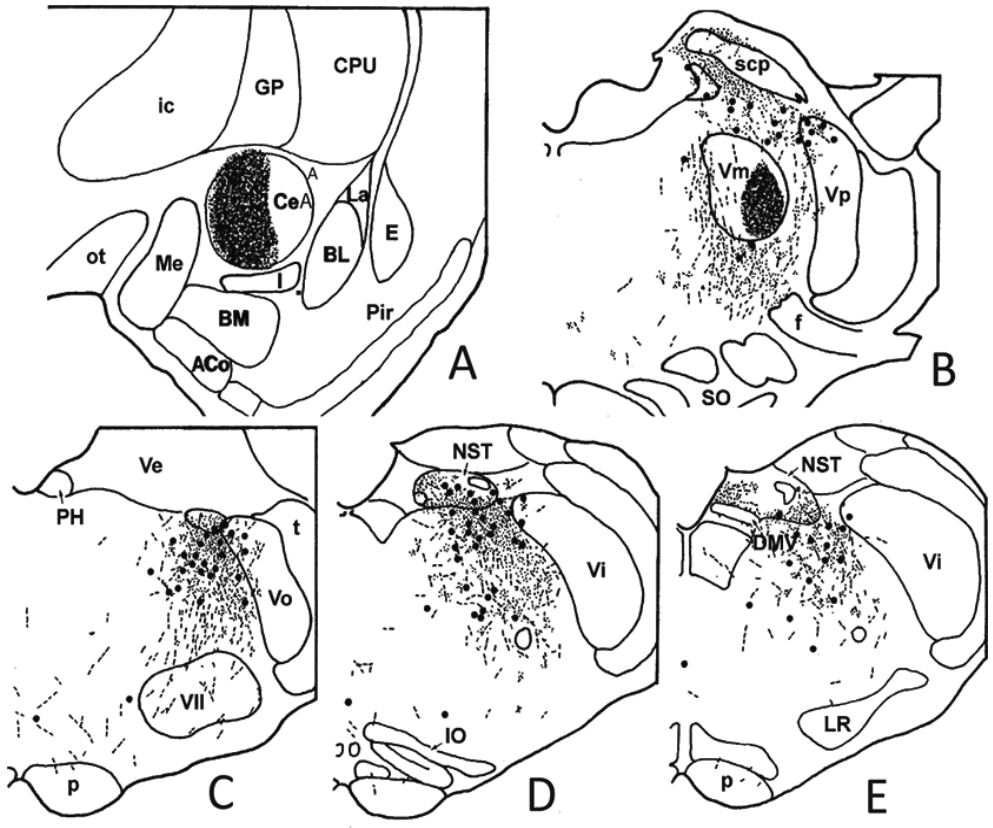

Figure 3. Projection drawings showing the sites of BDA injection into the CeA (shaded area in A) and CTb injection into the Vm (shaded area in B), and resulting anterograde and retrograde labeling in the lower brainstem ipsilateral to the injection sites (B–E, rostral to caudal). BDA-labeled fibers and terminals are presented by fine lines and fine dots, respectively. CTb-labeled cell bodies are presented by filled circles, each of which represents approximately two labeled cell bodies. ACo, anterior cortical amygdaloid nucleus; BL, basolateral amygdaloid nucleus; BM, basomedial amygdaloid nucleus; CPU, caudate-putamen; GP, globus pallidus; CeA, central amygdaloid nucleus; DMV, dorsal motor nucleus of the vagus nerve; E, endopiriform nucleus; Me, medial amygdaloid nucleus; I, interstitial amygdaloid nucleus; ic, internal capsule; La, lateral amygdaloid nucleus; LR, lateral reticular nucleus; ot, optic tract; PH, prepositus hypoglossal nucleus; Pir, piriform cortex; Ve, vestibular nucleus; Vi, interpolar subnucleus of the spinal trigeminal nucleus; Vo, oral subnucleus of the spinal trigeminal nucleus; VII, facial nucleus. Other abbreviations are as in **Figure 2** (modified from Yasui et al. [20]).

Stimulation of the LHA region including the PLH is known to promote not only a jaw-closing reflex [34] but also the activity of the masseter muscle [35]. However, there have been no anterograde-tracing studies, which demonstrate the direct hypothalamo-Vm projection, though the existence of peptidergic projections from the LHA to the Vm has been reported, as is described below. Further, after BDA injection into the PLH, we observed only some passing BDA-labeled fibers without bouton-like varicosities within the Vm, counter-indicative of the existence of a direct PLH-Vm pathway [36].

Within the hypothalamus, the majority of RFp-projecting neurons are localized in the PLH region [36]. Although Mascaro et al. [19] observed a large number of FG-labeled neurons in the PLH region after FG injection into the Vm, there is a high possibility that these labeled neurons project to the reticular formation around the Vm, but not to the Vm. A combined anterograde- and retrograde-tracing techniques have shown the prominent overlap of distribution of PLH fibers and Vm-premotor neurons in the RFp region just ventral to the nucleus of the solitary tract and medial to the spinal trigeminal nucleus (**Figure 4**); further, the PLH axon terminals make asymmetrical synaptic contact with dendrites and somata of the RFp neurons, some of which were labeled with CTb injected into the Vm [36]. The results of these recent and earlier studies raise the possibility of the existence of a CeA-PLH-RFp-Vm pathway, and that RFp-projecting PLH neurons are under the direct influence of the CeA.

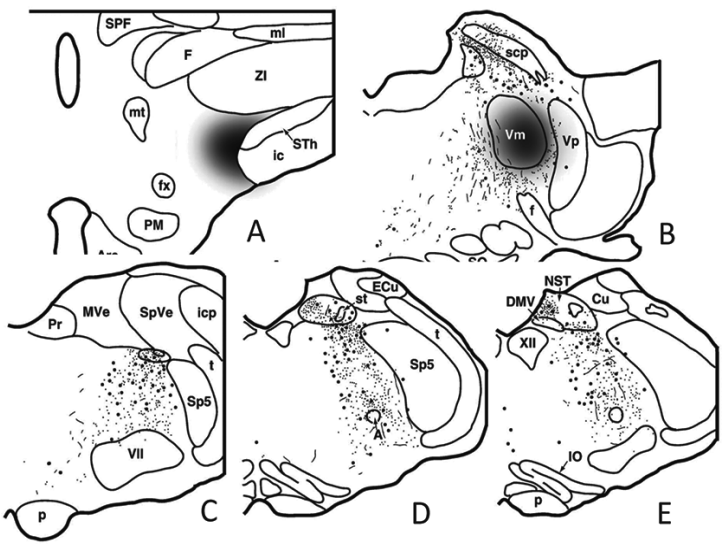

Figure 4. Line drawings showing the sites of BDA injection into the PLH (shaded area in A) and CTb injection into the Vm (shaded area in B), and resulting anterograde and retrograde labeling in the lower brainstem ipsilateral to the injection sites (B–E, rostral to caudal). BDA-labeled fibers and terminals are presented by fine lines and fine dots, respectively. CTb-labeled cell bodies are presented by filled circles, each of which represents approximately two labeled cell bodies. Arc, arcuate nucleus; Cu, cuneate nucleus; ECu, external cuneate nucleus; F, nucleus of the fields of Forel; fx, fornix; icp, inferior cerebellar peduncle; ml, medial lemniscua; mt, mammillothalamic tract; MVe, medial vestibular nucleus; PM, premammillary nucleus; Pr, prepositus hypoglossal nucleus; SPF, subparafacicular nucleus; SpVe, spinal vestibular nucleus; Sp5, spinal trigeminal nucleus; st, solitary tract; STh, subthalamic nucleus; ZI, zona incerta; Other abbreviations are as in **Figures 2** and **3** (modified from Notsu et al. [36]).

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5.1.3. CeA-SN/RRF-RFp-Vm pathways

A dense projection from the CeA has been observed in the lateral part of the substantia nigra (SN) pars compacta as well as in the SN pars lateralis, a part of the SN pars reticulata [37]. Both the lateral part of the SN pars reticulata and the lateral part of the SN pars compacta are known to contain neurons projecting to the reticular region around the Vm [38], as well as to the RFp of the medulla oblongata [39]. These data suggest the existence of a disynaptic pathway from the CeA to the RFp via the SN, which is responsible for the control of jaw movements.

CeA fibers are also densely distributed in the lateral portion of the retrorubral field (RRF) [37, 40] which is an area dorsal and caudal to the SN and is believed to be involved in orofacial motor function [41, 42]. Additionally, the RRF region contains a population of neurons that project their axons to the pontomedullary RFp [43, 44]. Taken together, these data indicate that the output signals from the CeA have a direct influence on the RRF-RFp pathway for the control of orofacial movements including the jaw movement. Following ipsilateral injections of BDA into the CeA and FG into the RFp, we noted a prominent overlap of the distribution of BDA-labeled fibers and FG-labeled neurons in the lateral part of the RRF ipsilateral to the injection sites, where BDA-labeled axon terminals make symmetrical synapses with somata and dendrites of the FG-labeled neurons [45] (**Figure 5**).

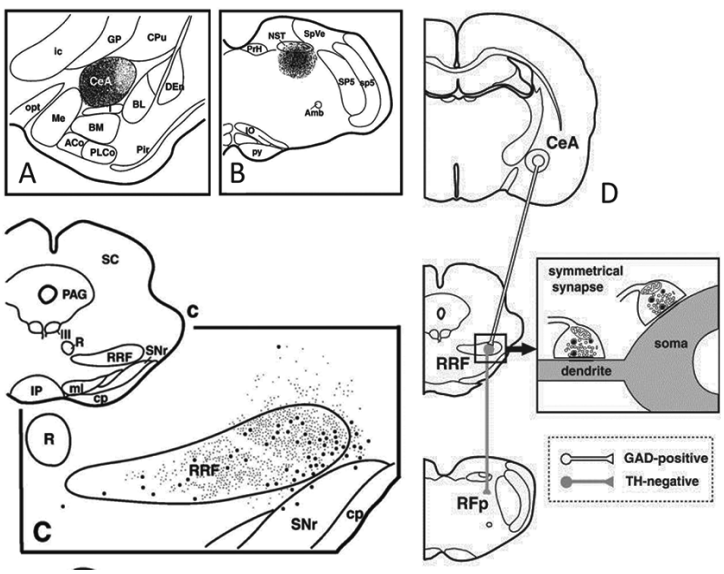

Figure 5. Line drawings illustrating the sites of BDA injection into the CeA (shaded area in A) and FG injection into the RFp (shaded area in B), and resulting distributions of BDA-labeled terminals (small dots) and FG-labeled neurons (large dots) in the RRF region (C). RRF region in the midbrain section (c) is enlarged in C. In D, the synaptic organization between CeA axon terminals and RFp-projecting RRF neurons is illustrated. Amb, ambiguous nucleus; cp, cerebral peduncle; Den, dorsal endopiriform nucleus; IP, interpeduncular nucleus; ml, medial lemniscus; opt, optic tract; PAG, periaqueductal gray; PLCo, posterolateral cortical amygdaloid nucleus; PrH, prepositus hypoglossal nucleus; py, pyramis; R, red nucleus; RRF, retrorubral field; SC, superior colliculus; SNr, substantia nigra pars reticulata; SP5, spinal trigeminal nucleus; sp5, spinal trigeminal tract; TH, tyrosine hydroxylase; III, oculomotor nucleus. Other abbreviations are as in **Figures 2–4** (modified from Tsumori et al. [45]).

5.1.4. CeA-Vmes-Vm pathway

The Vmes contains somata of primary afferent neurons whose peripheral processes are distributed in the muscle spindles of jaw-closing muscles and mechanoreceptors within the periodontal ligaments. According to Nomura and Mizuno [13], Vmes neurons that conduct jaw-closing muscle afferent are distributed throughout the whole rostrocaudal extent of the Vmes, while Vmes neurons, which conduct periodontal receptor afferent, are located mainly in the caudal part of the Vmes. The central processes of Vmes neurons terminate mainly in the Vm and additionally in the premotor reticular formation.

Recently, anterograde-tracing studies [21, 46] reported that the CeA sends projection fibers to the Vmes where CeA axonal varicosities are in close apposition to the somata of Vmes neurons. Further, Shirasu et al. [47] have shown that anterograde-labeled terminal buttons on Vmes neuronal somata are more abundantly present in the caudal than in the rostral Vmes after a wheat germ agglutinin conjugated to horseradish peroxidase injection into the CeA. The same study also indicated that a portion of these terminal buttons form axo-somatic synapses with Vmes neurons, and that the CeA has direct projections to the Me5, suggesting that the amygdala regulates bite strength by modifying neuronal activity in the Vmes.

5.2. Neurochemical organization

5.2.1. Neurotransmitter of CeA neurons

In situ hybridization studies demonstrated that almost all the CeA neurons are positive for glutamic acid decarboxylase (GAD) 65 mRNA [48] and GAD67 mRNA [49] but not for vesicular glutamate transporter (VGLUT) 1 mRNA [48] and VGLUT2 mRNA [48, 49] (**Figure 6**); GAD is an enzyme that converts glutamate to GABA and is utilized as a marker for GABAergic neurons, while VGLUT1 and VGLUT2 are used as markers for glutamatergic neurons. In an earlier study, we demonstrated that most CeA axon terminals in the RRF are immunoreactive for GAD [45]. Such GABAergic CeA axon terminals have been observed in other brainstem regions including the parabrachial nucleus [50] and nucleus of the solitary tract [51, 52], as well as in the forebrain regions including the parastrial nucleus [53] and LHA [32, 54]. These findings are all indicative of CeA projection neurons being GABAergic.

5.2.2. Neurotransmitter of hypothalamic neurons

Hypothalamic neurons labeled with CTb injected into the Vm region display glutamate-like immunoreactivity [55]. VGLUT is considered to represent the most specific marker for neurons using glutamate as a transmitter [56], and LHA neurons express predominantly VGLUT2 mRNA and additionally VGLUT1 mRNA [57]. It has also been reported that axon terminals with glutamate immunoreactivity [58–60] or with VGLUT2 immunoreactivity [61] make asymmetrical synapses with their target structures. We previously demonstrated that PLH axon terminals with VGLUT2 immunoreactivity make asymmetrical synapses with RFp neurons, suggesting that glutamatergic PLH neurons exert excitatory influence upon RFp neurons [36].

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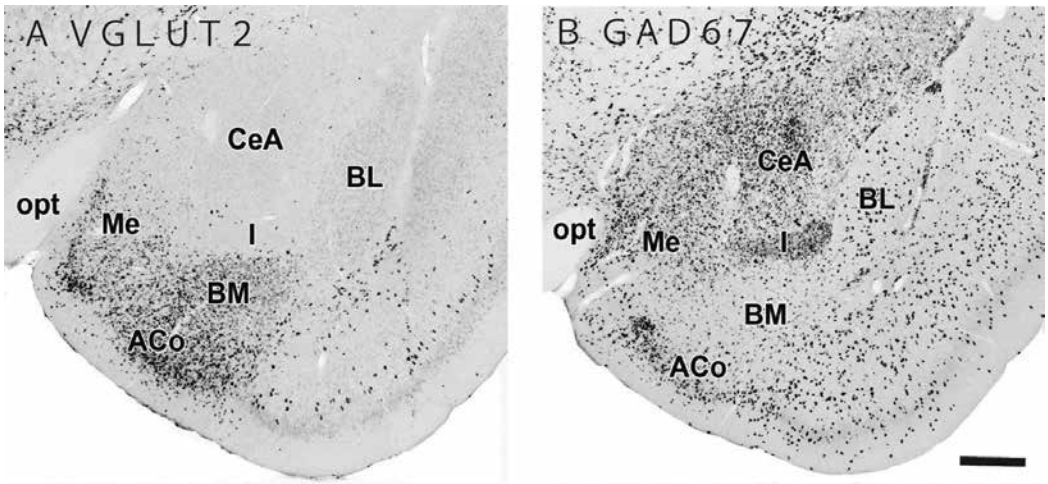

Figure 6. Photomicrographs showing the *in situ* hybridization staining for VGLUT2 mRNA (A) and GAD67 mRNA (B) in the amygdaloida. Scale bar = 500 μm (modified from Oka et al. [49]).

By using retrograde tracing in combination with immunohistochemical methods, studies [62, 63] have demonstrated at the light microscopic level that orexin (ORX)-containing hypothalamic fibers are in contact with Vm motoneurons, though there are only sparse ORXergic fibers in the Vm. More recently, Mascaro et al. [46] documented that ORXergic fibers are distributed in the Vm and that approximately one-third of the LHA neurons projecting axons to the Vm are immunoreactive for ORX. McGregor et al. [55], who also found ORXergic Vm-premotor neurons in the LHA, further indicated that Vm-premotor neurons in the LHA as well as in the perifornical nucleus are immunoreactive for melanin-concentrating hormone (MCH). Saito et al. [64] reported that MCH-immunoreactive fibers are distributed in the Vm where many neurons express MCH receptor mRNA. Interestingly, our previous study [54] indicated that GABAergic CeA neurons innervate MCH- and ORX-containing hypothalamic neurons.

Taken together, these data suggest that the CeA plays a role in the control of neuronal activity in the Vm by means of its inhibitory influence upon MCH- and ORX-containing hypothalamic neurons.

5.2.3. Neurotransmitter of SN and RRF neurons

The SN has two distinct parts: the SN pars compacta and the SN pars reticulata. Dopamine neurons are found predominantly in the SN pars compacta, while the pars reticulata is populated largely by GABA neurons. A densely packed group of dopaminergic neurons in the SN pars compacta is known as the A9 dopamine cell group. The RRF also contains many dopaminergic neurons referred to as the A8 dopamine cell group. SN neurons projecting to the brainstem regions, such as the inferior colliculus [65] and reticular formation around the Vm [38], are not immunoreactive for tyrosine hydroxylase (TH), which catalyzes the rate-limiting

step in the synthesis of catecholamine. We demonstrated in a previous study that RRF neurons projecting to the RFp are immunonegative for TH [45].

Such findings suggest that both the SN and RRF neurons sending their axons to the lower brainstem are non-dopaminergic and most likely GABAergic [66]. Future studies combining retrograde tract-tracing with immunolabeling for GABA or GAD, or with *in situ* hybridization for GAD mRNA, should help to demonstrate this.

5.2.4. Neurotransmitter of RFp neurons

As for neurotransmitter phenotypes, the RFp is heterogeneous and contains glutamatergic, GABAergic, cholinergic, and nitrergic neurons [67]. According to Pang et al. [68], VGLUT2-immunoreactive axon terminals, distributed in both the Vm-dl and the Vm-vm, originate from Vm-premotor neurons in the reticular formation such as the reticular region ventral to the Vm and RFp, as well as in the sensory trigeminal nuclei. They also observed VGLUT1-immunoreactive terminals within the Vm-dl only and demonstrated that these terminals originate from the reticular region ventral to the Vm as well as from the Vmes. Travers et al. [67] noted that approximately half of the Vm-premotor neurons in the RFp and IRt are immunoreactive for VGLUT2.

On the other hand, it has been reported that inhibitory Vm-premotor neurons immunoreactive for GAD or glycine are also distributed in the RFp [69]. In another study, Travers et al. [66] indicated that a quarter of the Vm-premotor neurons in the RFp and IRt are immunoreactive for GAD65/67, and that relatively few Vm-premotor neurons in the RFp and IRt are either nitrergic or cholinergic.

5.2.5. Neurotransmitter of Vmes neurons

It is known that Vmes neurons are glutaminergic [70, 71]. Also, a recent study [72] demonstrated that VGLUT1 mRNA is expressed in the cell bodies of Vmes neurons and showed VGLUT1 immunoreactivity in both the central axon terminals and peripheral sensory endings of Vmes neurons. Pang et al. [73] further noted that VGLUT1-immunoreactive terminals observed in the Vm-dl but not in the Vm-vm come from primary afferent Vmes neurons, whereas the VGLUT2-immunreactive terminals observed in both the Vm-dl and the Vm-vm come from Vm-premotor neurons, as previously stated.

6. Conclusion

The neuroanatomical and neurochemical organization of the control pathways from the CeA to the Vm is summarized in **Figure 7**. The CeA exerts its influence upon Vm motoneurons through its direct and indirect projections to Vm-premotor neurons, including RFp and Vmes neurons. These projections are responsible for the control of specific emotional motor activities of the trigeminal system. The RFp is a major premotor neuron pool not only for the Vm

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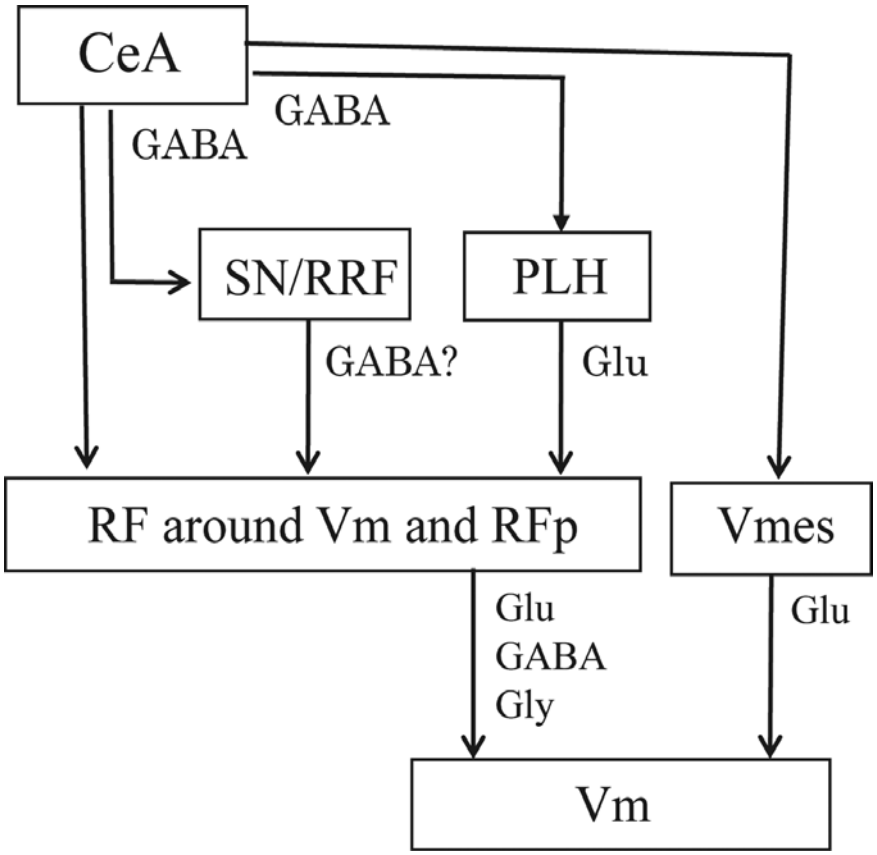

Figure 7. Summary diagram showing the control pathways from the CeA to the Vm, The CeA controls jaw movements through its direct projections to Vm-premotor neurons in the reticular formation (RF) around the Vm in the pons, RFp in the pontomedullary and medullary brainstem, as well as in the Vmes. In addition, the CeA control jaw movements through its indirect projections to these premotor neuron pools via the SN/RRF or via the PLH. The neurotransmitter(s) used in each pathway are also indicated. Glu, glutamate; Gly, glycine.

but also for the VII and XII. It is therefore likely that the amygdala controls orofacial movements during various emotional behaviors through its projections to the orofacial motor nuclei relayed by the RFp.

Acknowledgements

The authors are grateful for the support from Drs. R. Fujimoto, K. Fujita, T. Goeku, M. Kuzushima, T. Mukai, Y. Munenaga, T. Sakurai, Y. Sakurai, H. Taguchi, K. Tanaka, K. Urashima, H. Yamaoka, S. Yamazaki, and K. Yoshida. This study was partly supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Science, Sports, and Culture of Japan.

Author details

Yukihiko Yasui

Address all correspondence to: yyasui@med.shimane-u.ac.jp

Department of Anatomy and Morphological Neuroscience, Shimane University School of Medicine, Izumo, Japan

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Chapter 4

Looping Circuits: Amygdalar Function and Interaction with Other Brain Regions

Diana Peterson

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67836

Abstract

The ability to understand the relevance of environmental cues is necessary for animals to adapt and survive. How the brain interprets, understands, and reacts to stimuli is only partially understood. Such higher-order brain processes occur within series of highly interconnected brain circuits that allow the brain to alter its response appropriately to an ever changing environment. The amygdala is one of the brain regions that determine the significance of incoming environmental stimuli. Once the significance of a stimulus or set of stimuli is determined, other circuits utilize this information to initiate physiological and behavioral responses (e.g., alter the attention of the animal to relevant sensory cues, change the emotional state, initiate fight or flight responses, etc.). Because circuits between the amygdala and other brain regions are highly interconnected, dysfunctions in one region of the brain can influence several other brain regions. Such alterations in normal activity can induce psychiatric, psychosocial, or attentional symptomatology. Therefore, identifying the role of individual circuits as well as the interconnected nature of these circuits is essential for understanding how a normal individual survives and adapts to its environment. It also provides the knowledge necessary to devise therapies for both the cause and symptoms of psychosis.

Keywords: stimulus significance, sensory, learning, memory, psychiatric disorders

1. Introduction

To react appropriately within the environment, an individual must first gather information via sensory organs and systems (e.g., sound, sight, smell, touch, taste). Sensory information is then transmitted up to sensory cortex, where the sensation is perceived. Additional circuits route sensory information to the amygdala where it is combined with information from other sensory modalities as well as stored information from memory, association, and executive

cortices. Amygdalar neurons utilize these combinations of inputs to identify the significance of the various environmental stimuli [1–3]. For example, the call of a cat is significant to its prey (e.g., a mouse). Information about the cat vocalization (frequency spectrum, tone, and attenuation) is processed within the auditory system and perceived by the mouse within cortex. This information is sent to the amygdala where it is combined with other sensory information (e.g., cat smell or visualization of the cat's movements), as well as information from the mouse's prior experiences with the set of stimuli to provide an environmental context for a specific experience [4]. These sets of inputs (sensory, memory, and association) are combined within the amygdala to determine whether the given set of stimuli is significant to an individual [4].

Stimulus significance can influence both hormonal and behavioral responses through an intricate system of loops between the amygdala and other brain regions [4]. The amygdala projects directly or indirectly to sensory cortices, as well as modulatory, memory, motor, autonomic, stress, emotional, decision, and executive brain regions [1, 3–5]. Many of these areas of the brain have reciprocal projections back to the amygdala as well as connections between each other; therefore, small modifications in the excitation of one brain region can influence a cascade of adaptations throughout the brain. As each additional brain region is affected, projections from these regions influence both the original nuclei as well as additional looping circuits.

Dysfunctions within one or more of the interconnected circuits can lead to a dissonance within different brain regions or with the timing of inputs within a specific brain region. These alterations in firing patterns can alter processing and dramatically influence an individual's behavioral responses to their environment. Because numerous brain regions are affected, alterations in normal activity can present clinically as a group of seemingly disparate symptoms (e.g., hallucinations, loss of attention, alterations in decision making, working memory, emotional state, etc.) commonly observed in psychiatric disorders [6]. Identifying how brain circuits interact is crucial for understanding normal brain function as well as recognizing how variations can result in dysfunction. Because of the complexity of the circuit interactions, our current understanding is substantially incomplete.

2. Amygdalar circuits: sensory

The amygdala receives and sends projections to multiple regions of the brain (**Figure 1A**). Anterograde, retrograde, or autoradiography studies indicate that the amygdala receives both direct and indirect inputs from each sensory system (**Figure 1A**). Inputs include reciprocal projections from association cortices from each sensory modality (auditory [7, 8]; gustatory [9, 10]; olfactory [11, 12]; somatosensory [4, 13]; vision [14]). These association regions are known for higher-order sensory processing such as visualization of complex movements, facial recognition, and speech processing [15, 16]. Additional polymodal sensory regions have been shown to have reciprocal projections to the amygdala [17]. Such polymodal inputs provide complex sets of sensory information that are necessary components of environmentally relevant stimuli. Because the processing for these combined sensory stimuli are outsourced in

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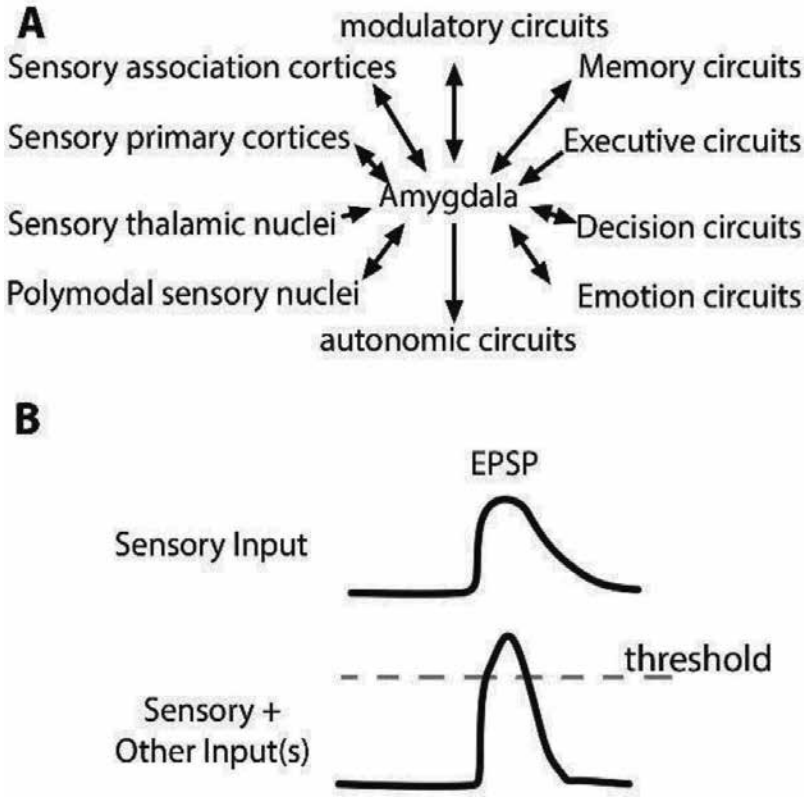

Figure 1. Numerous regions of the brain project to the amygdala (A). Amygdalar neurons may be combination sensitive, having facilitated neuronal responses to multiple inputs (B). EPSP = excitatory post-synaptic potential.

other brain regions, polymodal inputs may facilitate faster responses to these stimuli within the amygdala [18–21].

There is evidence that the amygdala receives some primary or simple sensory information from both cortical and thalamic nuclei. Auditory, somatosensory, and visual thalamic nuclei have direct projections to the amygdala [4, 22–25]. Because the regions of thalamus that project to the amygdala are known to receive both simple as well as polymodal sensory input, the type of sensory input (complex or simple) coming from these regions is not well understood.

Another source of simple sensory information is primary sensory cortices. The olfactory cortex has direct projections to the amygdala [26–28]. It is still debated whether the primary regions of other sensory cortices have direct projections or not. Several studies indicate that the amygdala receives no or few inputs from primary auditory, visual, or somatosensory cortices [17]. However, most of these studies utilized nonspecific techniques. Studies using fluorescent tracing techniques observe a small or moderate reciprocal connection with auditory cortex (preliminary data from my laboratory [8, 27]). Similar experiments in other primary sensory cortices are needed to confirm the existence of similar pathways for the other sensory modalities.

3. Amygdalar circuits: conditioned response

Classical conditioning is the pairing of an unconditioned stimulus (e.g., foot-shock) with a conditioned stimulus (e.g., sound of a bell). When the two are repeatedly paired together, the conditioned individual associates the two stimuli with each other (i.e., the foot-shock with hearing the bell). Once the pairing is learned, individuals exhibit a similar behavioral response any time they hear the bell whether the shock follows or not [2]. The memory of the rule allows individuals to respond faster to subsequent stimuli, and thus facilitates more timely, accurate, and environmentally advantageous behaviors.

Because the environment is constantly changing, conditioning can induce either long- or short-term associations. Short-term associations are important for allowing individuals to react to the fast-paced shifts in the context of environmental signals. Short-term plasticity can be mediated by altering the firing rate of cortical neurons through local circuit facilitation, inhibition, or disinhibition. Long-term associations are important for creating behaviorally relevant rules that will be important for the individual over extended periods of time. These longer associations can be created by altering receptors and dendritic spines to strengthen connections between regions of the brain [29].

Conditioning begins within the amygdala. Amygdalar neurons typically respond to significant or behaviorally relevant pairings of stimuli [18, 30–32]. Physiological recordings in the amygdala, for example, show an increase in neuronal firing to stimuli with positive or negative affect but no increased response to stimuli with neutral affect [18, 26]. During experimental conditioning, behavioral relevance can be created, and thus amygdalar responses provoked. During fear conditioning, a negative affect can be created by pairing a sensory stimulus (sound) with a noxious stimulus (foot-shock). Single-unit recordings in the amygdala indicate that amygdalar neurons have a higher rate of firing to the combined sound and foot-shock stimuli than to a sound alone [33]. They also respond with higher firing rates to novel combinations of inputs than to previously learned combinations [32]. Such deference to novelty information indicates that circuits from memory or association cortex are likely to provide additional inputs that influence the firing rate. Therefore, combinations of inputs from auditory and sensory cortex, as well as specific inputs from memory or association cortex, can increase the firing properties of AM neurons. These neuronal responses are created by combining inputs from multiple sensory stimuli with inputs that provide information about previous memories (context; e.g., hippocampus), prior motivation (rewards/punishments; prefrontal cortex), as well as other prior associations about similar sets of stimuli (association cortices; **Figure 1**).

The mechanism by which amygdalar neurons have higher rates of activity when presented with two distinct inputs (i.e., neuronal facilitation) is not known. It has been hypothesized that combinations of inputs alter amygdalar neuronal excitability and that these changes encode information about stimulus significance [2, 4]. This could mean that neurons within the amygdala are combination sensitive, a process by which combinations of inputs can facilitate or inhibit neuronal responses when presented within specific temporal delays. Combination sensitivity has been observed in several different brain regions (e.g., lateral lemniscus, inferior

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colliculus [34–37]). Within the auditory system, combination-sensitive neurons are thought to encode specific spectral-temporal features of sound combinations to facilitate functions such as deciphering speech patterns or echolocation information. These types of responses have been observed within the amygdala (preliminary data from my laboratory); however, only a few of these types of responses were observed. The lack of abundant responses may have been due to the selection of stimulus combinations we presented to our animals, or it may indicate that simple electrical summation (i.e., each input has a hyperpolarization influence on the targeted amygdalar neuron that when added together can surpass threshold) is more prevalent within the amygdala. In either scenario, it is likely that multiple inputs are required to hyperpolarize the amygdalar neuron (**Figure 1B**).

Longer-term associations within the amygdala are thought to occur through anatomical changes in receptors, transmission, activity, and dendritic spines of amygdalar neurons (long-term potentiation). This type of long-term change has been observed after electrical stimulation of the thalamus, external capsule, hippocampus, and entorhinal-amygdalar circuits [37–40]. Although the direct circuits that influence this type of amygdalar change are known, these types of experiments create extended excitatory influences from the stimulated brain region that could excite a plethora of secondary or tertiary looping circuits. For example, excitation of the medial geniculate nucleus (auditory thalamus) could influence amygdalar processing through direct excitation as well as indirect excitation through auditory cortex, auditory association cortices, polymodal cortices, as well as memory network circuits (i.e., thalamus-association cortex-hippocampus-amygdala), prefrontal cortex circuits (i.e., thalamus-association cortex-prefrontal cortex-amygdala), or modulatory centers (e.g., thalamus-auditory cortex-nucleus basalis-amygdala). Each of these circuits is likely to have heightened excitatory influences, and when combined results in long-term alterations in amygdalar activity.

Once significance is determined within the amygdala, this information is sent to a variety of brain regions to allow the brain to consolidate that new information within memory, decide how to respond, create or modulate rules about behavior, and provide appropriate physiological and behavioral responses. Projections from the amygdala to the nucleus basalis of Meynert, for example, initiates cholinergic modulation of cortical neuronal responses that are thought to shift the attention of an individual toward relevant sensory cues. Nucleus basalis projections are also sent to memory, association, and frontal network circuits to facilitate alterations in activity based on conditioning [1, 3, 41, 42].

Numerous experiments have shown that cholinergic projections from the nucleus basalis are involved with cortical conditioning. Lesions of the nucleus basalis have been shown to inhibit learning acquisition [43], while electrical stimulation studies induced significant changes in cortical activity as well as increases in the retrieval of behavioral learning tasks [44–49]. Electrical stimulation, in these experiments, was thought to facilitate acetylcholine release from the nucleus basalis, which in turn increased the animal's ability to consolidate learned information. This hypothesis was substantiated by experiments that applied atropine (acetylcholine antagonist) to auditory cortex to eliminate the influence of the nucleus basalis pathway. These experiments showed a complete inhibition of the conditioned responses, thus indicating that acetylcholine circuits are necessary for conditioned-based plasticity in

cortex [50]. Together, these results provide a substantial argument that amygdalo-nucleus basalis projections excite cholinergic neurons that project to auditory cortex, and that these neurons facilitate long-term plasticity in cortex that is based on conditioning.

Acetylcholine has modulatory effects; therefore, cholinergic inputs by themselves are not sufficient to induce long-term plasticity in cortex. Experiments that emphasize this point have examined the timing of cholinergic-cortical inputs at various temporal intervals surrounding conditioning. The results indicate that nucleus basalis stimulation before or within the first few minutes of conditioning augmented conditioned responses [48]. Similarly, atropine applied directly to the surface of cortex eliminated these changes [51]. In other experiments, similar atropine applications given 35 and 55 min postconditioning decreased observed plasticity by 56 and 66%, respectively [51]. These results indicate that acetylcholine is an essential component for conditioned response facilitation and that the timing of its inputs in cortex can alter plastic effects within cortex. It is unclear what additional inputs when combined with the cholinergic inputs are necessary to elicit the conditioning induced changes in cortex.

Potential circuits that could facilitate cortical plasticity based on conditioning include inputs from the ascending sensory system, other sensory cortices, association cortices, memory or decision cortices, direct inputs from the amygdala, or looping circuits between these regions [4, 49]. Cholinergic circuits from the nucleus basalis target many of the cortical input circuits mentioned above [42]. These cholinergic circuits could have a variety of influences that individually or combined may help facilitate cortical plasticity. They may enhance amygdalar stimulation (to strengthen learned stimuli significance), strengthen synapses between the thalamus and cortex, or improve memory consolidation within the hippocampus [52]. In addition, the hippocampus projects back to the amygdala, nucleus basalis, prefrontal cortex, and frontal cortex [53, 54]. These circuit loops can strengthen or diminish activity within each of these other regions [53, 54]. Because all of the regions above have either direct or indirect projections to auditory cortex, there are several cortical inputs that are likely candidates to generate long-term plasticity within cortex.

Although conditioning research has primarily focused on cholinergic projections, the nucleus basalis has additional GABAergic projections to auditory cortex [4, 55, 56]. The GABAergic projections from the nucleus basalis, as well as the direct amygdalo-cortical projection, have been shown to alter the spiking characteristic of cortical neurons. However, these changes are transient and do not seem to alter the frequency specificity of neurons [57, 58]. Because frequency shifts may indicate a long-term plastic change, it is hypothesized that the direct amygdalo-cortical and indirect GABAergic pathways from the nucleus basalis may function to alter the short-term attention of the animal, while the cholinergic projection from the nucleus basalis may have a longer-term plastic effect based on conditioned responses [1, 2].

4. Amygdalar circuits: memory

Several regions of the brain have been shown to interact during consolidation of long-term declarative memory (perirhinal cortex, entorhinal cortex, parahipocampus, and hippocampus [59]).

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The amygdala has extensive reciprocal connections with these nuclei [17, 59]. Because disruption of the circuits between the amygdala and this memory network can lead to deficits in contextual conditioning [60], it has been hypothesized that contextual information is determined within the memory network and then transmitted to the amygdala. However, environmental stimuli are not static. Therefore, another interpretation could be that the memory system provides information about an individual's prior experience with a set of stimuli (prior context). This prior information, when combined with current sensory inputs, could then help to shape the evolving (present context) of environmental cues within the amygdala.

Because the circuits between the amygdala and memory networks are reciprocal [17], these circuits have also been hypothesized to enable declarative memory information from the hippocampus to be integrated with emotional memories within the amygdala [61]. This hypothesis, while fundamentally sound, seems fairly limited in scope. It is reasonable to imply that emotional memories must include amygdalar processing, because emotion is not viable without salience information; however, the amygdala is not the only brain region involved with this process. Neurons within several brain regions have exhibited characteristics such as prolonged neuronal activity, formulate long-term potentiation responses, or exhibit altered dendritic morphology that are commonly believed to be neuronal forms of short or long-term memory (amygdala [31, 62]; ventral tegmental region [63]; prefrontal cortex [63, 64]; bed nucleus of the stria terminalis [65]; paraventricular nucleus [66]). Because each of these regions is highly interconnected, it is more likely that a larger network of circuits involving multiple looping circuits between executive, decision, emotion, and modulatory brain regions work with amygdalar and memory networks to perform these functions.

Like the amygdala, the memory network circuits have extensive set of inputs from multiple brain regions, and many of these regions are also interconnected. Memory regions receive either direct or indirect reciprocal inputs from sensory cortices, sensory association cortices, and polymodal sensory cortices that provide information about the environment. The reciprocal nature of these inputs is thought to be critical for long-term storage of memories in association cortices [8, 43, 53]. Additional inputs from frontal and prefrontal cortex provide higher-order information about the incoming sensory stimuli. Other inputs from the nucleus basalis, raphe, and hypothalamus provide modulatory influences over memory acquisition which in turn form reciprocal feedback loops to help maintain homeostasis within the system [43, 53].

5. Amygdalar circuits: decision and executive function

The amygdala has both direct and indirect connections with many regions of the brain involved with executive functions. These include functions such as selecting relevant information, planning how to act, deciding what type of action to take, initiating or inhibiting specific actions, evaluating the consequences of actions, and forming rules to guide future behavior [29]. Two brain regions important for these functions are the prefrontal and frontal cortices. Frontal cortex is a brain region that has been shown to encode task-specific meaning of stimuli during behavioral tasks [67]. It receives sensory input, thalamic input, as well as modulatory

inputs. Its primary projection is to the frontostriatal loops [68, 69]; however, it also interacts indirectly with association cortices, hippocampus, and the amygdala [68]. Because of the ability of neurons in this region to quickly adapt to various behavioral paradigms, it is thought that this region may contribute to decision processes that shift behavior based on changing environmental and reward salience [67].

Although the extrinsic circuitry of the frontal cortex is not as complex as prefrontal cortex circuits, manipulation of these circuits can significantly influence numerous regions of the brain through secondary looping circuits. Local field potential shifts within the frontal cortex, for example, resulted in alterations of neuronal responses within auditory cortex that correspond to the behavioral task [67]. Thus, descending circuit from the frontal cortex is likely to facilitate shifts in cortical activity (i.e., attention) toward behaviorally relevant stimuli.

Prefrontal cortex is a more highly connected executive brain area than frontal cortex. It has reciprocal projections to the amygdala, neuromodulatory centers (e.g., raphe, locus coeruleus, nucleus basalis, and ventral tegmental area), basal ganglia, cingulate cortex, hypothalamus, association cortices, striatum, thalamus, insula, nucleus accumbens [4, 17, 23, 70–76]. It also receives unidirectional inputs from the hippocampus [72].

The primary function of the prefrontal cortex is formulation of decisions; but it has also been implicated in assisting with emotional regulation [77]. These functions are highly dynamic due to rapid changes in environmental signals and contexts that require analysis for optimal behavioral responses. Therefore, it is not surprising that prefrontal cortex activity can be substantially impacted by various regions of the brain. Hippocampal inputs, for example, synchronize the activity of the hippocampus and prefrontal cortex during working memory tasks [78]. The synchrony is maximized during acquisition of new roles or during moments of decision [79]. During these synchronous moments, the prefrontal cortex exhibits increases in neuronal firing that through indirect circuits influence the hippocampus and further synchronize the regions [79]. Lesions or desynchrony of the pathways can dramatically impact the accuracy of decision and emotional processing [80].

The synchrony of the hippocampus and prefrontal cortex may also be instrumental for convergence of inputs from these regions within the nucleus accumbens. Experiments have shown that precisely timed inputs facilitate neuronal responses [81, 82]. Because this region has reciprocal projections with the prefrontal cortex, nucleus accumbens facilitation is thought to gate goal-directed behaviors. Increased synchrony between the hippocampus and prefrontal cortex induces augmented facilitation within the nucleus accumbens. This augmented response can then be sent back to the prefrontal cortex to increase the accuracy of behavioral responses [80, 83].

Neuromodulators (e.g., acetylcholine, dopamine, serotonin, noradrenaline, and histamines) can also alter prefrontal cortex activity. Infusion of these modulators into the prefrontal cortex has been shown to dramatically alter behavioral performance [76]. Because the influence of these modulatory circuits is fairly critical to prefrontal cortex function, it is reasonable to assume that the reciprocal nature of the circuit helps to tightly regulate modulatory output for optimal performance [76]. Such regulation can become fairly complex because the modulatory

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circuits have numerous input and output circuits that both influence and interact with each other [76].

Outputs of the prefrontal cortex to the amygdala alter amygdalar activity based on dynamic changes in perceived significance to the individual [77]. In animal models, lesions of the prefrontal cortex cause losses in an animal's ability to alter its perception of a stimulus as well as change its behavioral responses to the stimulus [84, 85]. In humans, similar lesions lead to additional symptoms that include the inability to reason and suppress impulses [86].

6. Amygdalar outputs: emotion

Stimulation of the amygdala in humans has been shown to produce emotional responses including hallucinations, fear, rage, and pleasure [17]. Emotion can be defined as physiological changes (e.g., hormonal changes, changes in heart rate, etc.) that occur as a result of conditioned responses. Another way of thinking about this concept is that emotions are alterations in physiology produced by positive or negative reinforcement. Positive reinforcement is the addition of stimuli that increase or maintain a behavior, while negative reinforcement is the removal of noxious or aversive stimuli [87].

An example of a positive reinforcement may be a food reward to an animal, such as Pavlov's dog [88]. In these experiments, dogs learned to associate a food reward with the presentation of a bell [88]. The positive influence of the food was caused by amygdalar activation of dopaminergic mesolimbic pathways (i.e., pleasure centers within the brain). These centers include the nucleus accumbens and ventral tegmental area reward systems [89]. Release of dopamine from pleasure centers leads to behavioral and physiological changes in an animal or individual (e.g., smiling, dancing, foot tapping, physiological arousal) [89]. In the case of Pavlov's dog, the drooling response occurs via activation of the reward circuits that release dopamine. Because dopamine has been shown to directly influence salivary glands [90], activation of pleasure centers induced the behavioral effect of drooling in the dog. In this way, stimulation of the amygdala can activate a wide array of circuits that in turn have measurable physiological and behavioral effects [17].

Another brain system that helps to control emotional physiology is the hypothalamus. The hypothalamus synthesizes neuropeptides that are able to modulate both brain and body physiology [91] as well as numerous reciprocal circuits (e.g., prefrontal cortex, nucleus accumbens, hippocampus, amygdala, nucleus basalis, raphe). The hypothalamus also receives input from the midbrain and brainstem, and helps to modulate autonomic function within the parabrachial nucleus and periaqueductal grey [91]. The connections with autonomic circuitry regulate breathing, blood pressure, heart beat, dilation and constriction of blood vessels, as well as fight or flight, and rest or digest responses.

Many researchers utilize auditory stimuli when studying emotional processing. In several studies, amygdalar circuits have been shown to respond to sounds with either positive or negative affect, but are not responsive to sounds with neutral affects [31, 92]. Other functional

magnetic resonance imaging (fMRI) human studies used visual stimuli (i.e., kissing scenes from romantic comedies) paired with an auditory stimulus (i.e., happy, sad, or no music). Results from these studies showed decreases in cortical activation to sad music and increases to happy music. The changes in cortical activation correlated to increases or decreases in transmission between the fusiform gyrus and the amygdala, respectively. Therefore, it was proposed that amygdalo-fusiform gyrus connections modulate the emotional experience of the viewer to the movie and thereby help to alter the attention of the individual to relevant auditory cues. In this way, the auditory stimuli may help to initiate an emotional response to the stimuli, while the emotional response strengthened the attention of the individual to the auditory stimuli [93].

The physiological changes that we classify as emotion can also occur via alterations in hormones. For example, mothers have a hormonal response to the cry of their own child, but not to the cry of other children [5]. We hypothesize that the maternal attention is facilitated by interconnected feedback loops from the amygdala, prefrontal cortex, and memory network. This response is likely augmented by hormonal release that interacts with each of these brain regions [5]. Depressed mothers, on the other hand, do not have the same hormonal response to the sound of the infant's cry [94]. They attend less to the sound, and are less likely to actively care for the needs of the infant [95]. It is possible that disruption of one or more of cortical, limbic, and executive loops may dampen the significance of the infant cry within the amygdala and thus either actively inhibit or dampen the influence within the hypothalamus (increasing hormonal release) and decreasing hormonal activity within other regions of the brain (e.g., frontal cortex, bed nucleus of the stria terminalis, and amygdala) [96]. Reduced circuit activation could also decrease facilitation within modulatory, memory networks, and executive centers. This overall dampening of excitation would lessen the inclination of the individual to respond appropriately to the infant cry.

7. Dysfunction and pathology

There are varying forms of psychosis (schizophrenia, affective disorders: bipolar disorder, depression, anxiety disorder) as well as behavioral disorders (e.g., autism, attention deficit disorder) in which the brain does not accurately decipher or convey appropriate behavioral responses to environmental stimuli [97–99]. These issues are caused by problems with one or more of the interconnected circuits between the limbic, memory, sensory, and executive centers of the brain [99]. Because of the multiple interactions of each circuit, a wide array of disparate symptomologies can occur: problems with attention, memory, incongruous decisions, hallucinations, problems identifying accurate significance to stimuli, and exaggerated or inappropriate reactions to stimuli [97–99].

Human populations with these disorders have shown various anatomical, electrical, and functional pathologies. The activity and overall brain size of the prefrontal cortex, hippocampus, and amygdala have shown to be altered in patients with schizophrenia, bipolar disorder, and depression [97, 99, 100]. Although these types of variations are common in psychiatric patients, the number of days that a depressed individual goes untreated has been shown to

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dynamically affect the size of their hippocampus [101]. Therefore, these forms of psychosis are dynamic.

The adaptable nature of brain systems associated with psychosis also allows for the development of successful therapeutic alternatives to help reset the synchrony of the system. However, finding effective therapies has been a challenge. Because of the progressive nature of several disorders, the original impetus is difficult to discern. Altered activity within the amygdala, hippocampus, and prefrontal cortex may either be resultant from, or the cause of, additional variations in the activity of the thalamus, [102]; disrupted gamma oscillations in cortex [103], inappropriate excitation within the anterior cingulate cortex [100], and more reactive dopamine circuits [99]. Understanding the interconnections and intricacies of the loops will help build a framework of potential pathology whereby more effective therapeutic strategies can be formulated in the future.

Because many psychiatric illnesses are progressive, the prognosis of affected individuals would be improved if they could be identified and treated earlier. Beyond genetic risk factors, there are several groups of individuals in the general population that are at-risk for mental dysfunction (i.e., individuals exposed to external stressors or various forms of mind-altering drugs).

Although stress and alcohol consumption, in moderation, do not directly lead to psychosis, they could increase desynchrony of brain circuits. In a normal system, the highly interconnected nature of limbic, sensory, associative, memory, and executive brain regions allows an individual to maximize appropriate behavioral responses to their environment. Feedback mechanisms between these regions help to keep the system functioning within normal rhythms when presented with external stressors or manipulations. These rhythms can be compromised by sudden changes in electrical activity.

Alcohol consumption, for example, has depressive influences on brain activity by temporarily potentiating GABAergic and glycine receptors while depressing N-methyl-D-aspartate receptors [104]. In normal individuals, the influence of moderate alcohol consumption can be overcome as the system rapidly adapts to the temporary insult. However, when the external stressor becomes more pronounced, it can destabilize the rhythms of these circuits to a degree that leaves them more vulnerable to the dysrhythmia of psychosis. This idea is supported by the fact that individuals that utilize alcohol chronically have been shown to be much more prone to the development of a psychosis (e.g., depression, schizophrenia) [105].

Other stressor can have similar effects. Stress can inhibit plasticity in the hippocampus, increase excitability in the anterior cingulate cortex, elevated cortisol release through the hypothalamic-pituitary-adrenal circuits, and modulate hormonally modulate executive and memory circuits [106–109]. Excessive exposure to stress over extended periods of time can lead to long-term alterations in brain patterns, de-optimize circuit function, and leave the brain more prone to additional desynchrony and psychosis [109]. Therefore, education of the general populous (including children) as well as military personnel about the importance of moderation and stress-relieving techniques may help to decrease the number of individuals that develop psychosis or behavioral disorders into the future.

8. Conclusions

Current research supports the idea that multiple looping circuits from sensory, memory, association, and executive brain regions are combined within the amygdala to determine the significance of environmental stimuli. This information is utilized to alter the firing characteristics of other brain circuits and support higher-order functions such as focusing attention on relevant sensory cues as well as learning and decision making. Because the targets of amygdalar circuits are highly interconnected with each other, changes in one region of the brain have widespread influences throughout the system. We hypothesize that the interconnected nature of the circuits facilitates flexibility within the system, which in turn enables the brain to respond and react quickly to environmental changes.

Although the complexity of the circuitry may allow rapid adaptations, dysfunctions in the firing rhythms, neurotransmitter release, or abnormalities in connections can influence numerous brain regions. This results in cortical processing and behavioral actions that are dissonant with the environment. Psychiatric, mood, and attention disorders are ideal examples of limbic circuit dysfunction. Symptoms from these disorders range from visual or auditory hallucinations, paranoia, delusions, inappropriate social or emotional responses, as well as learning and memory deficits [110–114]. The interconnected nature of the circuits involved make treatment of these patients more complex. Better understanding of the interconnections and functions of each circuit can help us identify the mechanisms and progression of these disorders and devise effective therapies for both the symptoms and psychosis.

Author details

Diana Peterson

Address all correspondence to: dpeterso@highpoint.edu

Department of Physical Therapy, High Point University, High Point, NC, USA

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Chapter 5

Brain Activity During Autobiographical Retrieval Is Modulated by Emotion and Vividness: Informing the Role of the Amygdala

Patricia J. Bauer, Jennifer Strafford Stevens,

Anaïs F. Stenson, Theodore Waters and

Felicia L. Jackson

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67583

Abstract

Growing evidence indicates that the amygdala contributes to processing both emotional stimuli and highly vivid episodic memories. The present research used event-related potentials (ERPs) to examine the individual and joint contributions of these dimensions on the neural responses to naturalistic stimuli, namely, autobiographical memories, which vary in terms of associated emotion and the vividness of recollection. In Experiment 1, participants recalled positive and negative personal memories, and memories for which no mention of emotion was made. Events recollected with high vividness showed no effect of emotion, whereas ERPs for events recollected with low vividness differed for both positive and negative memories versus non-emotional memories. The conjoint effects of emotion and vividness reflect the correlation of these variables in everyday life: more emotional memories are more vividly recalled. In Experiment 2, we pursued the interaction of emotion and vividness by asking participants to recall negative high-arousal, negative low-arousal, and emotionally neutral memories. Processing differed by vividness but not by emotion condition. The research implies that focus on the emotional valence associated with a memory, without conjoint consideration of how vividly it is recalled, neglects a critical determinant of neural processes that are modulated by the amygdala during recall of autobiographical memories.

Keywords: amygdala, autobiographical memory, emotion, ERP, vividness

1. Brain activity during autobiographical retrieval is modulated by emotion and vividness: informing the role of the amygdala

We experience emotion at virtually every turn, often within milliseconds of a precipitating event. We also frequently re-experience the emotion when we recall the event at a later time. Emotional content contributes to the vividness of recollection [1–4]. Investigation of the neural processing associated with emotional events and experiences has a long history. For example, Lifshitz [5] and Begleiter and colleagues [6] examined event-related potentials (ERPs) in response to picture stimuli containing pleasant or unpleasant content. Numerous subsequent studies have replicated their primary finding that emotional visual scenes elicit enhanced neural processing apparent at posterior scalp sites. Patient studies and fMRI investigations have elaborated our understanding of the neural substrate involved in encoding and retrieval of emotional scenes, indicating that the amygdala plays a central role (e.g., [7–11]). Yet surprisingly, little attention has been devoted to understanding the neural processing of personal emotional situations encountered in the course of everyday life, such as annoyance at losing a parking spot in a crowded lot, winning the championship game, or losing a loved one. Moreover, although there is growing evidence that the amygdala plays a central role in processing not only emotional (e.g., [12, 13]) but also highly vivid (e.g., [1, 3]) episodic memories, there are few studies that permit articulation of the respective contributions of these aspects to memory. In the current research, in two experiments, we used event-related potentials (ERPs) to examine neural processing of personally experienced affective stimuli, namely, autobiographical memories. We examined the responses as a function of both emotional valence and vividness.

Electrophysiology has been a method of choice for studying emotion processing because of its sensitivity to real-time processing of stimuli with emotional qualities. Indeed, electrophysiological responses to emotional stimuli become apparent even prior to conscious experience of emotion. In laboratory studies of processing of stimuli with emotional qualities, including scenes, faces, and words, an "emotion effect" emerges as early as 200–300 ms after stimulus onset. The effect is apparent in slow positive-going amplitudes to emotionally valenced stimuli that continue until stimulus offset. In contrast, responses to neutral stimuli return to baseline (e.g., [14–16]). Importantly, the amplitude of the late positivity covaries with the arousal (intensity) component of emotional responses [15, 17–19]. When elicited in response to affective visual stimuli such as scenes from the International Affective Picture System (IAPS [20]), the late positivity is most pronounced on centroparietal and occipital sites and has been linked with enhanced activity in visual processing areas of the occipital cortex [15, 21]. Such findings suggest that the component reflects an up-regulation of visual perceptual processing for arousing stimuli; the up-regulation of visual perceptual processing likely contributes to the vividness of recollection (see [22] for discussion).

A major benefit of using picture and other laboratory stimuli to examine patterns of neural processing associated with emotional events and experiences and vivid recollections is the control the approach affords. Most importantly, all participants experience the same stimuli. Yet, the gain in internal validity associated with use of such stimuli comes at a cost to external

http://dx.doi.org/10.5772/67583

validity. Specifically, the pictures, scenes, and words that are the subject of laboratory stimuli do not approximate the emotional events and vivid recollections of everyday life. As such, greater understanding of how emotion affects processing in life outside the laboratory can be gained by using stimuli that are personally relevant and significant to the participant. As well, personally relevant and significant stimuli can be expected to be associated with vivid recollections, making them ideal candidates for examining the respective roles in memory of emotion and vividness.

An excellent candidate source of stimuli that are relevant and significant to the participant is autobiographical memories (see [23] for a review). Autobiographical memories tend to be highly durable and long-lasting [24], and are accompanied by a sense of vivid reliving [25]. Because moments of great personal significance often are emotional; autobiographical memories tend to be rich in emotional content and have been shown to elicit powerful emotional responses. For example, participants recalling affectively positive or negative autobiographical events experience heightened physiological arousal, including changes in heart rate, breathing, and sweating response [26–28]. In fMRI studies, retrieval of autobiographical memories is associated with activation in emotion-related brain regions such as the amygdala and anterior cingulate cortex [29, 30]. Thus, autobiographical events and memories thereof are a potentially powerful vehicle for examining emotion processing.

Autobiographical memories also are a potentially powerful vehicle for examining neural processing involved in vivid recollection. As noted earlier, one of the characteristic features of autobiographical memories is that recollection of them is associated with a sense of vivid reliving [25]. Participants report traveling back in time as if re-experiencing the sights, sounds, and other sensory features of such events [31, 32]. Consistent with this phenomenology, in fMRI studies, retrieval of autobiographical memories is associated with activation in sensory and imagery-related regions of the brain, including posterior midline (precuneus, posterior cingulate), and visual processing (occipital cortex, ventral temporal cortex) regions (e.g., [29, 30, 33, 34]; see also [22] for a review). Thus, autobiographical memories are well suited to examination of the neural processing associated with vivid recollection.

Whereas the experiences that become autobiographical memories typically happened outside the laboratory; they can be studied in the laboratory using electrophysiology. For example, several studies have used scalp electrophysiology to study the timecourse of autobiographical retrieval, including ERP and slow cortical potential methods (e.g., [35–37]). In these investigations, participants' memories were measured as differences in the electrophysiological response to different types of retrieval cues, or for autobiographical versus imagined events. Autobiographical memories are consciously retrieved after an average of 5 seconds, yet variation in electrophysiological response related to autobiographical memory content begins as early as 400–600 ms after a retrieval cue and is associated with a negative-going slow wave on posterior sites.

In a developmental study, we extended this general approach to an ERP investigation of 7–10-year-old children's retrieval of emotional autobiographical events and experiences [38]. We elicited autobiographical memories using the cue word technique [39, 40], which has been successfully extended to children as young as 7 years of age [41, 42]. At the beginning of a

laboratory visit, children were given neutral concrete nouns (cue words—e.g., *dog, chair*), and for each one, they were explicitly directed to generate a personal memory that was positive, negative, or unconstrained by emotional valence. During ERP recording, children recalled each memory in response to the visual presentation of the corresponding cue word (e.g., DOG). Because the memories were generated earlier in the laboratory visit, the children could begin recalling the memories quickly after reading the cue, allowing us to timelock autobiographical retrieval to cue onset.

The investigation revealed differential processing as a function of emotional valence of the events. In contrast with the canonical late positive potential elicited by emotional visual stimuli, recollection of autobiographical memories produced a late negative-going waveform on posterior sites (1000–1500 ms); the effect was more pronounced for positive memories relative to neutral memories [38]. Because this was the first study of its kind, and because there was no adult comparison group, it was not possible to determine the source (or sources) of the difference between the observed pattern of findings and the canonical emotion effect reported in the adult literature. The departure from the typical adult pattern could have been due to the difference in stimulus modality (internally generated autobiographical memories vs. external visual stimuli), or could relate to the developmental status of emotion processing in school-aged children (see [43], for evidence of developmental differences). For present purposes, the more important point is that the cue word technique successfully elicited differential neural processing as a function of event valence.

In the present research, we conducted two investigations of ERP responses to autobiographical memories in adults. In both experiments, we used the cue word technique to elicit autobiographical memories. In Experiment 1, in advance of their laboratory visits, participants received a list of neutral concrete nouns (cue words) and were explicitly directed to generate personal memories to accompany each word. As in Ref. [38], some of the trials were unconstrained as to the valence of the memory to be retrieved, whereas others explicitly prompted recall of a positive or negative personal memory. During ERP recording, participants recalled each memory in response to the visual presentation of the corresponding cue word. They also rated their recollections of the events in terms of their vividness.

To anticipate the results of Experiment 1, we observed differential processing of positive relative to negative autobiographical memories as early as 400 ms. In comparison with the traditional "emotion effect," ERP responses to positive and unconstrained memories did not differ; both elicited more positive-going slow-wave responses relative to negative memories. Importantly, the emotion effect both interacted with and became more canonical when we took into account the reported vividness of the recollection. On trials on which participants reported highly vivid recollections, there was no effect of emotion. In contrast, on trials on which participants reported less vivid recollections, ERPs responses to both positive and negative events differed from those to emotionally unconstrained events.

To further pursue the joint effects of emotion and vividness, in Experiment 2, we focused on negative memories, and manipulated the level of arousal associated with them. Specifically, participants were explicitly instructed to recall negative autobiographical memories with different levels of arousal, based on the circumplex model of emotion [44]: low-arousal and

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high-arousal. That is, participants were instructed to recall events during which they experienced sadness, guilt, or loneliness (low arousal), and during which they experienced fear, anxiety, or anger (high arousal). On the balance of the trials, participants were instructed to recall events and experiences that were explicitly neutral in valence. We expected this manipulation to result in a stronger contrast between the emotion conditions and the non-emotion condition, relative to the unconstrained "neutral" condition in Experiment 1. The manipulation also permitted test for replication of the effects of vividness observed in Experiment 1.

From the standpoint of eliciting processing of emotion, using cue words to elicit autobiographical memories is ideal. First, autobiographical memories represent rich, naturalistic emotion stimuli, thus facilitating extension of our knowledge of emotional neural processing beyond the more typical laboratory scenario in which participants view a series of static visual stimuli. Second, in contrast with picture or word stimuli, memories are tailored to the individual participant and entail greater personal relevance. They also typically are associated with recollections that vary in their subjective vividness. Third, because the affective content and sources of vividness are internally generated, low-level stimulus confounds are reduced relative to the more common emotion-elicitation methods (e.g., pictures, scenes) for which the content is external. We further reduced confounding neural activity relating to the content of the retrieval cues by using affectively neutral cue words, which, across participants, were each assigned equally often to the different emotion conditions (positive, negative, and unconstrained in Experiment 1; negative high-arousal, negative low-arousal, and neutral in Experiment 2).

The current examination of emotional autobiographical memories represents an important extension of the literature on emotion processing, testing the extent to which "emotion effects" observed in previous ERP studies represent core elements of the emotional response, which generalize beyond emotions elicited by impersonal, external stimuli, to emotions associated with self-relevant, internally generated stimuli. Moreover, the examination permits assessment of the extent to which patterns of ERP activity associated with different emotion conditions are qualified by the vividness of recollection. Given that the amygdala plays a central role in processing both emotional (e.g., [12, 13, 45, 46]) and highly vivid [1, 3] episodic memories, there is reason to expect that both factors will influence neural responses.

2. Experiment 1

2.1. Method

2.1.1. Participants

Forty-six adults (23 women) age 18–28 years (M = 20.8 years) participated. Fifty-two percent of participants were Caucasian, 20% were African-American, 20% were Asian, 2% were of mixed race, and 7% did not indicate their race. The data from all participants were included in analyses of the behavioral data. The data from 39 participants (20 women) were included in analyses of the ERP data; seven participants were excluded from ERP analyses because of

excessive noise or artifact in their ERP data (See Section 2.1.3). Participants were compensated with either class credit or a gift certificate. The paradigm was reviewed and approved by the university Institutional Review Board.

2.1.2. Stimuli, materials, and procedure

The study involved elicitation of memories using cue words via an online survey, and a laboratory session during which event-related potentials (ERPs) were recorded while participants viewed cue words on a computer screen and retrieved the corresponding memories.

2.1.2.1. Elicitation of memories with an online survey

Five to seven days in advance of their laboratory visit, participants were e-mailed a direct link to a secure and encrypted online survey that was designed and managed using SurveyMonkey (www.surveymonkey.com). The first page of the survey outlined the study and its requirements and informed participants that by proceeding, they were consenting to participate in the study.

The survey consisted of 90 neutral concrete nouns that were selected from previous studies [e.g., 38, 40–42] and used as cue words. A complete list of cue words is given in Appendix A. The 90 words were used to elicit affectively negative and positive memories, as well as memories unconstrained as to emotional valence. Each participant selected 75 of the 90 words and described a memory for each word: 25 positive, 25 negative, and 25 neutral. Three different surveys were created so that each word was used in each emotion condition approximately equally often across participants (e.g., the cue word *paper* was used to elicit positive, negative, and unconstrained memories in equal numbers of participants). To aid in later recall (see below), participants also provided an additional keyword or phrase that would remind them of the target event. The additional keywords were restricted to 1–3 non-emotion words and typically represented a specific element of the event such as a person's name (e.g., Night and *Jessica*), the location of the memory (e.g., Night and *Las Vegas*), or what they were doing (e.g., Night and *Mini Golf*).

In the survey, the 90 cue words were divided into 15 sets of six words each. For each set of six words, participants were instructed to select five words and describe one specific event that occurred within the past year that related to the given word (e.g., for the word *dog:* "Last weekend I took my dog on a walk through the park"). This design allowed participants to select any combination of five words that were more easily associated with specific memories, and exclude the sixth. Participants were prompted to select events from the past year to control the age of memories across the different emotion conditions, while still allowing participants to select from a range of remembered events. The first five sets of words elicited memories without any mention of emotion. Responses to these unconstrained prompts constitute the *unconstrained* condition. Every set that followed alternated between the *negative* and *positive* conditions (i.e., sets 1–5 were unconstrained, sets 6, 8, 10, 12, and 14 were negative, and sets 7, 9, 11, 13, and 15 were positive). The survey began with the unconstrained prompt in order to omit information about emotion from the instructions, allowing participants to

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select memories in a manner unconstrained by emotion. The survey always ended with a set of positive memories so that participants ended the experience on a positive note.

For the unconstrained condition, participants were given the general prompt: "There are six words given below. Choose any FIVE of the words and describe an event of which each word reminds you." Participants were further instructed to describe the event in two to four sentences. For the negative condition, participants were given the more specific prompt: "There are six words given on the next page. Choose any FIVE of the words and describe a NEGATIVE event of which each word reminds you. These should be events when you felt UNHAPPY, ANGRY, SAD or UPSET." The instructions in the positive condition prompted participants to describe events when they felt "HAPPY, EXCITED, CHEERFUL or GLAD."

After writing a description and determining the additional keyword for an event, participants were asked to indicate the pleasantness of their emotional response thinking about the event now (i.e., while completing the survey). The rating was on a five-point scale, ranging from −2 (very unpleasant), to 0 (neutral), to 2 (very pleasant). Additionally, participants were asked to indicate their arousal by rating the intensity of their emotional response (a) "when the event first happened" and (b) "as you think about the event now." These ratings were on a five-point scale from 1 (no emotional response) to 5 (strong emotional response). Sample narratives are provided in Appendix B. The survey took approximately 2–3 hours to complete.

2.1.2.2. Review of events and retrieval of memories while recording event-related potentials (ERPs)

An average of 2 days after completing the online survey, participants visited the laboratory during which they were (a) interviewed by a researcher to review the memories provided on the survey and (b) retrieved the memories during collection of ERP data. Before the session began, participants gave informed written consent to participate in the ERP portion of the study. During the interview, participants were seated in a chair directly across from a researcher. One by one, the researcher gave the participant a cue word from the survey along with the additional keyword the participant herself or himself provided, and the participant briefly summarized the event as s/he had described on the survey. In most cases, participants confirmed in free recall that the memory was of one specific event. If that information was not provided, the researcher explicitly asked the participant for confirmation. If the memory was not specific, the researcher prompted the participant to narrow in on a specific element of the event. Additionally, if the keyword that the participants provided was emotional (e.g., "scared" or "happy"), the experimenter guided the participant in the selection of a neutral keyword to replace it.

While participants reviewed their memories with one researcher, two additional researchers applied an Advanced Neuro Technology (A.N.T.) WaveGuard 32-channel ERP cap (A.N.T. Software B.V., Enschede, The Netherlands). The elastic-lycra cap contained 32 electrodes placed according to the International 10–5 system, an adaptation of the International 10–20 system [47]. For all participants, impedances were between 0 and 10 kΩ, and most frequently below 5 kΩ. The data were referenced online to mathematically linked mastoids. Using ASA amplifier (A.N.T. Software B.V., Enschede, the Netherlands), the EEG data were sampled

at 256 Hz continuously and amplified 20,000 times. The interview and application of the ERP cap took 20–40 minutes to complete.

Immediately after the interview and capping, participants were seated approximately 60 cm from a 38-cm computer monitor where the ERP stimuli were presented. Participants silently engaged in autobiographical recollection in response to cue words. Individual cue words were shown at the center of a slide, and the participant's additional keyword(s) was shown directly below the cue word. Words were presented in black, size 54 Calibri font on a blue background. The cue word and additional keyword pair occupied approximately 0.8–2.5° × 1.25° of the visual field on either side of the visual midline. Trials were structured as follows (also see **Figure 1**). Participants first viewed the cue and keywords, displayed for 3000 ms. During this time, participants were instructed to recall the corresponding autobiographical memory: "Think about who was there, what you were feeling, and generally try to recreate the event in your mind." Participants then rated the vividness of their memory on a scale from 0 to 3 (0 = not recalled, 3 = high vividness) using a button box corresponding to the visual scale. The vividness scale was displayed for 3000 ms. This was followed by a fixation cross, displayed for 3000 ms, during which participants were instructed to clear their minds, stop thinking about the previous event, and prepare for the next cue word. A jittered inter-stimulus interval (blank screen) of 300–400 ms followed the recall and rating portions of the trial.

Before beginning ERP data collection, participants were shown two practice trials to orient them to the timing of the task, and the button press. During the ERP presentation, words were presented quasi-randomly, with no more than three words of the same emotion in sequence. Each word was shown twice (all 75 words were shown, and then the presentation was repeated) for a total of 150 trials, with 50 trials in each affective valence condition (25 individual memories per condition). Two ERP orders for each of the three surveys were created in this fashion (six orders total), and counterbalanced across participants. The full ERP data collection took approximately 25 minutes.

2.1.3. Electrophysiological data reduction

The raw EEG data were individually bandpass filtered (0.1–30 Hz, roll-off of 24 decibels/octave). Electrodes with off-scale measurements were removed prior to averaging, and

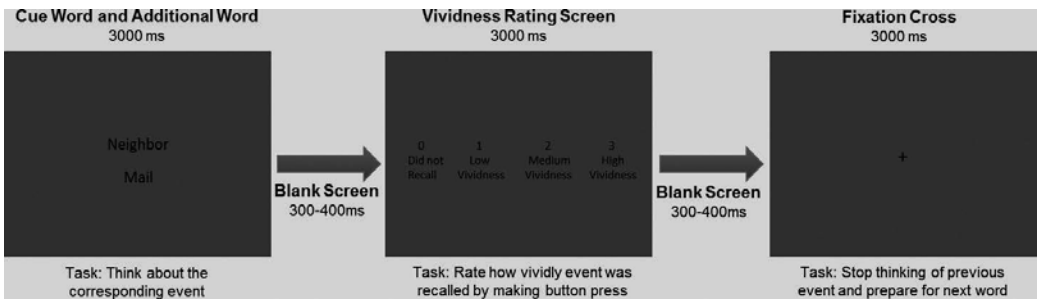

Figure 1. Structure of stimulus presentation for a sample ERP trial.

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excluded from analysis. No more than two electrodes were removed from any one participant's data (max loss = 6.25% of total electrodes), and electrodes were removed in hemisphere-matched pairs (e.g., if T7 was removed, T8 also was removed). Data from discarded electrode sites were not replaced. Eye-blink, saccade, and muscle artifacts were removed by independent component analysis using EEGLAB 13.4.4b ([48] http://www.sccn.ucsd.edu/eeglab) running under Matlab 8.4.0 (MathWorks, Natick, MA, USA). Additional artifacts that exceeded ±150 μV (typically caused by excessive movement or muscle activity) were excluded as well. Data were referenced to a 200 ms pre-stimulus baseline. Participants were included in final data averaging if at least 50% of their trials were usable (i.e., at least 25 trials per condition, $M = 47$ trials). These procedures resulted in exclusion of data from seven participants. Across participants, we created separate grand averages for trials in the positive, negative, and unconstrained conditions.

3. Results

3.1. Preliminary analyses

In preliminary analyses, we examined both the behavioral (pleasantness, arousal, and vividness) and ERP data for systematic effects associated with participants' gender. None was found. Therefore, gender was not included as a factor in the main analyses.

3.1.1. Behavioral data

We used within-subjects analyses of variance (ANOVAs) to test whether positive or negative memories differed from unconstrained memories in subjective pleasantness, arousal, and vividness. Post-hoc contrasts between emotion pairs were Bonferroni-corrected for multiple comparisons. Descriptive statistics are provided in **Table 1**.

3.1.1.1. Pleasantness

Average ratings of pleasantness varied significantly by emotion condition, $F(2, 88) = 456.17$, $p < 0.001$. Positive memories were rated as more pleasant than unconstrained ($p < 0.001$) and negative memories ($p < 0.001$). Unconstrained memories were rated as more pleasant than negative memories ($p < 0.001$). The ratings suggest that the emotional content of participants' memories was consistent with the target emotion.

3.1.1.2. Arousal

Average ratings of arousal at the time of the remembered event ("Arousal then") varied significantly by emotion condition, $F(2, 88) = 11.18$, $p < 0.001$. Positive and negative memories were rated as more arousing than unconstrained memories (ps < 0.0001); positive and negative memories did not differ ($p = 0.73$). Arousal when recalling the memory during the online survey ("Arousal now") also varied by emotion condition, $F(2, 88) = 9.02$, $p < 0.001$.

Scale/valence condition	Experiments				
	Experiment 1			Experiment 2	
	M	SD		*M*	SD
Pleasantness (scale −2 to +2)			Pleasantness (scale −2 to +2)		
Negative	−1.08	(0.05)	High-arousal negative	−0.93	(0.35)
Positive	1.14	(0.05)	Low-arousal negative	−0.83	(0.29)
Unconstrained	0.22	(0.05)	Neutral	0.14	(0.22)
Arousal (then) (scale 1–5)			Arousal (then) (scale 1–9)		
Negative	3.66	(0.09)	High-arousal negative	5.53	(1.61)
Positive	3.69	(0.07)	Low-arousal negative	4.60	(1.67)
Unconstrained	3.42	(0.07)	Neutral	2.49	(1.63)
Arousal (now) (scale 1–5)			Arousal (now) (scale 1–9)		
Negative	2.64	(0.10)	High-arousal negative	3.10	(1.41)
Positive	2.92	(0.09)	Low-arousal negative	2.91	(1.31)
Unconstrained	2.68	(0.07)	Neutral	1.84	(1.27)
Vividness (scale 0–3)			Vividness (scale 0–3)		
Negative	2.15	(0.06)	High-arousal negative	2.33	(0.39)
Positive	2.31	(0.06)	Low-arousal negative	2.23	(0.39)
Unconstrained	2.39	(0.05)	Neutral	2.17	(0.47)

Table 1. Subjective ratings of pleasantness, arousal, and vividness for autobiographical memories.

Positive memories received higher arousal ratings than unconstrained ($p = 0.001$) and negative ($p = 0.001$) memories. Unconstrained and negative memories did not differ ($p = 0.51$).

3.1.1.3. Vividness

After each ERP trial, participants indicated the vividness of their recollection of the cued event. The emotional valence of the memories was related to their vividness ratings, $F(2, 88) = 31.04$, $p < 0.001$. Unconstrained memories were rated as more vividly recalled than positive memories ($p = 0.005$); positive memories were more vividly recalled than negative memories ($p < 0.001$).

3.1.2. ERP data

Motivated by previous studies that found emotion effects on the ERP slow wave, we analyzed the mean amplitude of slow wave responses to the memory stimuli. We examined the mean amplitude of the slow wave within 400–1000 ms, and the mean amplitude of the sustained slow wave within 1000–2000 ms. Consistent with previous studies of emotion

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processing [14, 15, 17], visual inspection suggested that emotion effects were strongest on centroparietal and posterior lateral sites. Emotion effects were therefore quantified for posterior lateral (P3, P4, P7, P8) and centroparietal (CP1, CP2, CP5, CP6) clusters. In the analyses to follow, for violations of sphericity, we applied Greenhouse-Geisser correction to the *p*-value. Post-hoc contrasts between emotion pairs were Bonferroni-corrected for multiple comparisons.

3.1.2.1. Analysis by emotion condition

We analyzed ERP amplitudes using separate mixed-effects 3 (emotional valence of memories: positive, negative, unconstrained) × 2 (cluster: posterior lateral, centroparietal) × 2 (hemisphere: left, right) analyses of variance (ANOVAs) for the slow wave (400–1000 ms) and sustained slow wave (1000–2000 ms) windows. The overall waveform is illustrated in **Figure 2**. Given the focus of the analysis on emotion, we report only main effects and interactions involving emotional valence of memories.

As suggested by **Figure 2**, emotional valence significantly influenced mean amplitude during the slow wave window (400–1000 ms) across electrode sites and hemispheres, $F(2, 76) = 4.17$, $p = 0.019$. The mean amplitude for negative memories was less positive-going than for positive memories ($p = 0.025$, $M_{diff} = -0.479$); the difference between negative and unconstrained memories approached significance ($p = 0.064$, $M_{diff} = 0.390$). Positive and unconstrained memories did not differ ($p = 1.00$, $M_{diff} = 0.089$).

There was not an emotional valence effect in this sustained slow wave window (1000–2000 ms; $p = 0.749$). Thus beyond 1000 ms, there was no evidence that emotion influenced sustained processing on centroparietal and posterior lateral sites. In fact, there were no statistically significant effects in the sustained slow wave window.

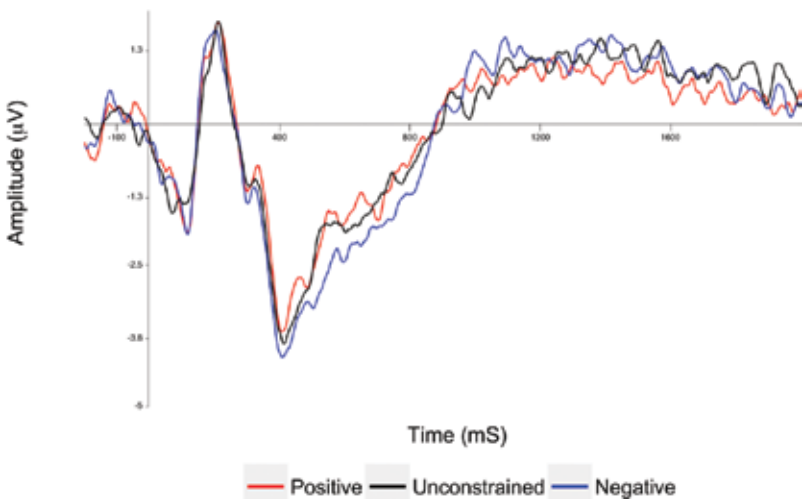

Figure 2. ERPs as a function of emotion condition (positive, negative, unconstrained) in Experiment 1.

3.1.2.2. Analysis by vividness

We analyzed ERP amplitudes using mixed-effects 2 (vividness: high [rating of 2 or 3], low [rating of 0 or 1]) × 3 (emotional valence of memories: positive, negative, unconstrained) × 2 (cluster: posterior lateral, centroparietal) × 2 (hemisphere: left, right) analyses of covariance (ANCOVAs), which took into account differences in trial counts for high- and low-vividness trials. We conducted separate ANCOVAs for the slow wave (400–1000 ms) and sustained slow wave (1000–2000 ms) windows. As above, given the focus of the analysis on vividness, we report only main effects and interactions involving subjective vividness of recollection.

In the slow wave window (400–1000 ms), although the main effect of vividness was not statistically significant ($p = 0.292$), vividness of recollection significantly interacted with cluster, $F(1, 38) = 29.75$, $p < 0.0001$. However, separate one-way ANCOVAs for each cluster (posterior lateral, centroparietal) revealed no effects of vividness.

Vividness also interacted with hemisphere, $F(1, 38) = 9.75$, $p < 0.003$; the two-way interaction was further qualified by the three-way interaction with emotion, $F(2, 76) = 3.40$, $p = 0.038$. To examine the interaction, we conducted separate ANOVAs for each level of vividness. As suggested by the top panel of **Figure 3**, for trials on which participants reported highly vivid recollections, there was no effect of emotion ($p = 0.915$). Although the interaction of

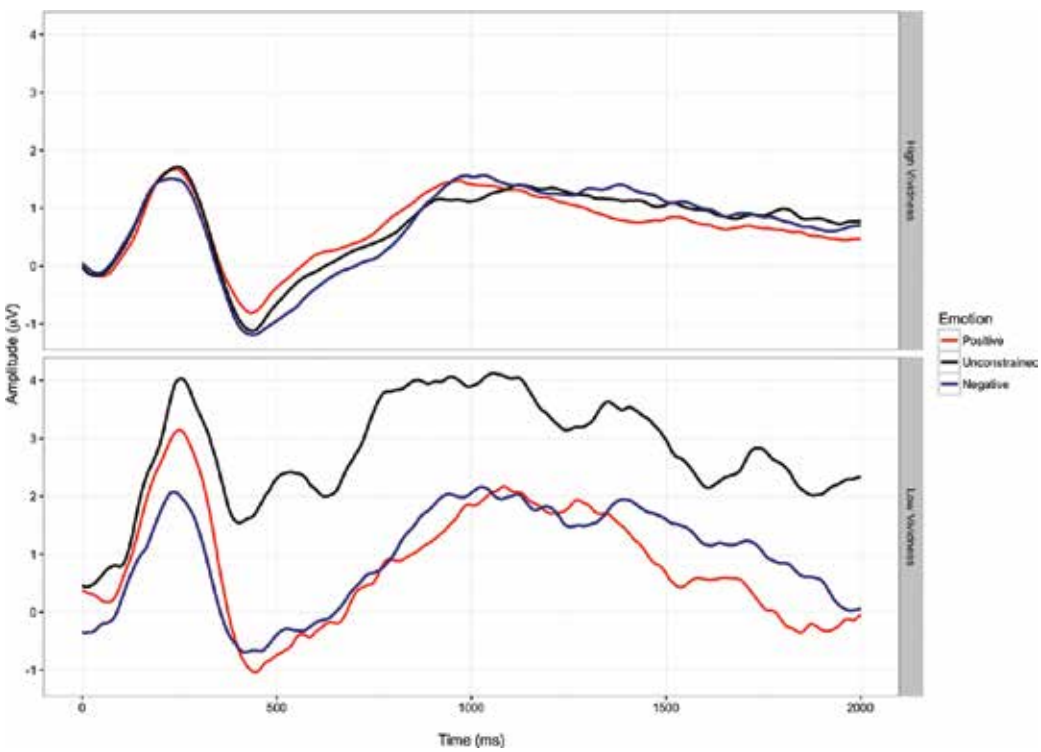

Figure 3. ERPs as a function of vividness in Experiment 1, for high-vividness trials (top panel) and low-vividness trials (bottom panel).

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emotion × hemisphere was significant, $F(2, 76) = 3.66$, $p = 0.030$, follow-up analyses revealed no emotion effects. For trials on which participants reported less vivid recollections, the main effect of emotion was significant, $F(2, 76) = 3.12$, $p < 0.05$. As suggested by the bottom panel of **Figure 3**, the mean amplitudes for emotional events—both positive and negative—were less positive, relative to unconstrained events ($M_{diff} = 1.043$ and 1.331, for positive vs. unconstrained and negative versus unconstrained, respectively; ps < 0.0001); mean amplitudes did not differ for positive and negative memories ($M_{diff} = 0.288$).

In the sustained slow wave window (1000–2000 ms), ANCOVA revealed a three-way interaction of vividness × cluster × hemisphere, $F(1, 38) = 4.45$, $p = 0.041$. Separate analyses by cluster revealed no significant effects in the centroparietal cluster. In the posterior lateral cluster, the interaction of vividness × hemisphere was significant, $F(1, 38) = 4.93$, $p = 0.032$. Over the right hemisphere, there was no effect of vividness. Over the left hemisphere, amplitude was more positive for high versus low vividness recollections ($M_{diff} = 1.222$), though the effect only approached statistical significance ($p = 0.14$).

4. Discussion

The primary goal of Experiment 1 was to use ERP's to examine the processing of autobiographical memories that varied in emotional valence and for which recollection varied by vividness. Based on their ratings, participants retrieved autobiographical memories that were positively valenced, negatively valenced, and more neutral in content, and which were differentially vivid.

As predicted, the emotional content of the memories influenced ERP responses, though the effect was short-lived and not entirely in line with the patterns observed in prior studies of external stimuli, such as stimuli from the IAPS. In the slow wave window (400–1000 ms), negative memories evoked less positive-going responses than positive memories. The difference between negative and unconstrained memories approached significance. In the sustained slow wave window (1000–2000 ms), no effects of emotion were observed. The patterns differed from the typical late positive emotion effects observed in previous studies. Commonly, the emotion effect in ERP is manifest as a slow wave that is more positive-going for emotional than neutral stimuli (e.g., [14–16, 21]). One likely source of the difference is the study design, in which we recorded ERPs to internally generated, personally relevant stimuli, in contrast to external, visual stimuli. Moreover, ERPs were recorded as participants recalled emotional events versus the more typical study design involving recognition. The departures from the canonical emotion effect in the literature suggest that the stimulus characteristics and retrieval conditions are critical in understanding emotion effects in neural processing, and thus some limits to the generalizability of previously identified emotion effects.

Analysis of the data considering the vividness of recollection as well as the emotional valence revealed another likely source of difference between the findings of the present research and previous studies in the literature. Specifically, for highly vivid recollections, there was no effect of emotion condition. In contrast, on trials on which recollection was rated as less vivid, ERPs to both positive and negative memories differed from those to unconstrained memories.

This pattern is a more typical "emotion effect," yet as noted above, ERPs to explicitly emotional memories were less positive, relative to unconstrained memories. The fact that the effect was only observed on low-vividness trials suggests that emotion and vividness may have additive effects on slow-wave amplitudes, effects that are not observed when vividness is at ceiling. The conjoint effect of emotion and vividness reflects the natural correlation of these variables in everyday life: more emotional memories are more vividly recalled.

One source of concern with the present research is that the comparison of memory for emotional stimuli to non-emotional stimuli was made not to a "neutral" stimulus category, but to a category "unconstrained" as to emotion. That is, there was no explicit mention of the emotional valence of the memories nominated in it. As intended, the valence of the memories featured in this category was intermediate between positive and negative, based on participants' ratings. However, participants' ratings also indicated that at the time of the memory report, the level of arousal associated with the unconstrained memories was not significantly lower than that associated with the negative memories. Moreover, at the time of the ERP recording, participants rated the vividness of their unconstrained memories as higher than both positive and negative. These features of the memories in the unconstrained category may have diminished differences in the neural processing of the stimuli, relative to explicitly emotionally valenced stimuli.

In Experiment 2, we extended the study of neural processing of emotional autobiographical memories, as well as addressed the limitations of Experiment 1. Specifically, we further investigated the potential additive effects of emotion and vividness, testing for replication of the effect of vividness on the slow-wave amplitudes in response to emotional memories. We also replaced the unconstrained condition with a condition that was explicitly affectively neutral. Participants were explicitly instructed to nominate memories of events during which they did not experience emotion. Further, we focused on events that were negative, and did not include positive emotional events. Exclusive focus on negative events is common in the emotion processing literature, and inclusion of positive stimuli in Experiment 1 may be one reason why the emotion effects were somewhat muted, relative to the literature as a whole. We included two classes of negative emotional stimuli, namely, low-arousal and high-arousal [44]. That is, participants were instructed to recall events during which they experienced sadness, guilt, or loneliness (low arousal), and during which they experienced fear, anxiety, or anger (high arousal). We anticipated that this manipulation would increase the opportunity for detection of differences between negative and neutral memories.

5. Experiment 2

5.1. Method

5.1.1. Participants

Forty-nine individuals (25 women) age 16–22 years (M = 19.8 years) participated. Forty-eight of the participants gave written informed consent to take part in this study. For the 16-year-old, consent was obtained from a legal guardian. Thirty-eight percent of participants were

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Caucasian, 18% were African-American, 36% were Asian, 6% were of mixed race, and 4% did not indicate their race. Two additional participants were excluded from analyses of the behavioral data because the length of the delay between phases of the experiment exceeded limits. Six additional participants were removed from ERP analyses due to (a) excessive noise in the ERP data (N = 5) or (b) a technical malfunction prevented the recording of electrophysiological data (N = 1). Participants were drawn from the same source and represent the same population as in Experiment 1. None of the participants had taken part in Experiment 1. Participants received either class credit or a gift certificate. The paradigm was reviewed and approved by the university Institutional Review Board.

5.1.2. Stimuli, materials, and procedure

The study consisted of the same two phases as in Experiment 1. In each phase, the procedure was the same as in Experiment 1, except as noted.

5.1.2.1. Elicitation of memories in response to cue words with an online survey

An average of 7 days before their lab visit, participants were e-mailed a direct link to a secure and encrypted online survey. The survey consisted of 84 neutral concrete nouns that overlapped with those used in Experiment 1 (which featured 90 potential cue words). The 84 words were used to elicit autobiographical memories that were affectively neutral, negative low-arousal (including emotions such as sadness, guilt, and loneliness), and negative high-arousal (including emotions such as fear, anxiety, and anger). Participants were instructed to select 54 of the 84 words and described a memory for each word: 18 neutral, 18 negative low-arousal, and 18 negative high-arousal. Each cue word was used in each emotion condition approximately equally often across participants. As in Experiment 1, participants also provided an additional keyword or phrase to facilitate later recall. The additional keywords were restricted as in Experiment 1.

In the survey, the 84 cue words were divided into 12 sets of seven words each. In the first six sets, participants were instructed to select five of the seven words, and describe one specific event that occurred within the past year that related to the given word. In the last six sets, participants were instructed to describe memories for four of the seven words.

For each set of words, participants were instructed to describe memories of one emotion condition. For the negative low-arousal condition, the prompt was: "This set of memories should be about emotionally negative events during which you felt SADNESS, LONELINESS, FAILURE, DISAPPOINTMENT, or GUILT." For the negative high-arousal condition, the prompt was: "This set of memories should describe emotionally negative events during which you felt ANGER, FEAR, DISGUST, ANXIETY, or HATRED." For the neutral condition, the prompt was: "Neutral memories should describe emotionally neutral events during which you felt NO SADNESS, NO ANGER, NO ANXIETY, NO HAPPINESS, and NO PRIDE." To further emphasize the distinctions, participants were also instructed to: "try to avoid describing events during which you were feeling emotions from more than one category."

After writing a description and determining the additional keyword for each cue word, participants rated the pleasantness of their emotional response and their level of arousal at the

time of the event and at the time of the survey. As in Experiment 1, pleasantness of the emotional response thinking about the event now (i.e., while completing the survey) was rated using the five-point scale (−2 [very unpleasant], 0 [neutral], 2 [very pleasant]). Whereas in Experiment 1, participants used a five-point scale to rate their level of arousal, in the present experiment, we expanded the range to nine points: 1 (little or no arousal) to 9 (extremely aroused). The entire survey took approximately 2–3 hours to complete.

5.1.2.2. Review of events and retrieval of memories while recording event-related potentials (ERPs)

As in Experiment 1, after an average two-day delay, participants took part in a laboratory visit that began with an interview to review the memories provided in the survey. The interview was conducted following the same procedures as outlined in Experiment 1. While participants reviewed their memories with one researcher, two additional researchers applied the ERP cap. The ERP hardware, software, and settings were the same as used in Experiment 1. Stimulus presentation, recording, and vividness ratings (0 = not recalled, 3 = high vividness), also was the same as in Experiment 1. One exception was that whereas in Experiment 1, each cue word was presented two times, in the present experiment, each cue word was presented three times (i.e., all 54 words were shown, and then the presentation was repeated two additional times) for a total of 162 trials, with 54 trials in each condition. Two ERP presentation orders for each of the three surveys were created in this fashion (six orders total), and counterbalanced across participants. To ensure that the session ended on a positive note, after all the cue words were presented, participants were shown 10 positively valenced images from the International Affective Picture System (IAPS) and were instructed to rate each photo on a four-point scale (0 = very unpleasant, 3 = very pleasant). These ratings were not analyzed. ERP data collection took roughly 25 minutes.

5.1.3. Electrophysiological data reduction

Data reduction procedures were the same as in Experiment 1. Participants were included in final data averaging if at least 50% of the trials from the first two presentations were usable (i.e., at least 27 of 54 trials in each condition, M = 52 trials). Across participants, we created separate grand averages for trials in each condition.

6. Results

6.1. Preliminary analyses

As in Experiment 1, we examined the behavioral (pleasantness, arousal, and vividness) and ERP data for effects associated with participant gender. None was found. Therefore, gender was not included in the main analyses.

6.1.1. Behavioral data

We used within-subjects analyses of variance (ANOVAs) to test whether memories of low- and high-arousal negative events significantly differed from neutral events, and each other, in

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terms of reported pleasantness, emotional arousal, and vividness. Post-hoc contrasts between emotion pairs were Bonferroni-corrected for multiple comparisons. Descriptive statistics are provided in **Table 1**.

6.1.1.1. Pleasantness

Participants rated the pleasantness of their memories differently as a function of emotion condition, $F(2, 94) = 305.38$, $p < 0.001$. Post-hoc tests revealed that pleasantness ratings significantly differed between all conditions such that negative high-arousal events were rated as less pleasant than both negative low-arousal ($p = 0.02$) and neutral events ($p < 0.001$), and that negative low-arousal events were rated as less pleasant than neutral events ($p < 0.001$).

6.1.1.2. Arousal

Participants' ratings of emotional arousal at the time the remembered event occurred varied significantly by emotion condition, $F(2, 94) = 107.86$, $p < 0.001$. Arousal levels significantly differed between all conditions such that negative high-arousal events were rated as more arousing than negative low-arousal events ($p < 0.001$); negative low-arousal events were rated as more arousing than neutral events ($p < 0.001$). Emotional arousal when recalling the event during the online survey also varied by emotion condition, $F(2, 94) = 48.07$, $p < 0.001$. The same pattern was observed, such that negative high-arousal events were rated as more arousing than negative low-arousal events ($p = 0.04$); negative low-arousal events were rated as more arousing than neutral events ($p < 0.001$).

6.1.1.3. Vividness

After each ERP trial, participants indicated the vividness of their recollection of the cued event. The emotional valence of the memories significantly influenced vividness ratings, $F(2, 94) = 5.10$, $p = 0.008$. Post-hoc tests revealed that that negative high-arousal event memories were rated as more vivid than both negative low-arousal and neutral event memories, respectively ($p = 03$; $p = 0.003$). The negative low-arousal and neutral memory conditions did not significantly differ from each other in terms of vividness ($p = 0.76$).

6.1.2. ERP data

As in Experiment 1, in analysis of the ERP data, for violations of sphericity, Greenhouse-Geisser correction was applied to the *p*-value. Post-hoc contrasts between emotion pairs were Bonferroni-corrected for multiple comparisons.

6.1.2.1. Analysis by emotion condition

We first conducted analyses parallel to those for Experiment 1, with emotion condition (negative high-arousal, negative low-arousal, neutral), cluster, and hemisphere as factors. ERP amplitudes for the different emotion conditions are reflected in **Figure 4**. Although there was some variability in ERP amplitude as a function of emotion condition, effects of emotion condition were not statistically significant in either the 400–1000 ms window, $F(2, 88) = 0.242$,

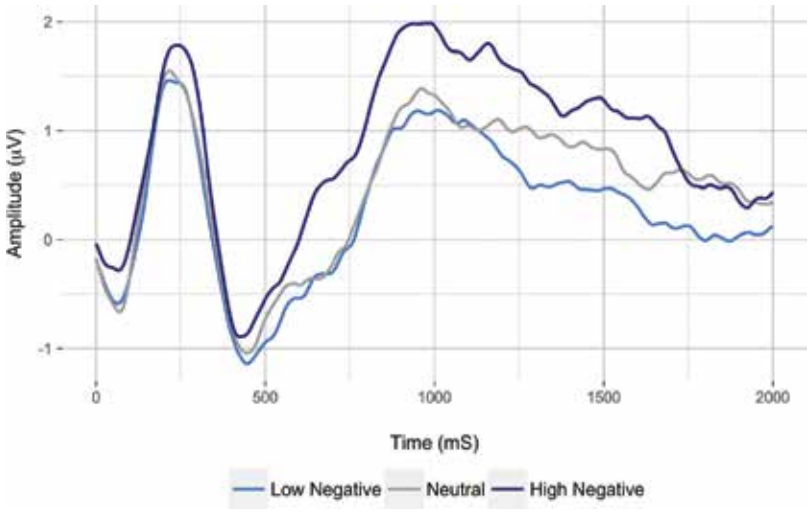

Figure 4. ERPs as a function of emotion condition (negative high-arousal, negative low-arousal, neutral) in Experiment 2.

$p = 0.785$, or the 1000–2000 ms window, $F(2, 88) = 0.332$, $p = 0.718$. There were no significant interactions with emotion condition.

6.1.2.2. Analysis by vividness

As in Experiment 1, we divided the data into high (ratings of 2–3) and low (ratings of 0–1) vividness. We then examined ERP amplitudes in mixed-effects 2 (vividness: low, high) × 3 (emotional valence of memories) × 2 (cluster) × 2 (hemisphere) ANCOVAs, which took into account differences in trial counts for high- and low-vividness trials. Separate ANCOVAs were conducted for the slow wave (400–1000 ms) and sustained slow wave (1000–2000 ms) windows. As in Experiment 1, given the focus of the analysis on vividness, we report only main effects and interactions involving subjective vividness of recollection.

In the slow wave window (400–1000 ms), the analysis did not reveal a main effect vividness ($p = 0.124$), yet the interaction of vividness × cluster was significant, $F(1, 44) = 44.839$, $p < 0.0001$. Follow-up analyses revealed an effect of vividness in the posterior lateral cluster, $F(1, 44) = 4.407$, $p = 0.041$. ERP amplitude was significantly greater on high-vividness trials ($M = 0.605$) relative to low-vividness trials ($M = 0.203$; $M_{diff} = 0.402$). In contrast, in the centroparietal cluster, there was not a significant difference as a function of the vividness of the recollection ($p = 0.321$). Emotion did not emerge as a significant effect nor participate in any interactions.

In the sustained slow wave (1000–2000 ms), the only significant effect was the interaction of vividness × cluster, $F(1, 44) = 4.822$, $p = 0.033$. Follow-up analyses did not reveal effects of vividness in either cluster.

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7. Discussion

As in Experiment 1, the conditions of the present experiment resulted in retrieval of autobiographical memories that differed in pleasantness, arousal at the time of the experience and at the time of recollection, and the vividness of recollection. In all cases, negative high-arousal memories were rated as having more of the attributes relative to negative low-arousal and neutral memories. In spite of the success of the manipulation, ERPs to the stimuli did not differ by emotion condition.

Whereas ERPs did not differ by emotion condition, they did differ by vividness. In the slow-wave window (400–1000 ms), memories that were rated as vividly recollected had greater mean amplitudes relative to memories that were rated as less vividly recollected. As was the case for Experiment 1, this pattern of findings implies that what is frequently referred to as the "emotion" effect may in some cases (such as the present research) more appropriately be described as a vividness effect. The effect emerges in response to emotional stimuli because memories of such stimuli typically are evoked with high levels of vividness. Yet when the contributions of emotion and vividness are jointly considered, the vividness of the recollection, rather than the emotion associated with the event, explains the greater amount of variance.

8. General discussion

Use of electrophysiology to examine neural responses to emotional stimuli has a long history [5, 6] revealing differential processing by emotion condition [12, 45]. Studies with patient populations and with fMRI have elaborated the findings, implying that the amygdala plays a central role in processing of emotion (e.g., [7–9]). More recently, the amygdala also has been implicated in memories that are subjectively rated as high on vividness [1, 3]. In the present research, we furthered these literatures, examining event-related potentials (ERPs) to stimuli that are both emotional and vividly recollected, namely, autobiographical memories. The findings revealed effects associated with both dimensions of difference.

In the present research, we used autobiographical memories as stimuli because they are personally relevant and significant to the participant. They also frequently are about emotional events that are recollected with varying degrees of vividness (e.g., [22], for discussion). As such, they could be expected to inform emotion effects as they occur in life outside the laboratory. We elicited the emotional autobiographical memories using neutral cue words. Neutral cue words provide an advantage relative to the more common methods of emotion elicitation via picture stimuli, short film clips, or words. The cue word stimulus itself is neutral, and across participants, the same word can be used equally often as a cue for memories from difference valence categories (see Appendix B, for example). This ensures that any effects specific to individual cue word stimuli are diminished after averaging across participants. In contrast, picture, word, and film stimuli all confound emotion condition with content (see [38] for discussion).

The unique methodology of the present research revealed unique patterns of neural processing. In Experiment 1, we observed differential processing of positive relative to negative autobiographical memories as early as 400 ms. In contrast to the canonical "emotion effect," ERP responses to positive memories did not differ from non-emotional memories; non-emotional memories did not differ from negative memories, though the effect approached significance ($p = 0.064$). In Experiment 2, in which we contrasted recall of negative high-arousal, negative low-arousal, and neutral memories, there were no effects of emotion. Similar patterns of processing of emotional and neutral memories were observed in spite of the fact that participants rated their memories as differentially pleasant and as associated with different levels of arousal. We speculate that the use of an explicitly "neutral" condition in Experiment 2 may have been responsible for the dampening of differences between valence conditions. Although participants were instructed to recall events that were not emotional, by virtue of evoking emotion in the context of recall, further processing of the memories may have been colored by emotion. In this regard, use of "unconstrained"—but not explicitly neutral—retrieval instructions may be the superior method.

ERP responses were influenced by the subjective vividness of participants' recollections. In Experiment 1, on trials on which participants reported highly vivid recollections, there was no effect of emotion. In contrast, on trials on which participants reported less vivid recollections, ERPs responses to both positive and negative events differed from those to emotionally unconstrained events. Thus, consideration of vividness and emotion in conjunction with one another resulted in a more canonical "emotion" effect than consideration of emotional valence alone. The effect could be interpreted to suggest that emotion effects are secondary to the vividness of recollection. They arise because typically, emotional stimuli are more vividly recollected, relative to non-emotional stimuli. When the latter are recalled with high vividness, there is no further enhancement of processing associated with emotion, per se. We also note the difference in patterns of neural processing when both emotion and vividness were taken into account, relative to that observed in more typical tests of recognition memory for emotional picture stimuli. Whereas the more typical effect is for emotional memories to elicit more positive-going ERPs, in the present research, ERPs to emotional stimuli were less positive-going. In future research, it will be important to test whether this difference is specific to retrieval-induced emotions. In Experiment 2, emotion and vividness did not interact, yet there were significant differences in processing on trials on which participants reported that they recollected the events with high and low degrees of vividness. As above, the possibility that in Experiment 2, all stimuli were "emotional," may explain the absence of an interaction with emotional valence.

The present research does not afford a direct test of the role of the amygdala in recollection of emotional events or in recollection that is especially vivid—the analyses we employed do not permit localization of the source of the EEG/ERP. Yet, the findings are largely consistent with those from neuroimaging studies that have examined relations between amygdala activity and both emotional valence and vividness. In Ref. [1], amygdala activity during encoding was linearly related to the subjective vividness of subsequent memory. That is, greater amygdala activity as stimuli were encoded was associated with greater subjective ratings of the vividness of the memory. The links were observed across all types of emotional items: positive,

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negative, and neutral. Thus, the activity was not restricted to items that were especially emotional. Kensinger and colleagues [1] speculated that the details of events that are enhanced by amygdala activity—including item-specific details—may be those that figure especially prominently in participants' evaluations of the vividness of their recollections. Maintaining the vividness of recollection over long delays may be especially dependent on connectivity between the amygdala and hippocampus [49].

One limitation of the current study is that it does not directly inform us on the manner in which emotion may influence the cognitive components of retrieval or how it influences subjective sense of vividness of recollection. Although mechanisms by which emotion influences memory encoding are fairly well established (e.g., [50, 51]), the manner in which emotion influences retrieval—and thus the vividness thereof—is less clear. Previous ERP studies of emotional retrieval have used recognition paradigms exclusively [52–57]. Using recognition tasks to examine the effects of emotion on retrieval presents multiple difficulties. The repetition of the emotional stimulus at retrieval may initiate new emotional responses that overlap with responses to the retrieved memory. One previous method for controlling the emotion of the retrieval cue has been to have participants read a neutral word within an emotional sentence context during encoding and then use the neutral word as a cue during later retrieval [53]. Similarly, the cue word paradigm used in the current research used neutral words to cue the retrieval of emotional memories. However, the cue word procedure probes recall, a more demanding form of retrieval than recognition in that the to-be-retrieved material is not present in the retrieval environment. For this reason, recall involves the recruitment of greater and more widespread neural resources than recognition [58] and is likely to be a more sensitive task for detecting the neural processes involved in event memory retrieval. Few previous studies have examined autobiographical memory using ERP [59–61]. The current research provides novel method for investigating the component processes of retrieval.

In conclusion, the present research revealed differential ERP responses to cue words associated with autobiographical memories that themselves differed in terms of the prevailing emotion and the vividness of recollection. Although the techniques employed cannot inform the particular roles of specific neural structures involved in memory, they are largely consistent with findings that the amygdala plays a central role in processing not only emotional but also highly vivid episodic memories. The research implies that focus on the single dimension of the emotion associated with an event, without conjoint consideration of the vividness of the recollection of it, misses important aspects of neural processing, thus undermining our understanding of memory and its determinants.

Acknowledgements

Support for this research was provided by Emory College of Arts and Sciences, Emory University. The authors also thank members of the Memory at Emory laboratory for their help at various stages of the research and the participants.

Appendix A. Cue words

airplane	camera	dream	hill	night	soda
baby	candy	drink	house	ocean	sofa
ball	car	field	internet	office	squirrel
band	card	fire	key	paint	star
bank	chair	flower	kitchen	paper	store
bed	child	friend	lamp	parent	sun
bicycle	city	fruit	letter	park	table
bird	class	game	library	party	teacher
bill	computer	gas	lunch	plant	telephone
book	cup	gift	mail	radio	television
box	dinner	grass	map	rain	toothbrush
bread	dirt	gum	milk	shirt	train
bridge	doctor	hair	money	shoe	tree
bug	dog	hand	music	snow	water
cake	door	hat	neighbor	soap	window

Appendix B. Sample narratives for the cue word *Park*

Park (Neutral):

I went to the Dogwood Festival at Piedmont Park, and lied down on the grass and watched the clouds.

Additional keyword for park: Festival

Rate the intensity of your emotional response…

How did you feel when it happened? (2) Little Emotional Response

How do you feel when thinking about the event now? (1) No Emotional Response

Rate the pleasantness of your emotional response to the memory when thinking about it now: Neutral

Park (Negative):

This is the place where my last girlfriend and I officially broke up. I felt very upset because she felt unprepared for the relationship.

Additional keyword for park: Breakup

Rate the intensity of your emotional response…

How did you feel when it happened? (5) Strong Emotional Response

How do you feel when thinking about the event now? (5) Strong Emotional Response

Rate the pleasantness of your emotional response to the memory when thinking about it now: Very Unpleasant

Park (Positive)

Cate, Yamini, and I went to Olympic park. Even though we were all wearing jeans, we decided to run through the fountains over and over again.

Additional keyword for park: Fountains

Rate the intensity of your emotional response…

How did you feel when it happened? (5) Strong Emotional Response

How do you feel when thinking about the event now? (4) Moderate Emotional Response

Rate the pleasantness of your emotional response to the memory when thinking about it now: Very Pleasant

Author details

Patricia J. Bauer[1]*, Jennifer Strafford Stevens[2], Anaïs F. Stenson[1], Theodore Waters[3] and Felicia L. Jackson[4]

*Address all correspondence to: patricia.bauer@emory.edu

1 Department of Psychology, Emory University, Atlanta, Georgia, USA

2 Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, Georgia, USA

3 Department of Psychology, New York University, Abu Dhabi

4 Department of Psychology, SUNY, Stony Brook, New York, USA

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Chapter 6

Revisiting the Role of the Amygdala in Posttraumatic Stress Disorder

Gina L. Forster, Raluca M. Simons and Lee A. Baugh

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67585

Abstract

Over the past 20 years, the reactivity of amygdala to emotive stimuli has been explored by emerging neuroimaging techniques in an effort to understand the role of amygdala in the pathophysiology of posttraumatic stress disorder (PTSD). A fear neurocircuitry model, whereby the amygdala is hyperactive due to poor top-down control from the anterior cingulate and ventromedial prefrontal cortices, has been supported by numerous experimental studies and meta-analyses. However, this model has not always been upheld by experimental data and clinical observations. In particular, many neuroimaging studies find that the amygdala fails to activate in response to negative stimuli in individuals with PTSD. Several technical and design issues may explain disparate results regarding amygdala reactivity in PTSD. However, biological and symptom-based factors emerge as possible mediators of amygdala function in PTSD, leading to the conclusion that symptoms of emotional disengagement and dissociation are associated with amygdala hyporeactivity, and symptoms of hypervigilance/hyperarousal and problems with fear conditioning and extinction are reflected by amygdala hyperactivity. Therefore, treatment of PTSD should take into account the nature of amygdala dysfunction in the individual to optimize treatment outcomes.

Keywords: posttraumatic stress disorder, dissociative PTSD, fear conditioning, neuroimaging, amygdala, prefrontal cortex

1. Introduction: posttraumatic stress disorder

Posttraumatic stress disorder (PTSD) is the most prevalent psychiatric disorder, affecting one-quarter of the world's population [1]. PTSD is characterized by four clusters of symptoms that

develop in response to a traumatic event, defined as exposure to actual or threatened death, serious injury or sexual violation [1, 2]. This can be directly experienced or witnessed by the individual, or the individual may learn that the traumatic event occurred to a close family member or close friend [2]. Clinical criteria include (1) intrusive symptoms related to reexperiencing the trauma, (2) avoidance of the traumatic memory or cues, (3) negative mood and thoughts including emotional numbing and anhedonia, and (4) altered arousal including hypervigilance, irritability, aggression, and sleep disturbances [1–3]. The Diagnostic and Statistical Manual of Mental Disorders version 5 (DSM-5) [2] also recognizes a dissociative subtype of PTSD in which dissociative symptoms additional to those typically included in the intrusive symptoms cluster occur, including depersonalization and derealization ('this is not happening to me'). PTSD symptoms result in significant social, personal, and vocational impairment [1]. Furthermore, PTSD is commonly comorbid with other anxiety or mood disorders and is also associated with increased risk for a number of negative behavioral and health conditions, including substance use disorder, chronic pain syndromes, cardiovascular disease, type II diabetes, and Alzheimer's disease [3–9]. Therefore, PTSD has wide-ranging mental and physical health implications for the individual across their lifespan. Despite concentrated efforts in behavioral sciences, neurosciences and medicine to better understand and treat the disorder, current treatment strategies are only effective in approximately half of the PTSD population (as reviewed by [10]). One way in which current and future research may contribute to improving outcomes for those with PTSD will be to develop greater insight into the neurobiological mechanisms that relate trauma to symptoms, and how these predict symptom trajectory, associated behavioral and medical problems, and treatment outcomes.

Research suggests that the symptoms of PTSD may be associated with dysfunction in regulating responses to negative emotions, as well as attentional bias for negative stimuli, altered encoding of trauma-related memories, enhanced fear conditioning and poorer fear extinction [11–17]. Therefore, PTSD can be thought of as disorder in which emotional and cognitive dysfunction intersect, which has direct implications for future efforts to improve psychological and pharmacological therapies for PTSD [17]. In terms of the neurobiology underlying the cognitive and emotional alterations observed in PTSD, experimental and meta-analysis studies implicate cortical (anterior cingulate cortex (or ACC), ventromedial and lateral prefrontal cortex, and insula) interactions with limbic structures such as the hippocampus and amygdala [11, 14, 17–19]. The core of the traditional neurocircuitry model of PTSD is illustrated in **Figure 1**, and posits that the ventral ACC and ventromedial prefrontal cortex normally provide top-down inhibition of the amygdala, which is impaired in PTSD [17, 18, 20, 21]. This model is based on converging evidence from both animal and human studies, although some human neuroimaging findings suggest that this model should be reevaluated or expanded [14, 21, 22]. Therefore, the goals of this review are to revisit the role of the amygdala in PTSD and explain disparate findings for amygdala reactivity in PTSD across neuroimaging studies; to ultimately update the neurocircuitry model of PTSD and understand how amygdala dysfunction can be helpful in the ability to predict and/or reveal treatment outcomes.

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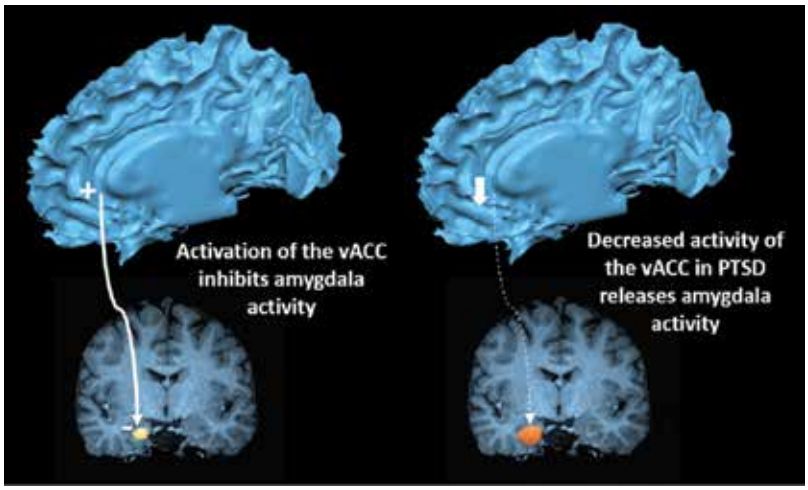

Figure 1. Fear neurocircuitry model of PTSD (based [20]). The ventral ACC and ventromedial prefrontal cortex are proposed to inhibit activity in the amygdala, and this top-down control is thought to be diminished in PTSD, leading to enhanced fear conditioning and poor fear extinction. PTSD, posttraumatic stress disorder; vACC, ventral anterior cingulate cortex.

2. Fear neurocircuitry and the amygdala in PTSD

Substantial evidence from animal experiments and human neuroimaging studies suggests that activity of the amygdala is related to the activation of fear responses and anxiety states, whereas the ventral ACC (or rodent equivalent within the medial prefrontal cortex) dampens or regulates amygdala activity and plays an important role in autonomic and behavioral inhibition [18, 20, 23–25]. Activity of specific subregions of the rat amygdala (e.g., basolateral and central nucleus) is required to respond to fearful stimuli and also to conditioned stimuli that predict or are associated with fearful stimuli [18, 20]. Similarly, the human amygdala is active during fear conditioning and this activity is positively correlated with skin conductance during conditioning [26–29], a psychophysiological index of a fear response. Projections from the rat ventral medial prefrontal cortex inhibit output from the central nucleus of the amygdala to brainstem regions involved in producing autonomic and behavioral fear responses [18, 23]. Furthermore, experimental lesions of the ventral medial prefrontal cortex of rats homologous to the primate ventral ACC prevent extinction of conditioned fear behavior while stimulation of this region promotes fear extinction [30, 31]. This, combined with observations that hypofunction or lesions of the primate ACC result in fear extinction deficits or emotional perseveration, suggests that ventral ACC inhibition of the amygdala is required for extinction of conditioned fear responses [18, 20].

In relation to the neurocircuitry model of PTSD (**Figure 1**), individuals suffering from PTSD show poor fear inhibition and reduced extinction of conditioned fear responses, indicative of disruption to ACC-amygdala circuitry [12, 13, 32, 33]. Furthermore, activity in the

ventral ACC or ventromedial prefrontal cortex within PTSD populations in response to trauma-related and nontrauma-related emotive imagery is often negatively correlated with amygdala activity [20, 34–36]. Overall, a reciprocal relationship between ventral ACC hypofunction and amygdala hyperfunction is thought to result in the inability to suppress or extinguish traumatic-related fear responses in PTSD [18, 20, 36].

The amygdala is not only critical for processes underlying fear conditioning, but also is thought to play a more general role in emotional salience and encoding emotional relevance or value, to influence attention and motivation as well as autonomic and behavioral responses [17, 22, 37]. Furthermore, it has been suggested that the amygdala may be more responsive to external threat rather than internal emotional state [38, 39]. There are numerous findings of increased amygdala activity in response to negatively valenced emotional stimuli in PTSD, and this amygdala activity is often positively correlated with the severity of PTSD symptoms [19] (**Table 1**). These findings lend support for the neurocircuitry model depicted in **Figure 1** where amygdala hyperactivity may result from poor top-down control in PTSD.

However, this model has been challenged by conflicting neuroimaging findings regarding amygdala reactivity to emotive stimuli in PTSD populations [11, 21] (**Table 1**). In particular, a meta-analysis of neuroimaging studies suggests that amygdala hyperactivity occurs more frequently in social anxiety disorder and panic disorder as compared to PTSD [11]. Although the review of relevant literature depicted in **Table 1** is not exhaustive, it is representative of amygdala-based findings in PTSD populations over the past 20 years. There are as many studies showing no effect of PTSD on amygdala reactivity as they are demonstrating hyperactivity within this region (**Table 1**). Notably, some studies actually suggest reduced amygdala activity in response to emotional stimuli in PTSD. For example, a recent study [21] shows that amygdala hyperactivity and hyporeactivity can occur within the same indi-

Population sampled	Task/stimuli	Task condition/contrasts	Amygdala activity	Reference
Civilian PTSD	Script-driven imagery	Trauma-related vs. neutral	Increased blood flow in right amygdala	[40]
Male Vietnam veterans with and without PTSD	Mental imagery	Combat-related	Increased blood flow in right amygdala in PTSD	[41]
Women abused in childhood, with and without PTSD	Script-driven imagery	Trauma-related vs. neutral	No difference in amygdala blood flow	[22]
Male Vietnam veterans with and without PTSD	Trauma-related auditory and visual stimuli	Combat-related vs. neutral	No difference in amygdala blood flow	[42]
Vietnam veterans with and without PTSD and nonveteran male controls	Trauma-related auditory stimuli	Combat sounds	Increased blood flow in left amygdala/nucleus accumbens region in PTSD group only	[43]
Women abused in childhood, with and without PTSD	Script-driven imagery	Trauma-related vs. neutral	No difference in amygdala blood flow	[44]

Population sampled	Task/stimuli	Task condition/contrasts	Amygdala activity	Reference
Male Vietnam veterans with and without PTSD	Masked faces	Fearful vs. happy	Increased BOLD signal in amygdala of PTSD vs. NoPTSD	[38]
Civilian PTSD and trauma exposed controls	Script-driven imagery	Trauma-related	No change in BOLD signal within the amygdala in any group.	[45]
Women with abuse-related PTSD—dissociative symptoms	Script-driven imagery	Imagery vs. implicit baseline	No different in BOLD signal in the amygdala	[46]
Women with childhood-related abuse PTSD and nontraumatized controls	Declarative memory task	Emotional vs. neutral words	No difference in amygdala blood flow	[47]
Israeli Defense Forces veterans with and without PTSD	Masked pictures	Combat or noncombat	Increased BOLD signal within amygdala in PTSD vs. NoPTSD group	[48]
Women with childhood-related abuse PTSD and nontraumatized controls	Stroop task	Neutral color vs. control	Increased blood flow in right amygdala in PTSD group (did not reach significance when directly compared to NoPTSD group)	[39]
		Emotional color vs. neutral color	No difference in amygdala blood flow	
Vietnam veterans with and without PTSD	Script-driven imagery	Trauma-related vs. neutral	Increased blood flow in left amygdala—males only	[34]
		Trauma-related vs. neutral	Increased blood flow in right amygdala with increasing symptoms (males and females)	
Motor-accident related PTSD (acute phase)	Masked and Unmasked faces	Unmasked fearful vs. happy	Decreased BOLD signal in right lateral amygdala with increasing CAPS	[49]
		Masked fearful vs. happy	Increased BOLD signal in right lateral amygdala with increasing CAPS	
Vietnam veterans with and without PTSD and nonveteran controls	Script-driven imagery	Trauma-related vs. neutral	No difference in amygdala blood flow in PTSD group (noncombat controls showed an increase).	[50]
Trauma-exposed men with and without PTSD	Unmasked faces	Fearful vs. happy	Increased BOLD signal in right amygdala of PTSD vs. NoPTSD; BOLD signal did not correlate with CAPS score	[35]
Civilian PTSD and nontrauma exposed controls	Masked trauma-related images	Trauma-related vs. neutral	No difference in BOLD signal in amygdala	[51]

Population sampled	Task/stimuli	Task condition/contrasts	Amygdala activity	Reference
Vietnam veterans with and without PTSD and nonveteran male controls	Rating IAPS (noncombat related)	Aversive vs. neutral and Aversive vs. blank screen	Absence of response (rCBF) in left amygdala of PTSD vs. both combat exposed and not exposed controls (i.e., stimuli elicited increased blood flow in controls)	[52]
Civilian PTSD and nontrauma exposed controls	Unmasked faces	Fearful vs. neutral	Increased BOLD signal in left ventral amygdala in PTSD vs. controls	[53]
Civilian PTSD and nontrauma exposed controls	Masked faces	Fearful vs. happy	Increased BOLD signal in right amygdala of PTSD vs. NoPTSD	[10]
Civilian PTSD—dissociative and nondissociative	Unmasked and masked faces	Unmasked fearful vs. neutral	No difference in BOLD signal in amygdala in dissociative vs. nondissociative PTSD	[54]
		Masked fearful vs. neutral	Increased BOLD signal in bilateral amygdala of dissociative PTSD vs. nondissociative PTSD	
Civilian PTSD, trauma-exposed and nontrauma exposed controls	Masked faces	Fearful vs. neutral	Increased BOLD signal in amygdala of PTSD vs. both NoPTSD groups, no sex differences	[55]
Civilian PTSD and nontrauma exposed controls	Matching faces	Emotional (angry/fearful) vs. neutral	Increased BOLD signal in right amygdala of PTSD vs. NoPTSD; Increased BOLD signal within the right amygdala with increasing PCL scores	[15]
OEF/OIF veterans (3.5 years on average return from last deployment)	Traumatic memory encoding (visual)	Combat-related vs. neutral pictures	No difference in BOLD signal in amygdala of PTSD vs. NoPTSD	[16]
Civilian PTSD and nontrauma exposed controls	Affective Priming Task	Sad + happy + neutral faces vs. fixation	Increased BOLD signal in left amygdala in PTSD	[56]
OEF/OIF veterans within 10 years of deployment	Trauma-related and nontrauma movies.	Civilian vs. fixation	Increased BOLD signal with increasing CAPS scores	[21]
		Combat vs. fixation	Decreased BOLD signal with increasing CAPS score	
Recently (8 weeks) returned military OEF/OIF personnel, trauma exposed but did not reach score of 50 in PCL-M	Affective stroop task (IAPs)	Positive vs. neutral	Increased BOLD signal with increasing PCL scores	[57]

Population sampled	Task/stimuli	Task condition/contrasts	Amygdala activity	Reference
		Negative vs. neutral	No significant relationship between BOLD signal and PCL score	
OEF/OIF veterans with and without PTSD	Masked faces	Fearful vs. fixation and Happy vs. fixation	No difference in BOLD signal in right amygdala, failure to activate left amygdala, compared to combat-exposed NoPTSD group	Figure 2
Civilian PTSD and nontrauma exposed controls	Masked and Unmasked faces and words	Masked fearful vs. neutral faces and Masked trauma vs. neutral words and Unmasked fearful vs. neutral faces and Unmasked trauma vs. neutral words	No difference in BOLD signal in amygdala in either PTSD or controls in any contrast	[58]

BOLD, blood oxygenation level dependent; CAPS, clinician administered PTSD scale; IAPS, international affective pictures set; OEF/OIF, operation enduring freedom/operation Iraqi freedom; PCL, PTSD checklist; PTSD, posttraumatic stress disorder; rCBF, regional cerebral blood flow.

Table 1. Representative studies testing amygdala reactivity to emotive stimuli in PTSD.

vidual in response to different stimuli. Specifically, veterans within 10 years of deployment were presented with either a combat-related or civilian-related movie. The civilian movie produced greater amygdala activity with increasing PTSD symptoms, while in the same participants, the combat movie elicited *less* amygdala activity with increasing PTSD symptoms [21] (**Table 1**). Therefore, the authors suggest that the standard model by which PTSD is associated with hyperactivity of the amygdala needs to be reassessed. This has broader implications for furthering our understanding of biomarkers for PTSD development and treatment, and our understanding of the neurobiology of the amygdala in general.

3. Understanding inconsistent functional neuroimaging findings for amygdala function in PTSD

A variety of design, analysis, trauma-based, pharmacological and biological factors could contribute to disparate findings regarding amygdala hyperactivity in PTSD. Each will be reviewed in turn here. First, the manner in which neuroimaging data are compared to control populations may be a key in observations of amygdala hyperactivity in PTSD. To illustrate, hyperactivity of the amygdala is more likely to be revealed in a PTSD group in the absence of statistical comparison to a control group, or often when PTSD groups are compared to a nontrauma-exposed control group (**Table 1**). A further issue is the possibility of false positives in whole-brain analyses, given that there is a great degree of variability across studies in whether multiple statistical analyses made across the brain have been corrected for multiple

comparisons to reduce the chance of type-I error [59]. The independence of samples between studies arising from the same research group is also often not clear. Consistency and transparency in the statistical comparisons made is essential to resolving whether these design-based differences may contribute to false positive outcomes for amygdala hyperactivity in PTSD studies.

With regard to lack of effects in the amygdala, the particular characteristics of this region may play an important role in the production of null effects within some PTSD studies. As reviewed by Etkin and Wager [11], the amygdala readily habituates to emotive stimuli, which may contribute to lack of differences seen between PTSD and control groups when time course analyses are not conducted. Furthermore, the amygdala is a small volume (~1.5 cm^3; [60]) region that is difficult to image largely due to its susceptibility to artifact [19, 61]. Each one of these problems singularly or combined may contribute to false negative outcomes, where hyperactivity of the amygdala in a given PTSD population is not observed.

On the other hand, a lack of an effect within the amygdala in a given PTSD study is not always due to technical difficulties associated with imaging this region. Often a null effect in the amygdala within a study is actually a failure of the PTSD group to increase activity in the amygdala in response to negative stimuli, where the control group shows amygdala activity (**Table 1**; **Figure 2**), and the analysis performed by Phan et al. [52] clearly illustrates this point (**Table 1**). A recent study [62] also sheds light on this issue, demonstrating that emotional numbing in PTSD results in a failure to activate the amygdala in all stimulus conditions, thus resulting in a 'null effect' when amygdala activity is compared across conditions in the experiment. This effect is also illustrated in **Figure 2**, where fearful and happy stimuli conditions result in a failure to activate the left amygdala in combat-related PTSD compared to combat-experienced controls. For the purposes of this review, the term 'hyporeactive' will be used to describe a lack of the effect in amygdala responses to emotive stimuli.

Differences in the paradigm and stimulus types can also play a role in whether the amygdala is hyperactive in PTSD. For example, amygdala hyperactivity in PTSD groups is more likely to occur with nontrauma overtrauma-related stimuli, or with masked rather than overt stimuli presentation, when these factors are compared across studies (**Table 1**). While a few studies compare these factors within-session, Armony et al. [49] show *decreased* activity in the amygdala with increasing PTSD symptoms for unmasked fearful faces but *increased* amygdala activity with increasing PTSD symptoms when that activity is elicited by masked fearful faces in the same participants. Furthermore, Brashers-Krug and Jorge [21] show a similar distinction between trauma-related and nonrelated stimuli; where *increased* amygdala activity with increasing PTSD symptoms is observed in nontrauma-related conditions but a *decrease* in amygdala activity with increasing PTSD symptoms occurs in response to trauma-related stimulus presentation in the same participants. While these conclusions will need to be confirmed with further direct within-session comparisons, it appears that hyperactivity in the amygdala following trauma may be best revealed by nontrauma-related stimuli that are presented outside the realm of the individual's conscious perception, whereas amygdala hyporeactivity is observed with overt stimuli. It has been suggested previously that amygdala hyporeactivity to overt trauma-related stimuli may be a compensatory mechanism specific

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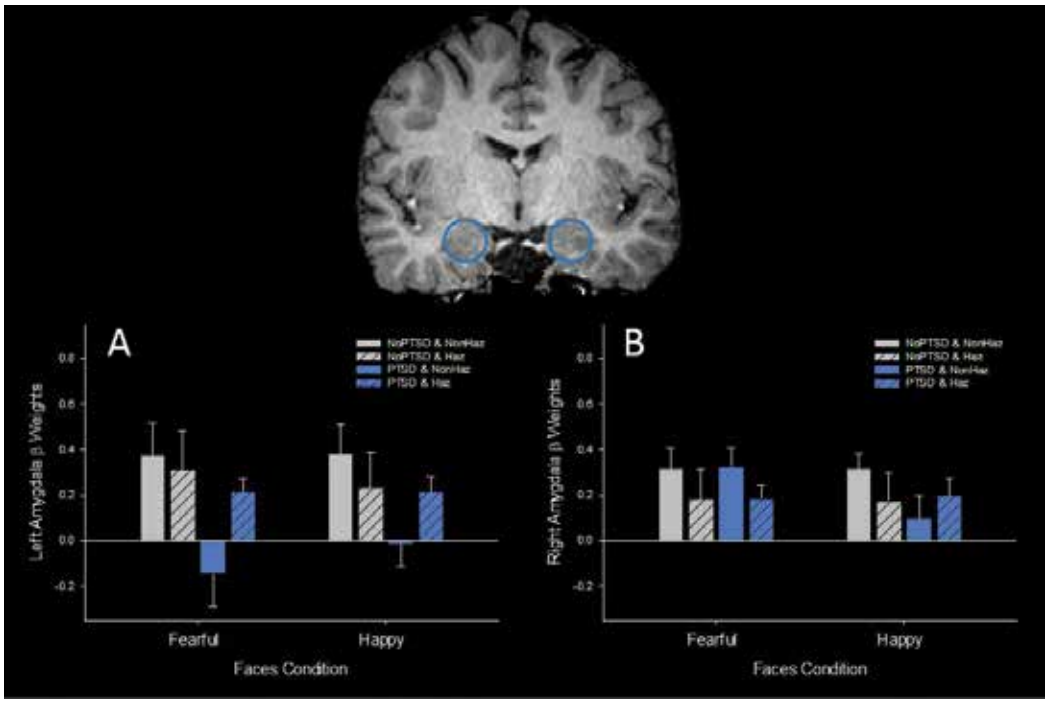

Figure 2. Activation (weights derived from BOLD signal) in the (A) left and (B) right amygdala during fearful and happy conditions of a backwards-masked faces paradigm (see [63] for full details on the paradigm and imaging parameters). One hundred and two OEF/OIF combat-exposed veterans (4.5 years from last deployment on average) were categorized by PTSD symptoms (29 or more score on PCL), and hazardous alcohol use (score of 8 or more on the AUDIT), resulting in 10–34 participants per group. (A) Three-way ANOVA revealed a significant interaction between PTSD and alcohol use in the left amygdala ($F_{(1,186)}$ = 4.696; P = 0.032), such that the PTSD without hazardous alcohol group exhibited significantly lower activation of the left amygdala in both fearful face and happy face conditions as compared to all other groups (H-S P = 0.006). (B) However, there was no significant effect of PTSD or alcohol use on activation within the right amygdala in any condition. Data represented as mean ±SEM. AUDIT, alcohol use disorders identification test; BOLD, blood oxygenation level dependent; Haz, hazardous alcohol use classification; H-S, Holm Sidak post hoc test for multiple comparisons; NonHaz, nonhazardous alcohols use classification; NoPTSD, PTSD symptoms score below 29 on PCL; OEF/OIF, operation enduring freedom/operation Iraqi freedom; PCL, PTSD checklist; PTSD, posttraumatic stress disorder.

to trauma exposure [50], which may in turn, mediate emotional disengagement or dissociation from the stimulus in PTSD reflective of emotional numbing [52]. A recent study directly testing the neurocircuitry of subliminal versus overt stimuli failed to activate the amygdala in any condition in control and PTSD groups [58], thus further research is needed to test whether stimulus type differentially activates the amygdala.

In addition to contributions made by variations in design and analysis, inconsistent findings regarding amygdala reactivity to emotive stimuli in PTSD may also result from biological phenomena or trauma characteristics that could inform our understanding of amygdala function. For example, a meta-analysis of PTSD neuroimaging studies demonstrated hyperactivity of the ventral anterior amygdala and hypoactivity of the dorsal posterior amygdala during emotional processing [11]. This suggests that the direction change in amygdala activity elicited

by emotional processing within PTSD populations could be related to the subregion of the amygdala analyzed. A second interesting biological difference within studies is presented in **Table 1**. The majority of studies that only include women fail to show changes in amygdala responsivity to emotional stimuli in PTSD, whereas the majority of studies that only include men demonstrate amygdala hyperactivity, and mixed-sex studies are mixed in their findings (either no change or increased amygdala activity with PTSD; see **Table 1**). In one of the few PTSD studies that directly compared amygdala reactivity among the sexes, increased blood flow in the amygdala in response to script-driven imagery was only noted in male Vietnam veterans [34]. However, increased amygdala activation in response to fearful masked faces was equal in male and female civilian PTSD groups in a more recent study aimed at determining potential sex differences [55]. Overall, there is mounting evidence that hyperactivity of the amygdala is more often associated with male PTSD victims.

Not surprisingly, there are sex biases in combat versus civilian PTSD prevalence, with males more likely to report combat-based PTSD and females more likely to suffer from sexual assault-related PTSD [1]. Therefore, if hyperactivity of the amygdala is more common in studies using males, amygdala hyperactivity may be more likely to be associated with combat-related PTSD. However, when comparing across studies in **Table 1**, hyperactivity of the amygdala in response to emotive stimuli is equally as likely to be found in studies of civilians, Vietnam veterans, and veterans from recent military operations. Related, participants from women-only studies are victims of childhood sexual abuse, which is associated with dissociative PTSD [14]. As discussed in detail in the next section, dissociative PTSD may be characterized by amygdala hyporeactivity to negative stimuli [14] and thus the putative sex difference in amygdala reactivity noted above may in fact be more related to differences in PTSD symptoms across those studies.

It is worth noting that the only studies to show decreased amygdala activity with PTSD were those conducted with acute civilian PTSD [49] or with veterans from recent military operations [21] (**Figure 2**). Furthermore, a recent study of OEF/OIF veterans only included participants that showed PTSD symptoms without meeting the diagnostic criteria for PTSD, who were within 8 weeks upon return from at least a 90-day deployment [57]. The rationale for this was that individuals who show significant functional impairment within 8 weeks upon return from a combat zone would provide important information as to biomarkers of a subsequent PTSD diagnosis [57], and follow-up studies of these individuals will be quite informative. In this initial study, PTSD symptom scores were not significantly related to amygdala activity elicited by the negative condition of an affective stroop task, although these scores were positively correlated with amygdala activity in the positive condition [57] (**Table 1**). While a decrease in amygdala activity was not noted, the study is supportive of a lack of hyperactivity in the amygdala to negative stimuli in individuals with preclinical acute posttraumatic stress symptoms.

Interestingly, the studies reported in **Table 1** either exclude participants based on substance/alcohol use disorder or include participants with alcohol use problems without factoring this into the analysis or conclusions. PTSD is associated with significantly elevated drinking behaviors in both men and women (range of odds ratios = 1.3–4.8) [5]. Alcohol misuse can result in

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increased amygdala responsivity to emotive stimuli [64], suggesting that excessive alcohol use may, at least in some cases, underlie hyperactivity of the amygdala in PTSD. To address this hypothesis in more detail, we recently conducted a study of veterans where activity of the amygdala to masked faces was analyzed based on PTSD symptom status as well as hazardous alcohol use [63]. Activity of the left amygdala in response to fearful or happy masked faces was significantly less pronounced in individuals with PTSD symptoms but only in the absence of hazardous alcohol use, as compared to combat-exposed veterans without such symptoms (**Figure 2**). It could be speculated that hazardous alcohol use increased amygdala activity in the PTSD participants, as would be suggested by the previously noted association between alcohol misuse and amygdala hyperactivity [64], thus seemingly normalizing amygdala activity. However, hazardous alcohol use in the absence of PTSD symptoms did not have any effect on amygdala reactivity to masked faces in our study (**Figure 2**), suggestive of an interaction between PTSD symptoms and hazardous alcohol use on amygdala reactivity. Clearly, further work is needed to understand the impact of substance misuse, especially excessive alcohol consumption, on amygdala reactivity in PTSD.

Related to issues of alcohol consumption, medications taken by participants in the studies outlined by **Table 1** could also play a role in whether hyperactivity of the amygdala is observed in PTSD groups. A meta-analysis addressing this issue found that serotonin-specific reuptake inhibitors (SSRIs) and benzodiazepines may have a confounding effect on cerebral blood flow within regions commonly imaged in PTSD studies [65] but more information is necessary to determine whether medications affect amygdala hyperactivity or lack thereof. Recently, Hayes et al. [16] found that PTSD's lack of association with hyperactivity of the amygdala did not change when including medication as a covariate in the model. Due to the sparsity of research examining the effects of medication, it is recommended that investigators include both medicated but symptomatic and unmedicated participants in neuroimaging studies of PTSD to ensure generalizability of the findings [65].

In summary, there are a range of design and analysis factors that could contribute to disparate findings regarding amygdala hyperactivity in PTSD. If these potential design and analysis confounds are equally represented among the studies listed in **Table 1**, then factors such as sex, chronicity of PTSD, PTSD symptom types, and alcohol or medication use all stand out as factors that could influence amygdala reactivity to emotional stimuli. Therefore, the importance of these factors on amygdala functioning in PTSD should be systematically explored in the future to better our understanding of amygdala function and dysfunction in PTSD.

4. Functional consequences of amygdala hyporeactivity and/or hyperactivity in PTSD

Both amygdala hyperactivity and a failure to activate the amygdala (hyporeactivity) appear to be outcomes associated with PTSD, with the direction of activity dependent upon biological and stimulus factors and symptom features. Therefore, it is important to consider the functional consequences and implications of either outcome for individuals with PTSD.

As mentioned earlier, lack of activation or hypoactivation of the amygdala to emotive stimuli may mediate emotional disengagement and autonomic blunting in PTSD ultimately leading to emotional numbing and anhedonia [11, 52, 62, 66]. Furthermore, it has been suggested that the dissociative subtype of PTSD may be characterized by reduced amygdala reactivity, as a result of inhibition by midline frontal cortex structures that are often hyperactive in PTSD, such as the medial prefrontal cortex and dorsal ACC ([14]; **Figure 3**). Indeed, trait dissociation is associated with reduced amygdala activity [67], and hypnosis-induced depersonalization results in dampened amygdala activity as elicited by nociceptive stimuli [68]. Dissociative symptoms are thought to include increased pain threshold, due to an increase in stress-induced analgesia often found in PTSD [67]. Related, thermal pain stimulation resulted in amygdala hypoactivity in participants with PTSD [69]. Combined findings suggest a link between amygdala hyporeactivity, disengaging and dissociative processing, and altered nociception among trauma-exposed individuals with PTSD. Reduced amygdala reactivity could also be associated with symptom maintenance. For example, hypoactivity of the amygdala and hippocampus during the encoding of trauma-related memories has been suggested to underlie distorted memory for trauma-associated events in PTSD [16]. Finally, it has been suggested that hypofunction of the amygdala may lead to failure to form associations between consumption and negative outcomes of drug or alcohol use [70]. Thus, amygdala hyporeactivity may increase the likelihood of future dependence issues. Overall, evidence suggests that hypoactivity of the amygdala during emotional processing may result in PTSD symptom development and maintenance, particularly in relation to emotional disengagement, dissociative processing, and comorbid substance use disorder.

Amygdala hyperactivity in response to emotive stimuli in PTSD may reflect an increase in activity within the amygdala itself or alternatively, a failure of the amygdala to habituate to the stimulus [71]. Regardless, the heightened activity in this region is often positively correlated with symptom severity (**Table 1**). Furthermore, negative affect in response to positive stimuli, and reexperiencing symptoms are both positively correlated with amygdala activity in PTSD participants [58, 66]. Amygdala hyperactivity is thought to facilitate acquisition and maintenance of fear responses in PTSD [11, 17, 19], in line with its critical role in fear neurocircuitry as described above. Hyperactivity of the amygdala could be associated with attentional bias often reported in PTSD, characterized by patients having difficulty disengaging from negative stimuli [15, 17]. This attentional bias is thought to occur because the amygdala is part of the limbic-cortical neurocircuitry active during tasks that assess negative attention (as reviewed by [17]), and a positive correlation exists between amygdala activity and negative attentional bias in PTSD [15]. Similarly, amygdala hyperactivity is often linked to hypervigilance in PTSD, where the heightened activity of the amygdala is thought to increase reactivity to emotional stimuli and enhance priming of emotion and emotional representations in the limbic system [72]. Therefore, like amygdala hyporeactivity, hyperactivity of this region is likely to contribute to distinct PTSD symptom development and maintenance.

In line with the above arguments, Lanius et al. [14] suggest that PTSD characterized by undermodulation of emotional responses (e.g., reexperiencing and hyperarousal) would be reflected by hyperactivity of the amygdala whereas PTSD characterized by overmodulation of emotional responses (e.g., dissociative PTSD) would be reflected by reduced activity of the

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amygdala. However, it is also conceivable that a given individual with PTSD will exhibit both hyperactivation and hypoactivation of the amygdala depending on context; with attentional bias and poor fear extinction being associated with hyperactivity in the amygdala, and dissociative processes being associated with hyporeactivity of this region. Therefore, we propose that PTSD should be defined as a general dysfunction of amygdala activity rather than a directional change in activity (**Figure 3**). This is supported by findings showing both hyperactivity and hypoactivity (or lack of activity) of the amygdala in the same participants with PTSD, depending upon the stimulus presented ([21, 39, 54]; **Table 1**).

With this in mind, an important question arises—is amygdala dysfunction a consequence of trauma or a predisposing factor that convers vulnerability to PTSD? This is a critical question given that over half of the population will experience a major traumatic event in their lifetime, but the lifetime prevalence for PTSD is only 7.8% in the USA [1, 3]. There is some evidence for preexisting alterations of amygdala activity predisposing individuals to social phobia [36]. In a study of Israeli Defense Forces pre and postmilitary-based trauma, amygdala reactivity increased with increasing stress symptoms and amygdala reactivity to negative stimuli pretrauma predicted increased stress symptoms posttrauma [73]. On the other hand, studies of twins discordant for combat exposure using heart rate responses as an indirect measure of amygdala activity or resting cerebral blood flow suggest that amygdala hyperactivity may be acquired with trauma rather than a preexisting condition [74, 75]. Clearly, further longitudinal studies that capture amygdala function prior to trauma and onset of PTSD symptoms are needed to clarify whether amygdala dysfunction is acquired due to a major traumatic event or predisposes individuals to posttraumatic stress symptoms, or both as might be expected with early life trauma.

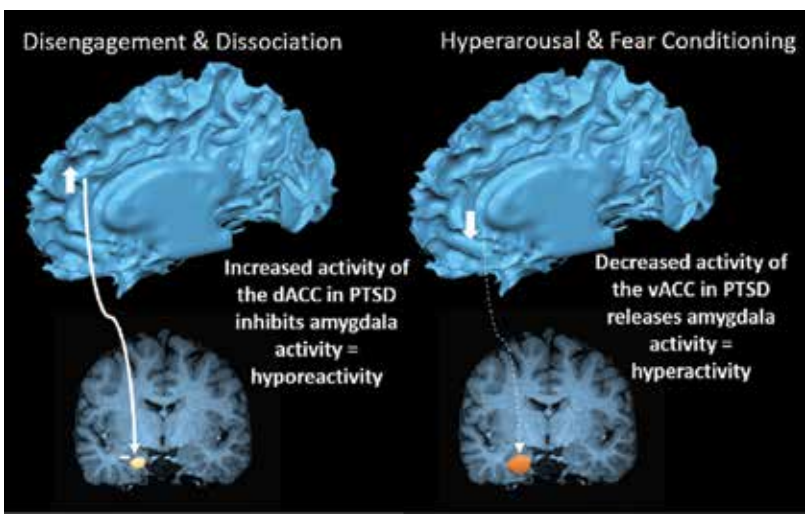

Figure 3. Refined neurocircuitry model of PTSD (based on [14]). Symptoms of emotional disengagement (emotional numbing, anhedonia) and dissociative PTSD are thought to be associated with reduced amygdala reactivity to emotional stimuli, due to increased top-down control from the medial prefrontal cortex and dorsal ACC. In contrast, hyperarousal, hypervigilance, and deficits in fear conditioning and extinction are thought to arise in part from a hyperactive amygdala, due to diminished top-down control from the ventral ACC and ventromedial prefrontal cortex are proposed. dACC, dorsal anterior cingulate cortex; PTSD, posttraumatic stress disorder; vACC = ventral anterior cingulate cortex.

A second important question arises from the conclusion that the amygdala may be dysregulated in either direction in PTSD. How does the traditional neurocircuitry model of PTSD (**Figure 1**) fit with findings that PTSD is not always characterized by amygdala hyperactivity? Many studies reported amygdala hyperactivity in PTSD also show reduced ACC or ventromedial frontal cortex activity [20, 34, 35], and a meta-analysis upholds the inverse relationship between the ventral ACC and amygdala in PTSD [11, 19]. However, it has been suggested that the 'poor top-down control of the amygdala' model of PTSD (**Figure 1**) should be reassessed, based on a failure to consistently evidence an inverse relationship between ventral ACC and amygdala activity in PTSD [21, 39, 45, 53]. Indeed, some studies show a positive association between the activity of the amygdala and ventral ACC, suggestive of either concurrent activation or positive feedback in individuals with PTSD (e.g., [21, 53]). Whether an inverse relationship between the ventral ACC/ventromedial frontal cortex and amygdala is apparent in PTSD may depend on the region of the amygdala sampled. The ventral ACC innervates the ventral amygdala to a greater degree [76] and a meta-analysis suggests that hyperactivity is more likely to be found in ventral anterior amygdala while and hypoactivity is more often observed in dorsal posterior amygdala during emotional processing in PTSD [11]. Therefore, it is plausible that the ventral amygdala is subjected to greater level of top-down control, thus becoming disinhibited with reduced cortical activity in PTSD, while the dorsal amygdala is relatively unaffected by these frontal cortex changes in PTSD. This possibility warrants further testing. Overall, an amygdala dysfunction model of PTSD would predict altered top-down control of the amygdala, with increased inhibition from dorsal ACC and medial prefrontal cortex resulting in amygdala hyperactivity accompanied by symptoms of disengagement and dissociation. Whereas decreased inhibition from ventral ACC and ventromedial prefrontal cortex would result in increased amygdala reactivity and hypervigilant and intrusive symptoms [14] (**Figure 3**).

5. The role of the amygdala in treatment of PTSD

A systematic review of amygdala function before and after psychotherapy suggests that reduced amygdala reactivity to trauma-related stimuli is associated with a decrease in symptoms in adult-acquired PTSD [77]. A similar effect has been noted in a more recent study using exposure therapy [78], and another when propranolol treatment was administered with traumatic memory reactivation [79], which the authors attribute to a norepinephrine-induced plasticity in the amygdala-cortical circuitry as outlined in **Figure 1**. Therefore, it is tempting to speculate that therapy-induced reduction in amygdala reactivity reduces symptoms of PTSD, in line with studies suggesting that hyperactivity of the amygdala is in part, responsible for symptoms of PTSD as discussed above. However, lower amygdala activity prior to treatment predicts better responses to cognitive-behavioral and trauma-focused therapy even when controlling for symptom severity [10, 80]. This suggests that reduced amygdala reactivity with decreasing PTSD symptoms after treatment may not be a direct effect of treatment on amygdala function, but instead that amygdala function prior to therapy predicts treatment efficacy. There may also be an important genetic component to this effect. Bryant et al. [81] show poorer

http://dx.doi.org/10.5772/67585

responses to cognitive-behavioral therapy in PTSD patients with short-form alleles (S and L_G) in the promoter region of the serotonin transporter (SERT) gene, even when pretherapy symptom severity was controlled in the analysis. The short-form alleles are associated with reduced SERT expression and function, and thus increased synaptic serotonin [82]. Of relevance is that short-form carriers have increased amygdala responses to negative stimuli [83, 84]. This, combined with the finding that short-form carriers do not respond as well to cognitive behavioral therapy [81], supports the idea that heightened amygdala activity is associated with poorer treatment outcomes. Interestingly, a recent analysis suggests that amygdala hyperactivity in short-form carriers may actually be a result of developmental effects of this polymorphism on amygdala structure and thus function, rather than current serotonergic status [85]. In addition to treatment implications, these findings suggest that differences in amygdala reactivity to emotional stimuli across PTSD studies, as discussed in detail above, could be influenced by the predominant SERT promotor polymorphism status of the study population.

The majority of longitudinal studies examining amygdala function before and after treatment have been conducted within the framework of the amygdala hyperactivity model of PTSD. It is worth noting that a recent study of adolescent sexual assault-related PTSD shows that higher amygdala reactivity to threat-based stimuli prior to trauma-focused therapy predicts *better* treatment outcomes, particularly related to emotional regulation [86]. The authors commented that those with lower amygdala reactivity prior to treatment and poorer treatment outcomes tended to have more complex symptoms with more comorbidities, although depression scores did not relate to amygdala changes over treatment [86]. Thomaes et al. [77] note that the fear neurocircuitry model of PTSD does not adequately explain dissociative PTSD that is often observed with child abuse, and as discussed earlier, the primary pathology of dissociative PTSD may be resulting from an inhibition of limbic structures like the amygdala rather than an overactivation [14] (**Figure 3**). There may be cases such as in dissociative-type PTSD, in which an increase in amygdala activity to threatening stimuli would be beneficial and therefore the goal of treatment. Thus, higher amygdala activity prior to treatment may in fact improve treatment outcomes in those cases. It is clear that future studies with large sample sizes and heterogeneous PTSD populations will allow comparison among PTSD symptom clusters, to draw conclusions about the ability of the amygdala to predict treatment efficacy. In the near future, it may be possible to utilize information about an individual's amygdala reactivity to personalize and guide their therapy, ultimately improving treatment outcomes for those with PTSD, thus reducing their risks of further health complications throughout their lifetime.

6. Summary and conclusions

A number of studies suggest that the amygdala is hyperactive in PTSD due poor top-down control from the ventral ACC and ventromedial prefrontal cortex (**Figure 1**). However, observations that the amygdala fails to activate in response to negative stimuli in individuals with PTSD in many studies (**Table 1**) suggest this model should be reevaluated. An examination of the neuroimaging literature conducted over the past 20 years suggests that technical and

design issues may explain disparate results regarding amygdala reactivity in PTSD. False positives could be a result of comparisons made in the absence of a control group, failure to correct for multiple comparisons in whole-brain analyses, and ambiguity in the independence of samples between studies. On the other hand, false negative findings could be due to rapid habituation of the amygdala to emotional stimuli, the small volume of the amygdala and its susceptibility to imaging problems. If one assumes that these issues equally affect studies over the years, several other important factors emerge as affecting amygdala function in PTSD. These include the amygdala subregion studied, sex of participants, PTSD symptoms—particularly dissociative PTSD, time between trauma and neuroimaging, substance and medicine use, and whether subliminal or overt stimuli are used to elicit amygdala function. Consideration of each one of these factors furthers our understanding of amygdala dysfunction in PTSD and leads to the model shown in **Figure 3** by which symptoms of emotional disengagement and dissociation are associated with amygdala hyporeactivity. On the other hand, symptoms of hypervigilance/hyperarousal, along with problems in fear conditioning and/or extinction, could be reflected by amygdala hyperactivity (**Figure 3**). Both amygdala states are proposed to be related to altered top-down control from the medial prefrontal cortical regions. Overall, it is unclear whether amygdala dysfunction predisposes an individual to PTSD or is acquired as a result of traumatic experiences, but clinical studies suggest that amygdala function prior to therapy can predict treatment outcomes for PTSD. Based on the refined amygdala dysfunction model of PTSD proposed here, it would be important to target amygdala function in a symptom-driven manner. That is, treatments should be designed to enhance or reduce amygdala function depending on the direction of pretreatment amygdala dysfunction in a given individual.

Acknowledgements

This work was supported by the Department of Defense grant W81XWH-10-1-0925. The authors would like to thank Vincent Magnotta PhD, Jeffrey Simons PhD, and Dawne Olson for their valuable assistance with the collection and analysis of data for **Figure 2**, and Jamie Scholl for editing assistance.

Author details

Gina L. Forster[1,2]*, Raluca M. Simons[1,3] and Lee A. Baugh[1,2]

*Address all correspondence to: gforster@usd.edu

1 Center for Brain and Behavior Research, Vermillion, SD, USA

2 Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota, Vermillion, SD, USA

3 Department of Psychology, University of South Dakota, Vermillion, SD, USA

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Section 2

The Role of the Amygdala in Emotional Learning - Animal Studies

Chapter 7

The Amygdala and Anxiety

Sergio Linsambarth, Rodrigo Moraga-Amaro, Daisy Quintana-Donoso, Sebastian Rojas and Jimmy Stehberg

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.68618

Abstract

The amygdala has a central role in anxiety responses to stressful and arousing situations. Pharmacological and lesion studies of the basolateral, central, and medial subdivisions of the amygdala have shown that their activation induces anxiogenic effects, while their inactivation produces anxiolytic effects. Many neurotransmitters and stress mediators acting at these amygdalar nuclei can modulate the behavioral expression of anxiety. These mediators may be released from different brain regions in response to different types of stressors. The amygdala is in close relationship with several brain regions within the brain circuitry that orchestrates the expression of anxiety. Recent developments in optogenetics have begun to unveil details on how these areas interact.

Keywords: amygdala and anxiety, central amygdala, basolateral amygdala, elevated plus maze, anxiety

1. Introduction

Anxiety is a physiological response that we encounter in everyday life. Although we normally associate it with a menace, anxiety can be elicited by a wide range of situations. Usually, we may feel anxious when the outcome of a future situation is uncertain. For example, when we are called to meet our boss, when we are preparing for a date, or when we stand in front of someone we like and we are trying to decide whether to talk to that person or not, even if we do not end up doing so. We also feel anxious when we want to do or have something excessively. Anxiety appears also as a common trait in our stressful daily living activities. When we are stressed, we become nervous, hyperresponsive, and sometimes easy to anger. Anxiety is in fact so common that it is one of the most frequent symptoms in psychiatric and neurological disorders, and it often appears in most chronic diseases.

The usual definitions of anxiety that can be found in Internet are "distress or uneasiness of mind caused by fear of danger or misfortune fear" (dictionary.com); "an uncomfortable feeling of nervousness or worry about something that is happening or might happen in the future" (Cambridge dictionary.org); "the vague, uneasy feeling you get when you're dreading something. Anxiety can also be a permanent state of nervousness that some people with mental illnesses experience, a milder version of panic" (vocabulary.com) or "Anxiety is an emotion characterized by an unpleasant state of inner turmoil, often accompanied by nervous behavior, such as pacing back and forth, somatic complaints, and rumination. It is the subjectively unpleasant feelings of dread over anticipated events" (Wikipedia.com).

Anxiety can also be operationally defined as an emotional response to potential unidentified threats and is characterized by sustained arousal, vigilance, worry, and apprehension that results in specific patterns of defensive behaviors and concomitant autonomic responses [1]. Arguably, in such cases, a threat may not necessarily mean a direct risk to our existence or a menace but may also include situations that threaten our emotional balance, like when we are anxious for meeting someone or when expecting something to happen, whether the outcome of such encounter is expected to be negative or beneficial.

Although we may agree or disagree with any or all the above definitions, clearly, we all have an opinion on what anxiety is from our own experience, as its description is highly subjective. We may also define anxiety based on its differences from fear. According to the American Psychiatric association, "Anxiety is not the same as fear, which is a response to a real or perceived immediate threat, whereas…anxiety is the expectation of a future threat" [2]. Other authors have gone deeper, suggesting that "Fear is defined as short lived, present focused, geared towards a specific threat…facilitating escape from threat; anxiety, on the other hand, is defined as long acting, future focused, broadly focused towards a diffuse threat, and promoting excessive caution while approaching a potential threat and interferes with constructive coping" [3].

When we try to understand the brain circuitry involved in anxiety, we need to distinguish it from the circuitry associated with fear. It is easier said than done, a large number of studies in animals have found that anxiety- and fear-related brain circuitry are quite similar, and much of what we know today of the brain circuitry associated with anxiety is actually extrapolated from that of fear.

A second issue that is critical for understanding the brain areas and mechanisms subserving anxiety is distinguishing anxiety from anxiety-related disorders. Here again, studies in both human and animals tend to extrapolate findings from anxiety-related disorders to anxiety per se. For example, in animals, most of what we know about the circuitry of anxiety comes from studies on fear conditioning, a model for posttraumatic stress disorder (PTSD), while in humans, most studies dealing with anxiety extrapolate their results from anxiety-related disorders, sometimes comparing levels of anxiety in patients with different disorders, including generalized anxiety, PTSD, or phobias.

In the present chapter, we shall try to describe the brain circuitry associated with anxiety per se, based on both human and animal studies. We shall include studies on fear or anxiety-related disorders only in exceptional cases where their findings may be critical for the general understanding of anxiety, or in cases where direct evidence of anxiety is lacking.

http://dx.doi.org/10.5772/intechopen.68618

2. Amygdala and anxiety

A large number of studies in humans and animals, using a variety of techniques including pharmacology, lesions, and imaging, suggest that the amygdala is a central orchestrator of anxiety responses. As previous chapters of this book deal in more detail with the anatomy and basic circuitry of the amygdala, we shall review succinctly the basic areas of the amygdala, only to the extent necessary to identify those that are believed to be relevant for anxiety.

2.1. The basic anatomy of the amygdala

The amygdala is an almond-shaped structure (hence its name; *almond* in Greek) that is buried deep within the temporal lobe. It was first identified by Burdach in the early nineteenth century. He originally described a group of cells that are now known as the basolateral complex. Subsequently, a large number of structures surrounding the basolateral complex have been identified, what is now dubbed the amygdaloid complex [4]. The nuclei within the amygdaloid complex may be broadly subdivided into the basolateral complex, the cortical nucleus, the medial nucleus, the central nucleus, and the intercalated cell clusters. The regions that appear to be more critical for anxiety are the basolateral amygdala (BLA) within the basolateral complex, the central amygdala (CeA) within the central nucleus, and the medial amygdala (MeA) within the medial nucleus. They differ in terms of cell types, functional organization, and connectivity. The BLA is a cortex-like structure that can be further subdivided into the lateral amygdala (LA), basal amygdala (BA), and basal medial amygdala (BMA) nuclei. The CeA can be further subdivided into the lateral (CEl) and medial central amygdala (CEm) [5]. Broadly, in rodents, it has been suggested that the BLA encodes the threat value of a stimulus, while the central nucleus is essential for the basic species-specific defensive responses associated with fear [6].

2.2. Amygdala studies in anxiety

Earlier studies suggested that the CeA may be involved in processing explicit cue information associated with fear, while less explicit information associated with anxiety may activate the bed nucleus of the stria terminalis (BNST) [7, 8]. The BNST is a component of the "extended amygdala," which plays a critical role in the integration of autonomic and behavioral responses to stress [7, 9]. Given the vast evidence suggesting a role of both areas in anxiety, this view has begun to be contested [10].

Fear extinction studies in animals support the idea that the amygdala plays a central role in the generation and experience of fear that can give rise to anxiety [6, 11]. In general, there is vast evidence that the amygdala plays an essential role in mediating emotions, such as anxiety [12, 13].

Excitotoxic lesions of the CeA [14], BLA [15], and MeA [13] induce changes in anxiety in rodents. Pharmacological studies suggest that activation of the BLA is anxiogenic, whereas its inhibition is anxiolytic [16, 17]. Likewise, pharmacological activation of the CeA and MeA is anxiogenic, whereas their inhibition is anxiolytic [18, 19].

Studies using functional neuroimaging in humans have shown elevated amygdala activity in anxious healthy individuals [20, 21]. Increased amygdala activity has also been reported to unattended fearful faces, which was in turn associated with higher levels of self-reported anxiety [20]. An increment in amygdalar activity has also been correlated with increased activity to neutral faces [22], suggesting that amygdala activity may reflect anxiety levels even in the absence of a threat [23]. In another study, it was reported that subjects with both low and high anxiety show increased amygdala activity in response to attended fearful faces, but only highly anxious volunteers showed increased amygdala response to unattended threat-related stimuli [20].

In consequence, current evidence suggests that the amygdala is involved in the generation of anxiety, with or without the presence of a threat.

The role of the amygdala in anxiety may be attributed at least in part, to its influence on the hypothalamic-pituitary-adrenal (HPA) axis. Both MeA and BLA are preferentially activated by psychological stressors [24–26]. Lesions of the MeA produce selective deficits in HPA axis responses to psychogenic but not homeostatic stressors [27], while BLA lesions dampen HPA axis responses to restraint stress [28]. The impact of the MeA and BLA on HPA responses is probably mediated by extensive interactions with paraventricular (PvN)-projecting neurons [29].

2.3. Amygdalar involvement in anxiety after stress

Current evidence suggests that both acute and chronic stress can induce anxiety [30, 31] and lesions in the CeA attenuate stress-induced anxiety [14]. This effect may be subserved by increased excitability in the BLA network, possibly mediated by a pronounced reduction in both spontaneous and evoked inhibitory postsynaptic potentials [32], neuronal remodeling of synapses, and dendritic branching in the BLA and MeA [33, 34].

Several studies have reported functional and anatomical changes in the amygdala following acute and chronic stress. Acute immobilization stress in rodents has been reported to increase spine density of principal neurons of the BLA only 10 days after stress, together with an increase in the level of general anxiety [35]. Chronic immobilization stress (e.g., for 10 consecutive days) can lead to greater anxiety-like behaviors in rodents, causing a significant increase in anxiety within 24 h after the cessation of the stress, and more robust and widespread increases in spine density, spanning both primary and secondary dendrites of BLA principal neurons [35], as well as robust dendritic growth in pyramidal and stellate neurons of the BLA [33, 34, 36]. The anxiety elicited by acute stress is also mediated by glucocorticoids (GCs) acting at the BLA, via signaling through nongenomic glucocorticoid receptors and the endocannabinoid CB-1 intracellular signaling pathway [37].

One of the most widely used tests to measure anxiety-like behaviors in rodents is the elevated plus maze (EPM) [38], which measures the avoidance of open spaces during spontaneous exploration. Lesioning studies have shown that permanent lesions of the CeA do not affect anxiety under baseline conditions, but attenuate the anxiogenic effect of restraint stress [39]. Moreover, lesions of the CeA produce “anxiolytic-like” effects on rats as measured by other paradigms that are sensitive to anxiety, including conditioned suppression of drinking (CSD),

conflict and defensive burying [40]. Using optogenetics (a technique that allows the activation or inhibition of specific cell targets or connections in the brain), Tye et al. showed that temporal stimulation of BLA terminals in the CeA was followed by an acute, reversible anxiolytic effect, while temporal inhibition increased anxiety-related behaviors, thus implicating specific BLA-CeA projections in the control of acute anxiety [41]. Stress-induced anxiety induces neuronal remodeling in the amygdala, which appears to be dependent on serine protease tissue-plasminogen activator [42].

2.4. Effects of chronic stress on amygdalar function and anxiety

A large bulk of evidence suggests that chronic stress (e.g., chronic restraint stress) and early exposure to stress (e.g., maternal deprivation) not only induce persistent anxiety even after 21 days from the end of stress [36], but also induce a series of functional and morphological changes in the brain. In the amygdala, changes in neural plasticity and electrophysiological responses including suppression of gamma-Aminobutyric acid (GABA) currents [43], as well as a persistent increase in dendritic arborization of spiny neurons and amygdalar hypertrophy, have been reported as a result of chronic stress, effects are not restored by ceasing the stress [36], but can be restored with short environmental enrichment [44]. Interestingly, some of these changes may be prevented by intra-BLA antagonism of corticotrophin releasing factor (CRF) [45], which is a critical hypothalamic activator of the HPA axis and mediator of stress responses in the brain.

Other brain areas involved in anxiety have also been reported to suffer changes and even damage as a result of chronic stress (reviewed in [46]). For example, in animal models of chronic stress, the hippocampus (HPC) and prefrontal cortex show atrophy, which may lead to memory impairments, whereas the amygdala hypertrophy may lead to increased anxiety and aggression (reviewed in [47]).

3. Neurotransmitters in the amygdala involved in anxiety

Given that stress induces anxiety, stress mediators in the brain, such as CRF, norepinephrine (NA or NE), and glucocorticoids (GCs) can all induce anxiety when microinjected into the amygdala. Other neurotransmitters and modulators known to be involved in anxiety include serotonin, dopamine, GABA, acetylcholine, endocannabinoids, neuropeptide Y (NPY), and orexins. Evidence of their role in anxiety while acting in the amygdala will be summarized succinctly.

3.1. Corticotrophin-releasing hormone

CRF (also known as corticotrophin-releasing hormone, CRH), is a 41-amino acid peptide produced primarily by the paraventricular nucleus of the hypothalamus (PvN) and released onto the medial eminence, triggering the release of ACTH from the pituitary, and activating the HPA axis. CRF is also released in synapses from the PvN innervating stress- and anxiety-associated brain regions, and hence, it mediates endocrine, autonomic, and behavioral responses to stress [48].

CRF microinjections into the amygdala induce anxiogenic effects [49], which appear to be mediated by CRF receptor type 1 and not type 2 [50–52]. The role of the CRF receptor type 2 in anxiety remains controversial [53]. Repeated local infusions of a CRF receptor agonist (urocortin) into the BLA increase anxiety-like behavior in rodents and induce synaptic plasticity [32]. CRF, when injected into the MeA, induces anxiogenic effects, and its antagonism, anxiolytic effects mediated by both CRF type 1 and type 2 receptors [54, 55]. Interestingly, the CeA is one of the brain regions with the highest number of CRF producing cells outside the hypothalamus. While knockdown studies using interference RNA have suggested that these CRF producing cells may have a role in the HPA axis, but not in anxiety-like behaviors [56], CRF overexpression in the CeA of nonhuman primates has anxiogenic effects [57]. Further studies are required to determine the role of CRF-producing neurons in the CeA.

Further evidence of a role of CRF in anxiety comes from studies showing that CRF concentration is markedly reduced in the amygdala after treatment with anxiolytics alprazolam and adinazolam [58], while a significant dose-dependent increase in CRF was found in the amygdala after cocaine injection [59], which is also known to induce anxiety-like behavior in rats [60]. The role of CRF in anxiety does not appear to be mediated solely by the hypothalamic-adrenal axis. In a recent study, selective knockout of hypothalamic CRF without affecting CRF to other brain areas was reported to induce a strong anxiolytic phenotype [61, 62]. Systemic administration of CRF and CRF-receptor 1 agonist, both had anxiogenic effects in the EPM, which was blocked by pretreatment with dynorphin/kappa-opioid receptor antagonist norbinaltorphimine, by a mechanism dependent on CRF receptor 1 signaling [63].

CRF within the extended amygdala has been implicated in the increased anxiety that occurs during prolonged abstinence from chronic opiates, cocaine, ethanol, and cannabinoids, and many of these stress-associated behaviors can be reversed by CRF antagonists administered systemically or into the extended amygdala [64].

3.2. Glucocorticoids (GCs)

Corticosterone (CORT) is the principal GC in rodents. Acute CORT administration can induce immediate anxiogenic effects [62], as do intra CeA microinjections [65]. Acute CORT may induce dendritic hypertrophy of BLA spiny neurons and heightened anxiety 12 days after CORT administration. Acute systemic CORT also induces dendritic atrophy of medial prefrontal cortex (mPFC) pyramidal neurons on day 6, concomitantly with impaired working memory [66], while chronic treatment with CORT also induces increased anxiety [67] and leads to dendritic hypertrophy in the BLA.

3.3. Norepinephrine (NE)

NE (also known as noradrenaline) is produced primarily by the nucleus coeruleus (LC) in the pons, although it is also produced in several other areas of the pons, medulla, and thalamus. Noradrenergic innervation from the LC is widespread, releasing NE in all stress and anxiety-related areas and inducing arousal and anxiety. In fact, acute restraint stress activates NE release in stress-related limbic regions, such as the CeA and MeA, lateral BNST, mPFC, and

lateral septum, and microinjections of adrenergic antagonists into those regions affect both stress-induced release of NE and anxiety [68]. Adrenergic manipulation in the amygdala has effects on anxiety [69] and adrenergic antagonists injected into the extended amygdala block the increased anxiety induced during prolonged abstinence from chronic opiates, cocaine, ethanol, and cannabinoids [64].

3.4. Serotonin

There is ample evidence of a role for serotonin in anxiety. Serotonin is produced in the dorsal raphe nucleus (DRN) and enhances fear and anxiety. Serotoninergic DRN projections activate a subpopulation of CRF neurons in the BNST via 5-HT 2C receptors in mice, engaging a CRF BNST inhibitory microcircuit that silences anxiolytic BNST outputs to the ventral tegmental area (VTA) and lateral hypothalamus [70]. In parallel, the PvN also receives serotonergic innervation from the median raphe nuclei in the midbrain, activating the HPA axis, with concomitant release of glucocorticoids [71], by activating serotonin 2A receptors on PvN neurons [72]. The anxiogenic effects of serotonin are further supported by the anxiogenic side effects of SSRI antidepressants that inhibit serotonin uptake [70].

Despite the clear role for brain serotonin in anxiety, studies reporting a role of serotoninergic transmission in the amygdala in modulating anxiety are somewhat inconclusive. Although the microinjections of serotonin receptors 3, 4 and 1A agonists and antagonists into the rodent BLA have no effects on anxiety, as measured using the EPM [73], compounds acting as 5-HT 3 receptor subtype antagonists microinjected into the BLA produce anxiolytic effects [74, 75]. Further support for a role of serotonin at the amygdala in anxiety comes from studies reporting that animals showing more anxious behavior in the EPM show greater serotonin content in the right amygdala [76].

3.5. Dopamine

Dopamine is another neurotransmitter that has long been associated with anxiety (for a review see [77]). Dopamine is produced by the VTA and the substantia nigra and appears to be critical within the reward system for motivation and anxiety. Both the VTA and substantia nigra release dopamine to all brain regions involved in anxiety (including the amygdala) via the mesolimbic, mesocortical and nigrostriatal dopaminergic systems (for a review, see [78]). Studies reporting a role for dopamine in the amygdala mediating anxiety suggest region specificity. Dopamine receptor D1 and D2 agonists show anxiogenic effects, while D1 and D2 antagonists induce anxiolytic effects when microinjected into the BLA [79] (but see Ref. [80]), yet they show no effects on anxiety when microinjected into the CeA [81].

3.6. GABA

The most commonly prescribed and well-known anxiolytics are benzodiazepines (BDZs), which are GABA agonists. GABA is the main inhibitory neurotransmitter in the brain and is released primarily by interneurons and astrocytes, which are widely distributed throughout the brain.

Microinfusions of GABA agonists have anxiolytic effects when injected into the BLA [15, 82–84] and CeA [85, 86], while microinjection of GABA antagonists produces anxiogenic effects when microinjected into the BLA [16, 82]. Microinjection of GABA agonists into the CeA produces anxiolytic effects in the social interaction test, which measures the interaction of unfamiliar rats [16]; however, a study by Zarrindast et al. [86] reported that intra-CeA injection of a $GABA_A$ receptor agonist had anxiogenic effects, while $GABA_A$ receptor antagonist had anxiolytic effects in the EPM.

In general, current evidence suggests that endogenous GABA acts at the BLA to inhibit anxiety responses [16]. However, the amygdala, or at least the CeA, does not appear to be critical for the anxiolytic effects of BZDs, as the anxiolytic effects of acute BZDs and barbiturates, such as chlordiazepoxide, phenobarbital, and carbamazepine, which are all GABA agonists, are not affected by CeA lesions [40].

3.7. Acetylcholine

Acetylcholine is produced by several areas in the brainstem collectively known as the mesopontine tegmentum area, the basal forebrain, the basal nucleus of Meynert, and the medial septal nucleus. The mesopontine tegmentum innervates the locus coeruleus, raphe nucleus, basal ganglia, and basal forebrain [87]; the basal nucleus of Meynert innervates the cortex and the medial septal nucleus projects to the hippocampus and cortex. Cholinergic agonist nicotine microinjected into the CeA induces anxiogenic effects, while intra-CeA injection of mecamylamine, a selective nicotinic acetylcholine receptor antagonist, produces anxiolytic effects [86].

3.8. The endocannabinoid system

Considerable evidence suggests that cannabinoids are anxiolytics and modulate the behavioral and physiological responses to stressful situations [88, 89]. There is vast evidence of a role of endocannabinoids in anxiety acting particularly in the amygdala. The primary constituent of cannabis, tetrahydrocannabinol (THC), when microinjected at low doses into the BLA produces anxiogenic effects [90]. Likewise, other cannabinoids agonists microinjected into the CeA induce anxiolytic effects [91], while cannabinoid antagonists induce anxiogenic effects [92].

3.9. Neuropeptide Y (NPY)

NPY is primarily produced by the arcuate nucleus of the hypothalamus and is released into the PvN. Local injection of NPY into the PvN increases acutely the release of CRF [93]. NPY and its receptor agonists microinjected into the CeA and the BLA have anxiolytic effects [94–96], while microinjection of NPY antagonists has anxiogenic effects [97]. The ablation of the Y2 gene—which encodes for the NPY receptor type 2—both in the BLA and CeA results in an anxiolytic phenotype, whereas deletion in the MeA or in the BNST has no effects on anxiety [98].

NPY also has an important role in feeding [99] and has been associated with stress-related obesity and metabolic syndrome [100].

4. Interactions between neurotransmitter and neuromodulatory systems in anxiety

It is critical to think of the brain as a dynamic system that is always changing and compensating to keep a balanced state, which may change over time with ageing, or chronic stress and could eventually lead to a pathological allostatic balance.

When we think of a neurotransmitter, we have the tendency to think of an individual synapse receiving a particular neurotransmitter and expressing receptors for this neurotransmitter only. In fact, the picture is much more complex, as a single neuron in cortex may participate in more than 10,000 synapses and receive different neurotransmitters, from glutamate, GABA, serotonin, or dopamine, to different peptides like orexins or neuropeptide Y. It may also respond to endocannabinoids, signaling fatty acids or microRNAs. In addition, released neurotransmitters can activate receptors located at the plasmatic membrane of neurons outside synapses. Moreover, astrocytes may also release their own transmitters (known as gliotransmitters) onto synapses, including glutamate, D-serine, and glycine, which are required for normal synaptic transmission and synaptic plasticity (for a review, see Ref. [101]).

It is assumed that single neurons may integrate information from several neurotransmitter systems by expressing different receptors in various locations of their cytoplasm. For example, a neuron may express receptors for some neurotransmitters in distant dendrites and for others located close to its soma, so that the effect of the activation of each receptor differentially influences the firing outcome (by spatial or temporal summation).

Interactions between neurotransmitter systems may not only occur at the single neuron level but may also take place between different neuronal populations, which may receive predominant inputs from different neurotransmitter systems. Alternatively, interactions may also take place not only at the site where neurotransmitters are released, but also at the site where they are produced. For example, chronic stress and chronic CORT administration both have anxiogenic effects. In a recent study, it has shown that chronic CORT treatment induces an increase in serotonin synthesis at the dorsal raphe nucleus, effect that is mediated by CRF within the nucleus [102].

There are many other relevant interactions between stress-mediators. Chronic CORT treatment mimicking chronic stress induces an increase in mRNA and protein levels of NE transporter (NET) and dopamine β-hydroxylase (DBH) in the locus coeruleus, amygdala and hippocampus, suggesting increased NE synthesis [103].

A recent study showed that delta opioid receptors and CRF co-localize in close proximity to NE-containing fibers in both BLA and CeA. In yet another example of such interactions,

the dopamine receptor agonist, SNC80, significantly attenuates the anxiogenic effects of α2 adrenergic agonist yohimbine, as measured in the rat on the elevated zero maze [104].

5. Different anxiety responses associated with different stress circuitries

To understand the different underpinnings associated with stress responses, some studies have suggested that different neurotransmitter systems may have differential roles in stress responses depending on the state of the animal or the type of stress. Recently, Smith et al. reported that while CRF receptor 1 antagonists have anxiolytic effects and allow escape in previously submissive animals, α2-adrenoreceptor antagonists have anxiogenic effects and hinder escape in nonsubmissive escaping mice [105].

Results obtained in a study using fMRI in rats receiving intravenous yohimbine, which induces stress and anxiety [106, 107], showed that the brain activity pattern found after yohimbine, which included activation of limbic structures including prefrontal, cingulate, orbito-frontal, and retrosplenial cortices, CeA, ventral hippocampus, BNST, and the shell of the nucleus accumbens, could be strongly attenuated by a α2-adrenoceptor agonist and by a dopamine (DA) D1 receptor antagonist [108]. Moreover, pretreatment with a CRF1R antagonist inhibited yohimbine-induced activation in the amygdala, striatum, and cingulate cortex, while an orexin type-1 receptor antagonist inhibited the response in fronto-hippocampal regions as well as the extended amygdala [108]. In summary, it appears that the behavioral choices in response to stress are the result of an interplay between different neurotransmitter systems in different brain areas involved in stress responses and anxiety.

6. Other brain areas within the circuitry of anxiety

6.1. Hippocampus (HPC)

The HPC is usually subdivided into ventral (vHPC) and dorsal (dHPC). There is a wide range of evidence showing that both regions of the HPC are central in the regulation of anxiety. This role appears to be mediated via several pathways, including direct amygdala-HPC and PFC-HPC interactions and regulation of the HPA axis. There are direct interconnections between the HPC and the amygdala (reviewed in [109]) and between the HPC and other limbic areas involved in anxiety [110]. Optogenetic activation of BLA-vHPC synapses increases anxiety, while their inhibition decreases anxiety [111]. This amygdalar-HPC interaction has been proposed as pivotal also for the regulation of emotions and cognition (reviewed in [112]).

Numerous studies link the hippocampus with inhibition of the HPA axis [113, 114], thus decreasing the release of glucocorticoids in response to stress and anxiety. Hippocampal activation decreases glucocorticoid secretion in rats and humans [115, 116], whereas HPC damage increases basal and stress-induced glucocorticoid secretion [113, 114]. Notably, lesion effects are most pronounced during the recovery phase of stress-induced glucocorticoid secretion, implicating the HPC in the termination of HPA responses. The inhibitory effects of the HPC

on the PvN are subserved by a relatively circumscribed population of neurons in the ventral subiculum [117] and lesions of this area result in increased corticosterone release following psychogenic but not systemic stressors [117], consistent with a context-specific modulation of stress responses.

The HPC also influences autonomic tone, as hippocampal stimulation decreases heart rate, blood pressure, and respiratory rate in awake rats, effects that are blocked by mPFC lesions [118].

Both vHPC and dHPC have been implicated in the action of several drugs that affect anxiety, including nicotine [119–121]. Nicotine elicits anxiety in mice as measured in the EPM [122]. In fact, mice deficient in the α5 nicotinic acetylcholine receptor (α5−/− mice) show high levels of anxiety in the EPM and in the dark-light box compared to WT. Interestingly the study showed that the reexpression of the α5WT gene in the VTA and the HPC of α5−/− mice restored WT levels of anxiety [123].

The HPC appears to mediate the effects of many other drugs in anxiety. Cholecystokinin (CCK) administration causes anxiety [124] and the injection of the CCK8S isoform into the dHPC has anxiogenic effects in the EPM [125]. The serotonin agonist meta-Chlorophenylpiperazine (mCPP) is another drug that produces anxiogenic effects in rodents when administered intraperitoneally [126] and shows anxiogenic effects when injected directly into the HPC [127]. Substance P (SP) is an endogenous neurokinin known to have effects on anxiety [128, 129] and the HPC contains a high density of SP containing fibers [130]. Injection of SP into the dHPC has anxiolytic effects [131]. Anxiolytics such as BZDs also show anxiolytic effects when injected into the HPC [132] and decreased BZD receptor binding in the HPC and PFC has been reported in patients with anxiety disorders [132].

6.2. Medial and lateral prefrontal cortex

Both medial and lateral prefrontal cortices (mPFC and lPFC, respectively) have direct connections with limbic structures involved in anxiety [133] including dense reciprocal connections with the amygdala and HPC [134].

The mPFC is a complex cortical structure with different subregions that may contribute to anxiety and stress responses. In general, lesions of the mPFC decrease anxiety in rats exposed to the EPM and the social interaction test [133, 135, 136]. The prelimbic mPFC preferentially inhibits HPA axis responses to psychogenic stressors [137–140]. It also regulates glucocorticoid secretion, particularly its duration. Inhibition of the prelimbic mPFC or local injection of NE enhances heart rate responses to psychological stimuli [141]. The infralimbic PFC is involved in initiating autonomic and HPA responses to psychogenic stimuli [140, 142]. Electrical stimulation of the ventromedial mPFC (including the infralimbic cortex) increases blood pressure in awake rats, while lesions or inactivation of the ventromedial PFC inhibits conditioned cardiovascular responses [143, 144] without affecting baseline heart rate or blood pressure, suggesting that it may be selectively involved in stress-induced cardiovascular regulation [145]. In a recent study using multi-site neuronal recordings with terminal optogenetic stimulation, it was shown that inhibition of the vHPC inputs to the mPFC induces anxiolytic effects [146].

Some studies have reported lateralization in the role of mPFC in anxiety. Activation of the right mPFC has been shown to increase anxiety, while its inhibition induces anxiolytic effects, whereas inhibition of the LmPFC elicits anxiogenic effects in a model of social defeat [147].

Imaging studies in humans support the notion that the mPFC is involved in anxiety. During sustained anxiety and high trait anxiety, amygdala activity has been shown to be positively coupled with dorsomedial PFC activity [148].

6.3. Paraventricular nucleus of the hypothalamus (PvN)

As stated earlier, the PvN is crucial for the regulation of the HPA axis, and hence, it is pivotal in stress responses and anxiety. The PvN secretes a number of factors or hormones that are released into the medial eminence to trigger the activation of specific neurosecretory cells in the pituitary, including CRF, which triggers the release of ACTH, which in turns activates glucocorticoid release from the adrenal cortex. It also releases other hormones, such as vasopressin and oxytocin through the neurohypophysis. Interestingly, these hormones also act as neurotransmitters and are released at synapses in limbic areas that are innervated by the PvN. Brain and peripheral oxytocin are released in response to stress and HPA activation (reviewed in [149]), release that is modulated by corticosterone [150], and it may have a role in gastric reflexes and penile erection [151]. Although it has not been proven so far, oxytocin may contribute to anxiety-related erectile dysfunction and, in an interplay with arginine vasopressin (AVP), to stress-induced gastric motility disorders [152]. Brain AVP acts synergistically with CRF on the pituitary, stimulating the release of ACTH and regulating the HPA axis. AVP is synthesized in the PvN and supraoptic nuclei of the hypothalamus and is involved in stress responses via HPA regulation (for a review see [153]). It is also released to the hypothalamus and limbic system (including the amygdala) and is involved in stress responses and anxiety. Notably, brain AVP and oxytocin appear to have opposite effects on anxiety; AVP is anxiogenic, while oxytocin has anxiolytic effects (for a review, see [154]). AVP-containing neurons have also been found in the rat's medial amygdala, innervating limbic structures such as the lateral septum and the vHPC [155]. AVP released within the brain in general has been proposed to modulate stress-induced anxiety [156] and AVP receptor V_{1b} receptor antagonists produce anxiolytic effects on mice [157]. The third neuropeptide released by the PvN is CRF, which was discussed earlier in this chapter.

6.4. Bed nucleus of the stria terminalis (BNST)

The BNST is a component of the "extended amygdala," which plays a critical role in the integration of autonomic and behavioral responses to stress [7, 9, 158]. Earlier studies suggested that the CeA may be involved in processing explicit cue information associated with fear, while less explicit information associated with anxiety may activate the BNST [7, 8]. As stated earlier, given the vast evidence suggesting a role for both areas in anxiety, this view has begun to be contested [10].

The BNST has numerous subregions that differ markedly in their contributions to stress integration. Anteroventral subregions are important in HPA axis excitation, as lesions there

reduce HPA axis responses and inhibit acute activation of PvN neurons following restraint stress [159, 160]. The anterolateral BNST contains CRF neurons that project to the PvN [161, 162], suggesting a mechanism for central excitatory actions of CRF on the HPA axis. Lesions of the posteromedial BNST increase ACTH and corticosterone secretion, PvN c-fos mRNA and PvN CRF mRNA expression [159, 160]. Tracing studies indicate that PvN-projecting neurons in the BNST are predominantly GABAergic [163], suggesting that, in contrast to the anterolateral PvN, posterior regions inhibit HPA responses to stress. Thus, it appears that different regions within the BNST may have opposing roles in anxiety. Further evidence to support this idea comes from a study showing that optogenetic stimulation of the oval BNST has anxiogenic effects, while activation of the anterodorsal BNST has anxiolytic effects [164].

Several studies have tested the effects of overall BNST modulation of different neurotransmitters in anxiety and in anxiety induced by acute or chronic stress. Changes in anxiety levels can be induced by intra-BNST manipulation of CRF [165, 166], glutamatergic AMPA receptors [167], serotonin 1A [168], calcium channel blockers [169], GABA synthesis [170, 171], calcitonin gene-related peptide [172], orexin A and B [173], and noradrenergic activity [174–176].

In awake animals, pharmacological activation of BNST elicits a rapid pressor response followed by bradycardia [177, 178], whereas inactivation exacerbates restraint-induced increases in heart rate [179]. This indicates that BNST signaling is necessary for inhibiting cardiovascular responses to stress. Modulation of heart rate by BNST stimulation or inhibition seems to be mediated by the parasympathetic nervous system [177, 178].

Chronic stress and chronic corticosterone both increase BNST volume [31] and modulation of cholinergic activity and galanin-mediated signaling in the BNST can block the anxiogenic effects of restraint stress [180, 181].

Finally, there are large inter-individual variations in fear responces of clinically anxious humans, who exhibit a tendency to generalize learned fear to safe stimuli. A study using lesions of the BNST in rodents showed that inter-individual variations in fear generalization and anxiety are determined by BNST influencing the amygdala and other limbic areas [182].

6.5. Septum

The septum is usually subdivided into the medial and lateral septum. Blockade of glutamatergic activity at the overall septum by microinjection of AMPA receptor antagonist CNQX induces anxiolytic effects [183]. Cholinergic antagonists microinjected into the medial septum have anxiolytic effects [184], while intra-septal histamine has anxiogenic effects [185].

CRF receptor type 2 agonist urocortin, when injected into the lateral septum, increases anxiety [53], while its antagonist has anxiolytic effects on mice exposed to stress and EPM [186]. Intraseptal microinjections of AVP has anxiolytic effects [187], while injection of an AVP receptor antagonist has anxiogenic effects on rats subjected to EPM [188].

6.6. Insula

The insula or insular cortex is located deep within the temporal lobe in humans and surrounds the rhinal fissure in rodents. The rat insula or insular cortex is reciprocally connected to several anxiety-related regions, including the paraventricular thalamic nucleus [189], infralimbic cortex [190], the BLA and CeA [191–194], BNST [195, 196], the lateral hypothalamic area (LHA) [192], and visceromotor regions in the brainstem including the vago-solitary complex [196–198]. There are massive reciprocal connections between the insular cortex and the amygdala [191, 192, 199, 200] to all amygdalar subdivisions [201].

Several studies have shown increased insular activity in patients suffering from different anxiety-related disorders including GAD [202], panic disorders [203], phobias [204, 205], OCD [204, 206], and PTSD [204, 207]. In all the above disorders, insular activity has been reported to decrease in response to effective treatments [202, 206, 208].

Despite the numerous studies demonstrating a relationship between insula activity and anxiety-related disorders, evidence regarding the role the insula in anxiety per se is still limited. Evidence in humans supports the notion that the insula may have a role in anxiety and a close relationship with the amygdala, as the severity of anxiety is positively correlated with CeA-insula functional connectivity [209], and the anxiolytic effects of lorazepam induce a dose-dependent decrease of activation in both the amygdala and insula during emotion processing [210].

The Insula receives interoceptive information, including pain, itch, muscular, and visceral sensations, as well as hunger and thirst [211]. Given its interoceptive inputs, it has been proposed that the insula may be crucial in determining the difference between the interoceptive sensation expected from a stimulus and the prediction of its outcome, represented as a prediction signal in the anterior insular cortex. Altered interoception would be the primary process underlying the initiation of an anxiety state, and the affective, cognitive, and behavioral components that characterize anxiety would be a consequence of this altered prediction signal, for which the insula would be pivotal [210]. There is presently not sufficient evidence to support this hypothesis.

Studies in rodents support the notion that the insula is involved in anxiety. Muscarinic cholinergic manipulation in the insular cortex in rats modulates anxiety in the EPM [212], while intrainsular modulation of adrenergic activity modulates arousal-induced increases in neophobia (reluctance to novelty), also known as hyponeophagia, which is used to measure of anxiety in rodents [213]. Direct studies to determine the areas of the insula involved in anxiety and its position relative to other brain regions associated with anxiety are a fertile ground for advancement.

6.7. Lateral hypothalamic area

The lateral hypothalamic area (LHA) has a critical role in sleep-wake states, feeding, energy balance, and motivated behavior. Cell populations in the LHA are typically defined

by neurochemical markers such as neuropeptides hypocretin/orexin, and melanin-concentrating hormone (for a review see [214]). Current evidence suggests that hypocretin/orexin neurons in the LHA integrate stress-related central and peripheral information [215–220] and produce hypocretin/orexin that is released at synapses in anxiety-related brain regions, including the amygdala, which shows reciprocal connections with the LHA [196]. Hypocretin/orexin neurons increase their firing rates *in vivo* during exposure to novel environments or other arousing situations [221]. Furthermore, *in vivo* optogenetic stimulation of hypocretin/orexin neurons results in hypercorticosteronemia [214]. Food deprivation enhances hypocretin/orexin-dependent HPA axis activation, while local infusion of leptin (a satiation signal produced by fat) into the LHA blunts hypocretin/orexin neuronal activity. Optogenetic activation of LHA leptin responsive neurons reduces both corticosterone release and suppressed hypocretin/orexin neuron activation in response to stress [214]. Further support for a role of hypocretin/orexin neurons in anxiety comes from studies showing that anxiolytic drug treatment with BZDs decreases c-fos activation of orexin neurons in the LHA [222]. Moreover, injection of orexin-A or orexin-B into the paraventricular nucleus of the thalamus increases anxiety, effect that can be blocked by an orexin 2 receptor antagonist [223]. For a review of the orexin system's role in neuropsychiatry see [224].

Given the critical role of hypocretin/orexins in increasing appetite, it is tempting to associate anxiety and appetite and proposes that the LHA is involved in eating disorders. Whether it is anorexia nervosa, obesity due to anxious eating or binge eating, eating disorders are characterized by changes in feeding behavior in response to anxiety.

There are other areas believed to play a role in anxiety, which include the paraventricular thalamic nucleus, periaqueductal grey, reward-related areas like nucleus accumbens and VTA (reviewed in Ref. [1, 225]). For a scheme of brain regions associated with anxiety, see **Figure 1**.

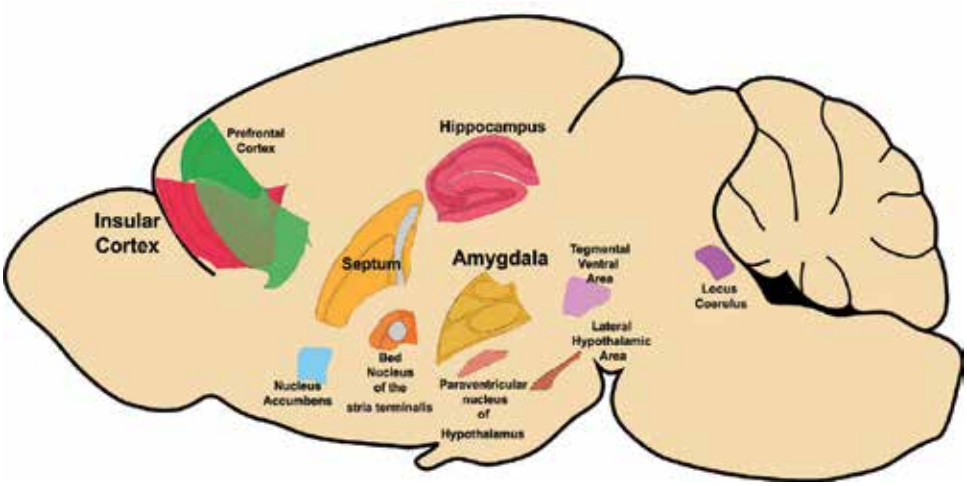

Figure 1. Scheme of main brain areas believed to be involved in anxiety.

7. Connections between the amygdala and other brain regions associated with anxiety

The recent development of optogenetic approaches allowing the activation or inhibition of specific cell types or neuronal projections using the inducible expression of channel rhodopsins has begun to allow a much greater understanding of the circuitries associated with anxiety. In a recent study, it was shown that inhibition of vHPC input to the mPFC and bilateral, but not unilateral inhibition of the input to the BLA disrupts anxiety [146]. In the same line, the activation of BLA inputs to the mPFC produces anxiogenic effects in the EPM and openfield tests, whereas inhibition of the structure produces anxiolytic effects [226]. Furthermore, systemic activation of Kapa opiod receptors was shown to inhibit glutamate release from BLA projections to the BNST and prevent the anxiolytic effects induced by optogenetic activation of BLA-BNST projections, while deletion Kapa opiod receptors from amygdala neurons induces anxiolytic effects [227]. In yet another study, it was reported that the stimulation of VTA-projecting BNST glutamatergic neurons has anxiogenic effects, while the activation of VTA-projecting BNST GABAergic neurons has anxiolytic effects, similar to the effects of direct inhibition of VTA GABAergic neurons [228].

In an elegant study by Kim et al., it was reported that the stimulation of the efferent projections from the anterodorsal BNST to the LHA reduced risk-avoidance, while the stimulation of the projections to the parabrachial nucleus reduced respiratory rate, and to the VTA, increased positive valence, all features associated with anxiolysis [164]. **Figure 2** shows a simplified scheme of the effects of stimulating the different brain regions associated with anxiety, and their

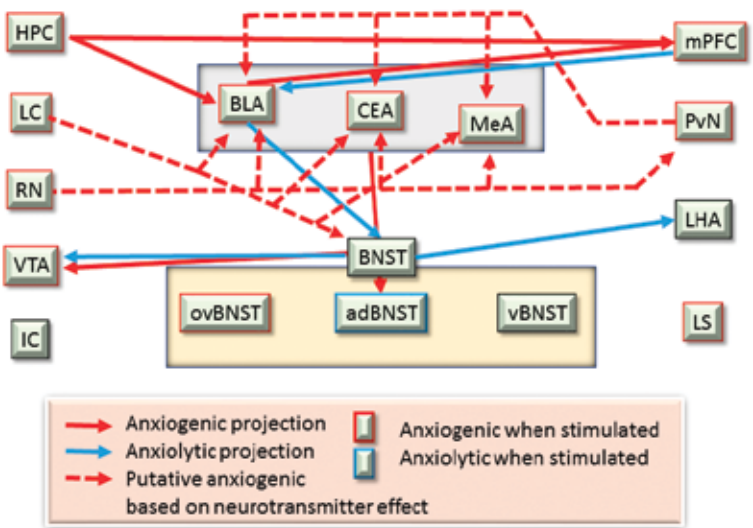

Figure 2. Effects in anxiety of the pharmacological activation of the different brain regions involved in anxiety and their projections. HPC, hippocampus; LC, locus coeruleus; RN, Raffe nucleus; VTA, ventral tegmental area; BLA, basolateral amygdala; CEA, central amygdala; MeA, medial amygdala; mPFC, medial prefrontal cortex; PvN, paraventricular nucleus of the hypothalamus; LHA, lateral hypothalamic area; IC, insula (insular cortex); LS, lateral septum; BNST, bed nucleus of the stria terminalis; ovBNST, oval BNST; adBNST, anterodorsal BNST; vBNST, ventral BNST.

projections, including those projections known to have specific effects on anxiety, and those that may have a role based on the effects of the neurotransmitters microinjected into those regions.

8. Conclusive remarks

The amygdala appears to be a pivotal orchestrator of anxiety, particularly through its subregions CeA, MeA, and BLA. Many of the other brain regions involved in anxiety have been identified, and it is quite clear that the interactions between these areas through a large number of different neurotransmitters and neuropeptides fine tune anxiety levels in response to diverse stressful situations (for a scheme of some of the known projections involved in anxiety see **Figure 2**). Recent optogenetic approaches allow a more detailed description for the role of the different connections among anxiety-subserving brain regions. Further research using more specialized tools, enabling the activation or inhibition of more specific cell populations, will allow us to understand with greater detail how different cell populations within and between brain areas interact to orchestrate behavior and anxiety in particular. There are many difficulties ahead. Distinguishing, for example, anxiety from stress responses—being anxiety a part of the stress response—is difficult in animal models where anxiety is attained through arousal or stress. From what we know so far, there are many stress mediators and brain areas that may trigger anxiety in response to different stressors, whether they are feeding-related, pain-related, acute- or chronic stress-related. The dysfunction of the brain circuitry subserving anxiety and stress, and the neurotransmitter systems involved, may be critical for the development of anxiety and stress-related pathologies. It is interesting to note that pharmacological activation of most anxiety-related areas has anxiogenic effects (see **Figure 2**), and their inhibition, anxiolytic. Which of those areas are downstream or upstream from each other within the circuitry of anxiety still remains elusive. It is easy to speculate that visceromotor areas should be downstream. Yet emotions require constant sensory feedback. Most visceromotor areas are also viscerosensory and as explained in the chapter, most areas involved in anxiety, including the amygdala, when activated, can elicit direct activation of the HPA axis. So, the pieces of the puzzle are all there, but it may still require some time to put them all together.

Funding and acknowledgements

This chapter was funded by Grant FONDECYT 1160986. We wish to thank the Book Editor, Barbara Ferry, for her useful comments.

Author details

Sergio Linsambarth, Rodrigo Moraga-Amaro, Daisy Quintana-Donoso, Sebastian Rojas and Jimmy Stehberg*

*Address all correspondence to: jstehberg@unab.cl

Laboratorio de Neurobiologia, Centro de Investigaciones Biomedicas, Universidad Andres Bello, Santiago, Chile

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Chapter 8

The Role of the Amygdala in Regulating the Hypothalamic-Pituitary-Adrenal Axis

Joseph Weidenfeld and Haim Ovadia

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67828

Abstract

We investigated the regulatory role of the amygdala upon the function of the hypothalamic-pituitary-adrenal (HPA) axis as measured by median eminence corticotrophin releasing hormone (CRH) content and serum levels of adrenocorticotrophic hormone (ACTH) and corticosterone. Our findings showed that (1) lesions of the central amygdala inhibited the HPA axis responses to a variety of stressful stimuli. (2) Depletion of norepinephrine or serotonin in the amygdala and hypothalamus and local injections of norepinephrine and serotonin receptor antagonists into the central amygdala inhibited the HPA axis responses to neural stress. Norepinephrine and serotonin agonists injected into the amygdala caused an increase in HPA axis activity. The activation of the amygdala facilitated the *in vivo* release of serotonin from the paraventricular nucleus following electrical stimulation of the brainstem raphe nuclei. (3) Electrical stimulation of the amygdala impaired the glucocorticoid negative feedback action following neural stressful stimuli probably via a decrease in hippocampal corticosteroid receptors.

Keywords: amygdala, HPA axis, stressful stimuli

1. Introduction

One of the major responses to various stressful conditions is the activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in hypersecretion of glucocorticoids (GC) (cortisol in humans and corticosterone in rodents). These hormones affect a wide spectrum of body functions, and in particular, they have an essential role in regulating energy body requirements by acting on glucose, protein, and fat metabolic pathways. GC also has a

cardinal role in many aspects of immune system function. The effects of GC on body tissues, including the brain, are mediated by two main types of intracellular GC receptors known as type 1 and type 2 [1]. The HPA axis consists of corticotrophin-releasing hormone (CRH) containing neurons that are located in the paraventricular nucleus (PVN) of the hypothalamus, which send projections to the median eminence (ME). In response to stressful stimuli, CRH is released into the portal circulation, causing the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary gland that in turn stimulates the secretion of the GC from the adrenal cortex [2]. The HPA axis can also be activated by various cytokines, including IL-1β, IL-6, and TNF-a [3]. Intracerebroventricular (ICV) administration of each of these cytokines stimulated the secretion of CRH, ACTH, and GC into the bloodstream. By using specific neurotoxins and specific agonists/antagonists, it was found that the responses of the HPA axis to various stressful stimuli, including these inflammatory cytokines, were regulated by central neurotransmitters and, in particular, norepinephrine (NE) and serotonin (5-HT) [4].

It is now well established that the activity of the HPA axis is modulated by extrahypothalamic limbic structures and in particular the hippocampus and the amygdala (AMG) [4, 5]. While hippocampal neurons exert an inhibitory effect on the activation of the axis, the activity of the AMG exerts a significant facilitatory effect [4]. Indeed, data have shown that acute electrical stimulation of AMG in several animal species activated the HPA axis [4, 6], while bilateral AMG lesions inhibited the adrenocortical responses to somatosensory stimuli caused by sciatic nerve electrical current and olfactory stimuli caused by exposure to amyl acetate fumes and immobilization stress [4]. We have previously provided evidence that AMG may regulate the HPA axis responses to hypoglycemia and cytoglucopenia, known also to activate HPA axis. Complete hypothalamic deafferentation in rats that disrupts the neural pathways between the hypothalamus and limbic structures including the AMG, caused a marked inhibition of the adrenocortical responses to insulin-induced hypoglycemia and 2-deoxyglucose-induced cytoglucopenia [7, 8]. The AMG has two direct and one indirect efferent connection with the hypothalamus: (1) The stria terminalis directly connects the AMG with the preoptic area in the hypothalamus. (2) The ventral amygdalofugal pathway situated in the medial forebrain bundle directly connects the central and basolateral AMG with the hypothalamus [6]. (3) An indirect pathway consists of projections from the AMG central nucleus to the bed nucleus of the stria terminalis the efferents of which retroproject to CRH cells in the hypothalamic PVN [9]. These three connective pathways form the anatomical basis for the neural communication between the AMG and the PVN of the hypothalamus.

In this review, we have focused mainly on the studies done in our laboratory for almost 35 years under the direction of the late Prof. S. Feldman. The aim of these studies was to elucidate the influence of the AMG on the activity of the HPA axis, and they can be subdivided into three topics: (1) Determining the role of the AMG on the HPA axis responses to various stressful stimuli. (2) Determining the role of central NE and 5-HT in mediating the effects of the AMG upon the HPA axis. (3) Determining the role of AMG in regulating the negative feedback action of GC upon the HPA axis.

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2. The role of the AMG on the HPA axis responses to various stress modalities

2.1. Neural stimuli

Male rats were exposed to either one of the following neural stimuli: photic stimulation that consisted of a 4-min exposure to flashes of light emitted by a photostimulator and an acoustic stimulation that consisted of a 4-min exposure to a ringing bell [10]. In intact rats, these two neural stimuli caused a significant increase in serum ACTH and CS associated with a marked depletion of ME CRH content due to the release of CRH into the hypothalamic-pituitary portal circulation. A similar activation of the HPA axis was obtained in rats with lesions in the basal nucleus of the AMG, indicating that this amygdaloid region does not participate in the modulation of the HPA axis responses. On the other hand, bilateral lesions of the medial and central nuclei of the AMG inhibited the rise in serum ACTH and CS following photic and acoustic stimuli. These results suggest that a tonic input from the medial and central AMG to the PVN is essential for the release of CRH from the ME and the subsequent pituitary-adrenocortical responses following these neural stimuli. Interestingly, while lesions of the central AMG inhibited the HPA axis responses following a short (4 min) photic and acoustic stimulation, lesions of the central AMG did not affect the HPA axis responses following longer (30 min) of neural stimulation [11]. This apparent discrepancy may be explained by the fact that activation of the AMG mediates the response to short stressful stimuli, while prolonged adrenocortical activation may involve both neural as well as systemic mechanisms. It is possible that the prolonged stimulus causes an increase in the peripheral secretion of cathecholamines that are known to stimulate both the release of CRH from the PVN and ACTH from the pituitary gland [12].

In conclusion, these results demonstrate the differential effects of various AMG nuclei in modifying the HPA axis responses to neural stimuli, and the role of CRH in this mechanism. A facilitatory input from the medial and central AMG nuclei to the hypothalamus seems to play a role in the activation of the HPA axis response.

2.2. Surgical stress

Surgical stress is the combined result of tissue injury, anesthesia, and postoperative pain. It is characterized by elevated levels of serum ACTH, CS, and prostaglandin E_2 (PGE_2). PGE_2 and other eicosanoids are produced from arachidonic acid by the cyclooxygenase pathway and are involved in inflammatory responses, nociception as well as hormones secretion [13–15]. We were also interested in studying PGE_2 measurements in brain tissue as a part of the evaluation of mechanisms involved in the responses to stressful stimuli. During the previous experiments, we examined the role of AMG activation on HPA axis responses in rats that underwent laparotomy and perioperative pain management. In addition, we tested the effects of surgical stress on the production of PGE_2 in several brain regions including the AMG. The results show that surgery is associated with activation of the HPA axis and bilateral lesions of the central AMG nuclei that blocked this response [16]. The results also indicate, for the first time,

that surgical stress is associated with elevated production of PGE_2 in the AMG. Furthermore, pre-emptive pain management (as described in details in Ref. [16]) extended into the immediate postoperative period that attenuated the production of PGE_2 in the AMG and the adrenocortical response. We have shown that the analgesic procedure used in our study (consisting of pre-emptive intrathecal and continued by postoperative sustained release of morphine) provided an effective long-lasting pain relief.

The mechanisms involved in the activation of the HPA axis by surgical stress, and its attenuation by perioperative analgesia is not clearly understood. Previous studies have shown that the activation of the HPA axis following laparotomy is mediated by central mechanisms. This includes the secretion of CRH from the hypothalamic PVN into the pituitary gland that depends on noradrenergic neural input from brainstem nuclei. Thus injection of 6-hydroxydopamine (6-OHDA) into the PVN, which significantly depleted NE content in the PVN markedly inhibited the HPA axis response to laparotomy. Also, ICV injection of IL-1β receptor antagonist inhibited the laparotomy-induced HPA axis response. This may indicate that the activation of the HPA axis may be mediated also by IL-1β [13, 17, 18].

Previous studies demonstrated that AMG is one of the brain regions, which expresses neuronal cyclooxygenase 2 (COX-2) and PGE_2-binding sites [19]. It has been shown that COX-2 maybe induced in brain endothelial cells and constitutively expressed in brain neurons in the cerebral cortex, the hippocampus, AMG, and glial cells [20]. PGE_2, which is elevated in the brain during surgical stress, is involved in the activation of the HPA axis [21]. These reports lend support to the hypothesis that the decreased PGE_2 production in the AMG following preemptive analgesia could be involved in the attenuated surgery-induced HPA axis activation. It should be emphasized that our findings show a correlative relationship between changes in amygdalar PGE_2, the HPA axis activity, and analgesic treatment. It may be assumed that one mechanism involved in this correlative relationship is the rise of nociceptive inflow including IL-1β from the inflamed tissue. This cytokine is known to increase both brain PGE_2 and the activity of the HPA axis. The pre-emptive analgesia may attenuate these responses.

2.3. Adrenalectomy

Elimination of the negative feedback exerted by GC following adrenalectomy (Adx) causes hypersecretion of hypothalamic CRH and, consequently, ACTH from the pituitary gland. We were interested in examining the effect of lesions of the central AMG nucleus on serum ACTH following Adx. Our data demonstrated that this lesion inhibited, ACTH hypersecretion following Adx [22]. Thus, our results suggest that AMG activation is involved in the ACTH hypersecretion following Adx. The nature of the neurotransmitters that mediate Adx-induced ACTH hypersecretion has not been fully elucidated. However, we demonstrated that depletion of hypothalamic NE caused by 6-OHDA, inhibited the rise of ACTH following Adx [23]. This supports the notion that an intact noradrenergic system of the hypothalamus is important in mediating this mechanism. In summary, the central AMG nucleus, which has a facilitatory effect on the HPA axis, can also modulate Adx-induced ACTH hypersecretion.

http://dx.doi.org/10.5772/67828

2.4. Viral infection of the brain

It is now well established that there is a bidirectional communication between the nervous system and the immune system via three different pathways: direct neural circuits reaching lymphoid organs, circulating humoral factors such as GC and neuropeptides, and cytokines released by lymphoid cells and neurons. This communication is mediated by specific receptors found on both immune cells and neurons. Since the HPA axis activation plays a major role in the bidirectional communication between the nervous and immune system, we sought to examine the role of the AMG in mediating the activation of the HPA axis following an immune challenge caused by herpes simplex type 1(HSV-1) infection of the brain [24]. This neurotropic virus is the most common cause of acute non-epidemic viral encephalitis. HSV-1 encephalitis typically manifests with fever and behavioral changes, including hyperactivity and aggression due to the predilection of the virus to limbic areas. We have previously shown that corneal or ICV inoculation of HSV-1 in rats results in the activation of the HPA axis and induces fever and typical behavioral changes. These effects of HSV-1 are probably mediated via toll-like receptors found on neurons and microglia, which activate signal transduction specific for the increased expression of IL-1β and other proinflammatory mediator genes [25]. The increased expression of these genes was observed in the brainstem, hypothalamus, and AMG and depends on intact noradrenergic transmission connecting the brainstem with the hypothalamus. We showed that impairment of the noradrenergic transmission caused by 6-OHDA inhibited the HPA axis responses following this viral infection [25]. One study showed that lesions of the central AMG markedly reduced ACTH secretion, hypothalamic CRH, as well as the expression of c-Fos gene in oxytocin cells in response to systemic injection of IL-1β [26]. We found that a bilateral lesion of the central amygdaloid nuclei markedly attenuated the HPA axis responses as well as fever, motor hyperactivity, and aggressive behavior that are induced by HSV-1 infection [27].

A plausible mechanism of reduced brain responses to HSV-1 in lesioned animals may be due to damaged neural pathways that regulate neuroendocrine and behavioral functions. The neurotransmitters that mediate the modulatory effect of the central AMG on the neuroendocrine and autonomic responses to HSV-1 infection are not entirely clear. The central AMG can influence the hypothalamus both via indirect and direct pathways. Indeed, this structure has rich projections to brainstem nuclei, including the noradrenergic and serotonergic nuclei [28, 29]. It has been demonstrated that these projections include neurons containing CRH that in this case function as a neurotransmitter in the AMG. These neurons activate, noradrenergic and serotonergic output from brainstem nuclei [26, 28, 30, 31]. These nuclei play an essential role in the activation of the HPA axis upon exposure to stressful stimuli, including immune challenges [32]. Another indirect pathway by which the central AMG can regulate hypothalamic functions is via its connection to the bed nucleus of the stria terminalis (BNST), which is known to regulate the HPA axis responses. In addition, the central AMG contains a small number of cells that project directly to the PVN. Regarding HSV-1 and HPA axis interactions, we have previously reported that HSV-1 infected the brainstem and induced IL-1β gene expression in this region [24, 33]. In turn IL-1β activates noradrenergic output to the hypothalamus via the ventral noradrenergic bundle (VNAB). We have shown that HSV-1-induced HPA

axis activation depends on endogenous IL-1β, as its receptor antagonist completely blocked ACTH and CS responses to the virus [33].

We have also shown that a neurotoxic lesion of the VNAB with 6-OHDA completely prevented the HPA axis responses to HSV-1 [25]. Furthermore, central AMG lesions attenuated the recruitment of noradrenergic neurons of the brainstem and BNST and the HPA axis responses to IL-1β [26]. Altogether these findings may suggest that the attenuation of the responses, including ACTH, CS, hyperactivity, and aggression, to HSV-1 infection in animals bearing lesions in the central AMG is due to a reduction in noradrenergic activity in the brainstem and BNST.

2.5. Direct adrenergic and glutamate stimulation of the hypothalamus

The involvement of the central AMG in the HPA axis responses to a variety of stimuli may be mediated by direct and indirect neural pathways, which at present are not fully characterized. We have previously demonstrated that the activation of central AMG has a facilitatory effect on the function of the dorsal raphe nucleus 5-HT neurons, which project to the PVN, suggesting a mechanism by which this structure may modulate the HPA axis responses [31]. In particular, we examined the role of the central AMG in modulating the HPA axis responses to specific hypothalamic noradrenergic and glutamate stimulation. Previous studies indicated that the excitatory neurotransmitter glutamate is also involved in neuroendocrine regulation. Thus, direct injection of glutamate into the PVN caused CRH release from the median eminence and consequent increase in serum ACTH and CS levels [34, 35].

Several *in vitro* and *in vivo* studies produced evidence for a reciprocal interaction between the action of NE and glutamate at the hypothalamic level. For example, the effect of hypothalamic NE on CRH release is mediated by intrahypothalamic glutamatergic interneurons [36]. Also, the activation of the HPA axis by electrical stimulation of the VNAB, or by direct PVN injection of, phenylephrine (an agonist with high affinity to α1-adrenergic receptors) was markedly inhibited by PVN administration of selective ionotropic glutamate receptor antagonists [37].

We attempted to further elucidate the mechanisms by which the activity of the AMG regulates the function of the HPA axis. To this end, we examined the effect of bilateral lesions of the central and medial AMG on serum ACTH and CS in response to the activation of the adrenergic neurons. Our results showed that the ACTH and CS responses to electrical stimulation of the VNAB, or local PVN administration of the α1-adrenergic receptor agonist phenylephrine, were markedly attenuated following central or medial AMG lesions. In addition, the pituitary-adrenal response to PVN injection of the excitatory neurotransmitter glutamate that is known to interact with NE at the hypothalamic level was also inhibited significantly following central or medial AMG lesions [38]. The exact neural mechanism involved in the modulatory role of the amygdaloid nuclei in the HPA axis responses to local adrenergic and glutamate stimulation is not clear at present. One mechanism may involve neural projections from the central or medial AMG to brainstem noradrenergic and/or serotonergic nuclei, which in turn project back on the hypothalamus, including the PVN [6]. These monoaminergic neurons are known

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to play an essential facilitatory role in the HPA axis responses. The central and medial AMG are also targets of innervation from brainstem NE neurons [39]. This innervation was found to play an important role in the activation of the HPA axis to stressful stimuli. It has been demonstrated that lesions of the central AMG greatly reduced the noradrenergic activity, in response to stress, within the hypothalamus including the PVN and in the bed nucleus of the stria terminalis. Similarly, another study demonstrated that bilateral central AMG lesions resulted in a significant reduction of c-*fos*-positive noradrenergic cells in the A1 brainstem region and in the bed nucleus of the stria terminalis. All together, these findings suggest that lesion of the central AMG impairs the hypothalamic adrenergic activity that results in inhibition of the adrenocortical response to direct adrenergic stimulation.

At present, the mechanisms by which the central AMG regulates the HPA axis responses to PVN glutamate administration are not known. Previous studies suggested an interaction between NE, 5-HT, and glutamate systems that may be involved in the effects of glutamate. In a recent study, it was found that the NE-induced increase of excitatory postsynaptic potentials was blocked by ionotropic glutamate receptor antagonists. This result suggests that spike-mediated transmitter release in the hypothalamus resulted from presynaptic effect of NE on glutamate neurons [36].

These findings suggest that impaired ACTH and CS response to PVN glutamate administration may be due to reduced hypothalamic NE or 5-HT activity resulting from the central or medial AMG lesions.

3. The role of central neurotransmitters in mediating the effect of AMG on adrenocortical responses

The responses of the HPA axis to a variety of stimuli depend on stress-sensitive neural circuits. Among them, NE and 5-HT containing neurons in the brainstem play a major role. The role of these neurotransmitters in mediating the effect of the AMG activation upon the HPA axis is not entirely clear. We attempted to elucidate the role of NE and 5-HT systems in both the AMG and the hypothalamus by using specific neurotoxins, agonists, and antagonists to NE and 5-HT receptors and by measuring the local secretion of 5-HT by microdialysis. Direct injection of 6-OHDA into the AMG caused a marked depletion of NE in this region [40], but no change in hypothalamic NE content was found. Our results also showed that 6-OHDA injection in the AMG inhibited the release of CRH from the ME following photic stimulation and, consequently, the secretion of ACTH and CS. However, this effect was specific to photic stimulation as depletion of amygdalar NE did not affect the HPA responses to acoustic stimulation. A similar differential effect of NE system damaged either by 6-OHDA or electrolytic lesions in the medial forebrain bundle (MFB), which serves both as an afferent NE and an efferent amygdalofugal pathway from the AMG to the hypothalamus was observed in our previous studies [4]. Similarly, in these experiments, we observed a greater inhibitory effect of NE depletion in the MFB on adrenocortical and ACTH responses to photic stimulation than to acoustic stimulation.

The central nucleus of the AMG receives an important catecholaminergic innervation from the ventrolateral medulla and the nucleus of the solitary tract. A number of studies indicate that exposure to stressful stimuli activates NE terminals in the AMG [4]. For example, some experiments have demonstrated that immobilization increases synthesis, release, and metabolism of NE in the AMG in conscious rats [41, 42]. The mechanism by which NE in the AMG activates the HPA axis following photic stimulation is not entirely clear. However, it is of interest that stressful stimuli cause an increase in NE in both the PVN and the AMG. As the presence of NE in the PVN is essential for the release of hypothalamic CRH, it can be assumed that NE also plays a stimulatory role within the AMG in the activation of the HPA axis.

To examine the nature of adrenoceptors in the AMG which mediate the effects of NE on the HPA axis responses following neural stimulation, rats were injected with prazosine (α1 blocker) or atenolol (β1 blocker) [43]. We showed that administration of the alpha1 but not beta-adrenergic antagonist into the central AMG blocks the responses of the HPA axis to photic stimulation. These findings indicate the importance of α1 adrenoceptors in the AMG in the mediation of HPA axis responses following neural stimuli.

We have previously demonstrated that hypothalamic 5-HT plays a role in the facilitatory effect of AMG activity on the HPA axis. To elucidate the role of 5-HT in the AMG in mediating the effect on the HPA axis, the neurotoxin 5,7-dihydroxytriptamine (5,7-DHT) was injected into the central nucleus of the AMG [44]. This treatment caused almost a complete depletion of 5-HT content in the AMG, but there was no effect on its concentration in the hypothalamus. The results indicated that 5-HT depletion in the AMG inhibited the effect of short photic stimulation on ME CRH content and ACTH and CS plasma levels. Also, in rats pretreated with ketanserin, a 5-HT_2 receptor antagonist, the rise in ACTH and CS following photic stimulation was significantly inhibited. These results suggest that the presence of 5-HT in the AMG is involved in the activation of the HPA axis by photic stimulus. All regions of the AMG have significant 5-HT innervation, which comes from the dorsal raphe nucleus with additional input from the medial raphe nucleus. Since these responses were also blocked by the direct injection of ketanserin into the AMG, it can be assumed that this subtype of 5-HT receptor is involved in the effect of the AMG function in regulating HPA responses.

Next we attempted to substantiate the importance of amygdalar NE and 5-HT in mediating the HPA axis responses. To this end, we examined the effect of direct AMG injections of phenylephrine (NE agonist) and 8-OH-DPAT (a specific 5-HT_{1A} serotonergic receptor agonist [45]. These agonists activated the HPA axis attested by increased secretion of ME CRH and a significant rise in serum ACTH and CS. We also showed that rats with hypothalamic depletion of NE and 5-HT failed to activate the HPA axis in response to electrical stimulation of AMG. Thus, direct stimulation of NE and 5-HT systems in the AMG activates the HPA axis and that this effect depends on the presence of these excitatory neurotransmitters also at the hypothalamic level [46].

To explore a possible mechanism by which the AMG affects the HPA axis function, we examined the specific role of the serotonergic system in mediating the effect of the AMG on the activity of the HPA axis [31]. Bilateral lesions of the AMG in rats reduced ACTH and CS responses to electrical stimulation of the dorsal raphe nucleus, where the cell bodies of serotonergic neurons are located. AMG lesions had no effect on the ACTH and CS responses

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to administration of a 5-HT_{1A} receptor agonist directly into the PVN of the hypothalamus, indicating that there was no impairment in the activity of the postsynaptic 5-HT_{1A} receptors in the hypothalamus. *In vivo* microdialysis showed that AMG lesions markedly attenuated the effect of electrical stimulation of the dorsal raphe nucleus to increase extracellular secretion of 5-HT in the PVN. These results show that activation of the AMG influence the activity of the dorsal raphe 5-HT neurons that project to the PVN and suggests a mechanism by which the AMG may modulate the function of the HPA axis.

4. The role of AMG in regulating the negative feedback effect of GC on adrenocortical responses

The activity of the HPA axis is negatively regulated by the feedback system exerted by corticosteroids that act predominantly at the level of the hippocampus [47]. The effect of these hormones is mediated by two types of intracellular cytosolic corticosteroid receptors [1]. It was previously reported that repeated electrical stimulation of the AMG resulting in kindling caused a transient decrease in hippocampal GR mRNA expression, and this effect was associated with increased fearful behavior [48]. Many studies showed that downregulation of hippocampal GR activity, caused by exposure to severe stress or administration of high doses of CS, may affect the responses of the HPA axis due to impaired feedback action of GC [47].

In view of these observations, we attempted to examine the effect of repeated electrical stimulation of the AMG on the responses to stressful stimuli and on the function of the negative feedback exerted by GC [49]. We found that repeated electrical stimulations of the central AMG significantly attenuated the inhibitory action of dexamethasone on the HPA axis responses to both acoustic and photic stressful stimuli. We have also shown that electrical stimulation of the AMG attenuated the decline in serum corticosterone to its basal levels, suggesting that the negative feedback exerted by circulating corticosterone was impaired. To examine whether the impaired feedback caused by AMG stimulation may result by a decrease in hippocampal GR, we measured the binding of ^{3}H-dexamethasone by the cytosolic fraction of hippocampal tissue. We found that electrical stimulation of the AMG caused a significant decrease in the binding capacity of dexamethasone to hippocampal cytosol. In summary, we showed that impaired GC feedback induced by repeated AMG electrical stimulations may be involved in the regulatory role of this limbic structure on the HPA axis.

5. Conclusions

The evidence indicates that activation of the AMG and, in particular, its central nucleus induces a facilitation of the HPA axis responses to a variety of stressful stimuli such as neural, surgical, adrenalectomy, and immune challenges. This facilitatory effect is mediated by adrenergic and serotonergic neurotransmission via α1 and 5-HT_2 receptors. In addition, activation of the AMG may enhance GC secretion by impairing the negative feedback of this hormone via a reduction in hippocampal GC receptors. **Figure 1** illustrates the possible pathways by which

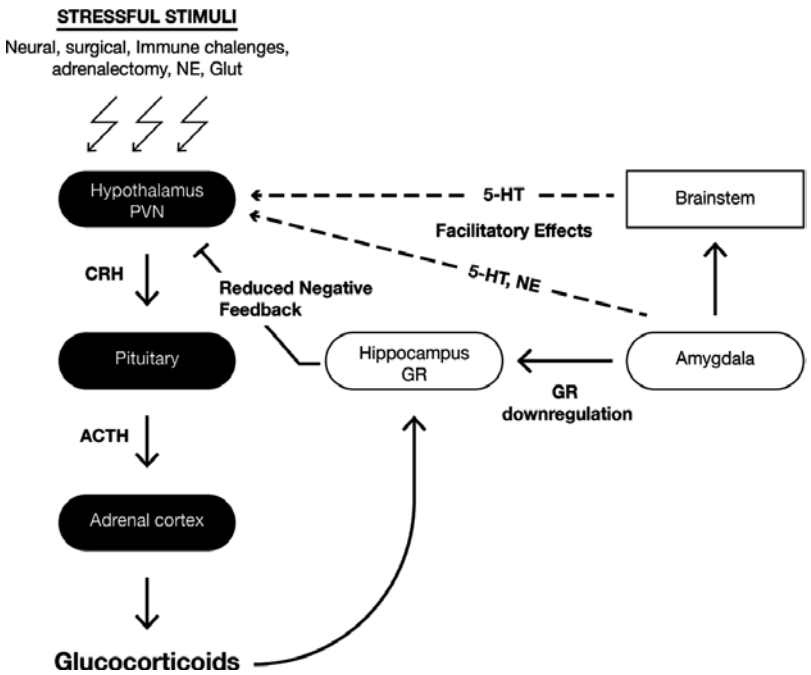

Figure 1. The facilitatory role of the amygdala on the HPA axis responses to various stressful stimuli. Various stressful stimuli activate the PVN to release CRH that causes the pituitary to release ACTH into the bloodstream, which in turn causes the secretion of glucocorticoids from the adrenal gland. The stimulatory effect of the amygdala upon the HPA axis is mediated by amygdalar and hypothalamic NE and 5-HT neurotransmission mediated by α1 and 5-HT_2 receptors, respectively. Neuroendocrine effects of NE in the hypothalamus are mediated by intrahypothalamic glutamatergic interneurons. Also, the activation of the HPA axis by electrical stimulation of the VNAB or by direct PVN injection of a α1 adrenergic agonist is markedly inhibited by PVN administration of selective ionotropic glutamate receptor antagonist. In addition, the amygdala facilitates the release of 5-HT from the PVN in response to electrical stimulation of brainstem raphe nucleus. The amygdala attenuates the negative feedback exerted by glucocorticoids probably by reducing hippocampal glucocorticoid receptors and thus facilitating the activation of the HPA axis.

the AMG regulated the function of the HPA axis. It is possible that the stimulatory effect of the AMG result in an increase in circulating GC may enhance the known modulatory effect of the AMG on the encoding and storage of hippocampal dependent memories.

Acknowledgements

This article is dedicated to the memory of Prof. Shaul Feldman (1927–2011). Prof. Feldman, former chairman of the Department of Neurology, was the founder of the field of neuroendocrinology of stress in our laboratory. The support of the Department of Neurology and Feldman family is greatly appreciated. The authors would like to thank Mrs. Miriam Ben-Yashar for critical reading of the manuscript and Mr. Ilai Ovadia for graphic design.

http://dx.doi.org/10.5772/67828

Author details

Joseph Weidenfeld and Haim Ovadia*

*Address all correspondence to: ovadiafam@gmail.com

Department of Neurology, The Agnes Ginges Center for Human Neurogenetics, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

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Chapter 9

The Key Role of the Amygdala in Stress

Diego Andolina and Antonella Borreca

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67826

Abstract

Several data highlighted that stress exposure is strongly associated with several psychiatric disorders. The amygdala, an area of the brain that contributes to emotional processing, has a pivotal role in psychiatric disorders and it has been demonstrated to be highly responsive to stressful events. Here we will review evidences indicating how the amygdala changes its functionality following exposure to stress and how this contributes to the onset of anxiety disorders.

Keywords: amygdala, stress, anxiety disorders

1. Introduction

The brain is a very complex organ and it establishes through complicated processes, which experiences are stressful, therefore determining behavioral and physiological responses.

Several clinical and preclinical data highlighted how acute or prolonged stress exposure may cause changes in brain that contribute to the onset of some psychiatric disorders.

The first effect of the stress response is the immediate activation of the hypothalamic-pituitary-adrenal (HPA) axis with release of specific hormones.

Specifically, HPA axis activation causes the secretion of neuropeptides, which are quickly released in the brain regulating the activity of some structures, and, among these, the amygdala plays a leading role in mediating the stress response.

The amygdala, an area of the brain that contributes to emotional processing, was seen to be very susceptible to stressful events, modifying its functionality and morphology. These modifications play an important role in stress-induced psychopathologies including anxiety,

depression, and addiction. These alterations involve genetic, epigenetic and molecular mechanisms as well as dendritic and synaptic reorganization processes.

Stress exposure increases the release of amygdala neurotransmitters including glutamate, GABA, noradrenaline, and serotonin. This immediately activates a signal transduction pathway with a downstream molecular cascade involved in the strengthening of postsynaptic neurons resulting in the instant regulation of specific genes engaged in neuroplasticity processes.

Furthermore, epigenetic mechanisms, including noncoding RNA, have been proposed to be involved in the rapid, long-term dynamic gene expression regulation during stress response.

For instance, many microRNAs (miRs), small RNA molecules that regulate gene expression at posttranscriptional level, modulate the synaptic plasticity and neurotransmission processes and for this reason they are considered important for the neuronal response to external stimuli. Recent studies show that the stress is able to alter the expression of some miRs in amygdala pointing to a role of these small molecules in regulating the stress response and some stress-related behaviors.

Although synaptic plasticity occurs within the amygdala, this structure obviously regulates stress response by interacting with other brain structures. The amygdala is specifically connected to a number of downstream and upstream regions that play a key role in emotional and stress-related behavior. Several data have highlighted the neurocircuits associated with stress response resulting in connections between different brain areas such as amygdala, prefrontal cortex.

In this chapter, we will review clinical and preclinical evidences indicating how this structure modifies its "shape" and functionality following exposure to stress and how this contributes to the onset/expression of anxiety disorders. In particular, we will focus on literature regarding stress-induced changes in neuroplasticity in terms of dendritic remodeling of neurons, as well as the molecular and epigenetic mechanisms involved. Moreover, we will discuss briefly how the amygdala, through connections with the prefrontal cortex, modulates stress response and stress-induced anxiety behavior.

2. Stress-induced changes in neurotransmission in the amygdala: evidence from microdialysis studies

Neurotransmission is the process by which the neurotransmitters are released by a neuron (the presynaptic neuron) and bind to and activate the receptors of another neuron (the postsynaptic neuron). Thus, neurotransmission is essential for communication between neurons, regulating behavior, emotional functioning, and cognition.

The *in vivo* microdialysis technique allows one to measure neurotransmitters in neuronal extracellular fluid in discrete regions of the brain in humans and laboratory animals [1–3]. The marked stress-responsiveness of several neurotransmitters in the amygdala has been

demonstrated using this approach, including glutamate (GLU), γ-aminobutyric acid (GABA), noradrenaline (NE), and serotonin (5-HT).

In the following section, we review microdialysis studies on the stress-induced release of neurotransmitters in the amygdala in animals and their putative function in mediating the stress response.

2.1. Noradrenaline

A variety of stressful events, including physical and psychological stimuli, increase noradrenaline (NE) release markedly in several regions of the brain, such as the amygdala. The ascending noradrenergic neurotransmitter system is activated by stress [4, 5] and provides dense innervation to the extended amygdala [6]. Microdialysis studies have shown that stress exposure enhances the release of NE in the basolateral amygdala (BLA), medial amygdala (MeA), and central amygdala (CeA) [7–15], thus that NE transmission is linked to the onset of negative emotions, such as anxiety and fear, in individuals who are exposed to stress [7, 16–19]. Consistently, benzodiazepine has been reported to attenuate this increase [7, 20, 21].

The MeA is innervated by noradrenergic neurons that arise primarily from the locus coeruleus [10, 22, 23]. An *in vivo* microdialysis study demonstrated that immobilization stress elevated NE levels in the MeA over threefold versus baseline [10].

Moreover, the administration of α1- or β-adrenergic receptor antagonists directly into the MeA mitigates the adrenocorticotropic hormone (ACTH) response to immobilization stress [10]. These data support the hypothesis that greater release of NE in the MeA, acting primarily through ACTH receptors, facilitates activation of the HPA axis in response to acute stress [10].

Stress-induced noradrenergic activity in the MeA, through projections to the bed nucleus of the stria terminalis (BNST) and preoptic area, is one possible mechanism by which the MeA modulates the stress-induced activation of the HPA axis.

The effects of stimulation of the MeA on increases in plasma corticosterone levels are partially blocked by lesioning the preoptic area or BNST alone but inhibited to a greater extent following the development of lesions in both structures and are blocked completely by bilateral lesions to the stria terminalis [24].

Immobilization stress also enhances NE release in the BLA [13–15, 21, 25]. Notably, in rats, long-term administration of citalopram, an antidepressant that belongs to the class of selective serotonin reuptake inhibitors (SSRIs), decreases the extracellular levels of NE in the BLA, suggesting that the therapeutic effect of citalopram is attributed to the loss of the NEergic stress response in the BLA that is caused by supersensitivity of α2-adrenoceptors in this region [13].

Immobilization stress affects a robust increase in NE release in the CeA [5, 26]. This release appears to be involved in stress-induced gastric ulcer formation [27, 28]. The expression of aggression during stress exposure attenuates stress-induced elevations in NE release in the

CeA and the development of gastric ulcer [27], whereas another study has indicated that ß-adrenoreceptor-mediated NEergic mechanisms in the CeA are important for the maintenance of gastric mucosal integrity during immobilization stress [28].

2.2. Serotonin

Several serotonin (5-HT) receptor subtypes are expressed in the amygdala, particularly in the basolateral regions [29–32]. The amygdala receives dense projections from the dorsal raphe nucleus (DRN) [33], and psychological stress activates ascending serotonergic neurons from the DRN to the BLA [34]. Injection of 5-HT into the amygdala evokes anxiogenic effects in various test situations [35–37]. However, the stress effects depend on features of the stressors and the genetic makeup of individuals. Regarding the former, for example, controllable stressors tend to have a less measurable impact than those that are uncontrollable, and the lack of behavioral control over stress might be critical to the development of mood disorders [38–40].

Exposure to uncontrollable stressors often increases anxiety behavior in humans and rodents, whereas controllable stress drastically reduces these effects [38]. An *in vivo* microdialysis study found that 5-HT neurotransmission in the amygdala—specifically in the BLA—is sensitive to the controllability of stress. In rats, inescapable stress (IS) activates DRN 5-HT neurons to a greater extent than escapable stress (ES), increasing 5-HT release in the BLA [35].

Moreover, serotonergic neurotransmission in the amygdala undergoes sensitization (a process in which there is progressive amplification of a response due to repeated administration of a stimulus) in response to stressful stimuli following IS. For instance, Amat and colleagues reported that two footshocks were sufficient to increase 5-HT efflux in the BLA in subjects who had experienced IS 24 h earlier but not in rats that had been subjected to ES [35]; a separate study found that 5-HT2C receptor in the BLA has significant function during this process in rats [41].

5-HT transmission in the BLA is also influenced by sex differences in the stress response. In rats, restraint stress significantly elevates extracellular 5-HT levels in the BLA in both genders, but females develop a greater response [42]. The authors suggest that this difference is related to sex-specific emotional responses to stress [42]. As proposed by Mitsushima and colleagues, the mechanism that underlies sex differences in the 5-HT response to restraint stress in the BLA is attributed to disparities in gonadal steroid hormone receptor expression on DRN 5-HT neurons, the major site from which 5-HT axons extend to the BLA in rats [42].

Consistent with these findings, androgen receptors abound in the DRN in male rats, whereas little or none is expressed in female rats [43]. Because several steroid hormones are released in the brain during stress exposure [for review, see 44], it is possible that sex-related differences in steroid hormone receptors govern 5-HT neurons in the DRN gender-specifically, differentially regulating extracellular 5-HT levels and the 5-HT response to stress in the BLA [42].

The DRN also provides 5-HT innervation to the CeA [45], and preclinical studies have shown that the upregulation of 5-HT in the CeA is related to the expression of stress-induced anxiety and depression [46].

In rats, stressful stimuli enhance the release of 5-HT in the CeA [47], and serotoninergic receptor stimulation in the CeA is sufficient and necessary for stress-induced activation of the HPA axis [48, 49]. Agonist-induced stimulation of 5-HT1A receptors (8-OH-DPAT) in the CeA stimulates the HPA axis [49], whereas depletion of 5-HT in CeA or infusion of 5-HT2 receptor antagonists in the CeA blocks its excitatory effects on the HPA axis [48]. Electrical stimulation of the CeA raises plasmatic ACTH and corticosterone levels [50–53]. 5-HT in the CeA has been suggested to have an important function in the stimulatory effects on the HPA axis through 5-HT in the paraventricular nucleus of the hypothalamus (PVN) [49].

Feldman and colleagues showed that hypothalamic lesions that were induced by 5,7-DHT, a neurotoxin that is used to decrease the concentrations of serotonin in the brain, prevented the stimulatory effects of a 5-HT1A agonist (8-OH-DPAT) that was injected into the CeA on plasmatic ACTH levels [49].

In conclusion, 5-HT release and activity in the CeA appear to be important for behavioral and endocrine responses that are related to stress exposure.

2.3. GABA

γ-Aminobutyric acid (GABA) is the chief inhibitory neurotransmitter in the mammalian brain and has significant function in reducing neuronal excitability in the nervous system. GABAergic transmission in the amygdala is an important pathway by which the flow of information, activity, and function can be controlled [54–56], and considerable evidence has shown that this neurotransmitter in the amygdala is critical in mediating several aspects of the stress response. Studies in rats have demonstrated that acute restraint stress increases GABA efflux in the BLA [57–60]. Conversely, GABAergic transmission in the BLA declines the following exposure to chronic or repeated stress [57]. It has been demonstrated that, by *in vivo* microdialysis, acute restraint stress enhanced GABA outflow in the BLA, whereas efflux in the CeA was unaffected [57]. Animals that were subjected to repeated stress (10 days of restraint) showed no acute stress-induced rise in GABA release in the BLA and did not experience any effects on GABA outflow in the CeA [57].

This evidence suggests that reduced GABAergic activity underlies the relationship between exposure to repeated stress and excessive fear responses to certain stimuli, characteristic of several anxiety disorders, such as posttraumatic stress disorder (PTSD). Manzanares and colleagues reported that previous restraint stress increases the fear response in a contextual fear paradigm in rats [61]. They also showed that infusion of midazolam, an agonist of GABAA receptors, into the BLA or systemic pretreatment with it prevents facilitation of the fear response that results from previous stress exposure [61]. Also, repeated stimulation of corticotropin-releasing factor receptors in the BLA enhances anxiety-like behaviors, which are associated with decreased GABAergic inhibition [62].

The impact of stress is also determined by the ability of the organism to cope with its situation [63]. Several reports have highlighted the function of GABAergic transmission in the mouse amygdala, particularly the BLA, in shaping an individual's coping style to stress [58, 59], which, with other factors, can in turn affect one's predisposition to affective disorders, such

as anxiety (for review, see [64]). Rats having more passive strategy of coping with an aversive event (i.e., a longer freezing response in the conditioned freezing test) are associated with upregulation of c-Fos (an index of neuronal activation) in the BLA and CeA, as a result of lower GABAergic activity in the amygdala [65]. With regard to individual coping styles to stress, GABArgic transmission in the BLA has been shown to function in the response of C57BL/6 and DBA/2 mice in the forced swimming test. C57BL/6 mice exhibit the highest levels of passive-coping behavior [58, 59, 66–68]. We have found that C57BL/6 mice show greater immobility in the forced swimming test (an index of passive-coping behavior), likely due to greater GABA outflow in the BLA, compared with DBA/2 mice [59].

Thus, the evidence from the animal studies above implicate BLA GABAergic neurotransmission in individual differences in stress-coping behavior, helping us understand the neurobiological mechanisms that underlie the susceptibility to stress-induced psychopathologies.

2.4. Glutamate

The amygdala receives glutamatergic afferents from several areas of the brain, including cortical and thalamic regions [69–71].

The function of glutamate (GLU) in acute rapid neurotransmission and processes that are related to long-term synaptic plasticity implicates extracellular GLU as a significant mediator of the effects of stress on amygdalar activity. Microdialysis studies have shown that acute restraint stress increases extracellular GLU levels in rat BLA and CeA complexes [72–74], which in turn activates the HPA axis [75, 76].

The release of GLU in the amygdala also increases with other types of stress and is modulated by fear responses. For instance, in rats, the expression of fear that is conditioned to a context that has been paired to shock induces a rapid increase in GLU in the BLA [77].

As for GABA, the effects of acute stress on GLU efflux differ fundamentally from those in individuals who have been subjected to repeated stress and challenged with acute stress. Whereas acute restraint stress elicits the quick and robust release of GLU in the BLA and CeA [72–74], the glutamatergic response to an acute stress challenge is diminished in the BLA and CeA following exposure to repeated restraint stress in rats [78].

The changes in GLU release following the administration of certain classes of psychotropic drugs during a stressful experience are notable. For instance, agomelatine (an antidepressant that acts as a melatonergic receptor agonist and a 5HT2C antagonist) and tianeptine (a tricyclic antidepressant that functions through indirect alteration of glutamate receptor activity and release of BDNF) blunt the increase in GLU that is elicited by acute stress in the BLA and CeA and prevent the stress-induced decline in GLU efflux in the CeA in repeatedly restrained rats, thereby reestablishing the responsiveness of glutamatergic neurons [78, 79]. These data suggest that stress-induced alterations in amygdalar glutamatergic systems have clinical relevance as potential therapeutic targets in stress-related psychopathologies, including anxiety.

http://dx.doi.org/10.5772/67826

3. The amygdala, stress, and dendritic alterations

The brain shows remarkable structural and functional plasticity in response to stressful experiences, including neuronal replacement, dendritic remodeling, and synapse turnover, and several studies have demonstrated that these events occur in the amygdala following stress exposure.

Neuroplasticity can be evaluated using various functional and morphological endpoints, ranging from molecular and cellular indices and changes in synaptic transmission to neurochemical alterations and changes in dendritic architecture and spine density.

The most significant evidence on stress-induced modifications in amygdalar neuronal plasticity refers to the morphological changes in dendrites. Currently, microscopy methods and associated algorithms permit one to perform a comprehensive dendritic neuronal morphological analysis, from 3D dendritic reconstruction to the estimation of spine numbers and density [for review, see 80].

In response to stress, dendritic branches extend or retract, on which dendritic spines emerge, disappear, or change in shape or size. Stress affects the morphology of neurons, primarily in the hippocampus, medial prefrontal cortex (mpFC), and amygdala. Furthermore, neurons in these regions are highly plastic and undergo dramatic transformations following traumatic experiences. In response to stressful conditions, amygdala neurons undergo differential changes compared with other structures that are implicated in the stress response. For instance, in the mpFC and hippocampus, stress triggers the dendritic atrophy and reduces spine numbers. Conversely, in the amygdala—in particular, the BLA—it increases dendritic length and spine density (for review, see [81, 82]).

Several studies have demonstrated that the effects of stress on amygdalar structural plasticity correlate with behavioral changes, such as the manifestation of anxious behavior [83–87].

The BLA can undergo structural reorganization in response to several stressors, such as immobilization, maternal stress, and external application of the stress hormone corticosterone [83, 85, 88, 89]. In rats, chronic stress causes hypertrophy of pyramidal neurons in the BLA. Specifically, repeated restraint increases the total dendritic length, the number of branch points, and spine density in BLA pyramidal neurons—effects that are accompanied by greater anxiety-like behaviors [83, 90]. Notably, compared with mpFC and the hippocampus, the structural changes in the BLA after repeated stress persist, even after a stress-free recovery period of 3 weeks [84], suggesting the high sensitivity of amygdalar neurons to the long-term effects of stress.

The changes in BLA dendrites likely involve higher stress-induced corticosterone levels. Chronic exposure of rats to corticosterone increases the spine density in BLA pyramidal neurons and anxiety-like behavior [91]. Similarly, acute stress worsens anxiety and induces dendritic hypertrophy in the BLA. A single episode of immobilization stress in rats and mice raises the spine density in BLA neurons, which is accompanied by anxiety-like behavior [60, 90, 92–95]. Pharmacological interventions for the treatment of mood disorders, including anxiety, reduce the stress-induced morphological changes in the rat amygdala. Specifically, the mood-stabilizer lithium prevents hypertrophy of BLA pyramidal neurons

that is elicited by stress [96]. This evidence highlights how the amygdalar morphological alterations that are induced by stress underlie the pathophysiology of neuropsychiatric disorders, such as PTSD, major depressive disorder, and anxiety.

Nevertheless, as discusses, individuals respond to stress and trauma differently. For example, traumatic experiences might lead to PTSD in certain individuals while others are less affected by the same incidents [97–99], and it appears that dendritic amygdala neurons are sensitive to individual variations in stress coping and stress responsiveness. One study demonstrated that 2 weeks after stress exposure (predator exposure stress), maladapted rats (i.e., animals that showed high anxiety following the stress exposure) harbored longer dendrites and more highly branched dendrites with greater spine density in the BLA compared with well-adapted animals (those with low anxiety after stress exposure) [86]. These data suggest that disparate patterns of plasticity in BLA neurons in response to stress account for individual differences in coping responses to stress and trauma.

The findings in these preclinical studies are consistent with human neuroimaging evidence. Clinical studies have demonstrated enhanced responsiveness of the amygdala in patients with PTSD and other psychopathologies that are related to stress, including major depression. This is discussed below.

4. The amygdala, stress, and epigenetic mechanisms: the function of miRs

Epigenetics is the study of heritable changes in gene expression (active versus inactive genes) that do not involve alterations to the underlying DNA sequence, which in turn affects how cells process the genes. Epigenetic mechanisms rely on specific gene sequences that normally lie in the 5′UTR or 3′UTR (regulatory sequences upstream and downstream of the coding sequence, respectively). These sequences regulate the expression of genes, based on the activities of various proteins (e.g., RNA-binding proteins) and short RNAs, which recognize, bind, and directly regulate the synthesis of specific genes. Such epigenetic modifications include histone modification, DNA methylation, and noncoding RNA mechanisms [100, 101].

Several studies have recently provided significant insight, suggesting that microRNAs (miRs)—small noncoding RNAs—are central in the epigenetic regulation of stress-induced psychopathologies, including anxiety disorders [102–105]. miRs influence chromatin structure and protein binding to DNA and directly affect transcription and translation. Most frequently, mRNA stability is governed through the binding of miRs to the 3′UTR of target mRNAs, decreasing mRNA stability and mRNA cleavage and thus impeding protein assembly [106]. Most mammalian miRs are encoded by RNA polymerase II-transcribed genes, which can be tens of kilobases in length and are frequently spliced [107]. Approximately one-third of known miRs is embedded within introns of protein-coding genes and is cotranscribed with the host gene, allowing coordinate regulation of miR and protein expression.

In the brain, miRs are critical in modulating many neurobiological processes, including changes in neuronal morphology and neurotransmitter homeostasis.

The ability of miRs to selectively and reversibly silence mRNAs and their involvement in neuronal plasticity and neurotransmitter release render miRNAs well suited as fine-tuning regulators of the complex and extensive molecular network that drives stress responses [108]. Consistent with this model, miRs are altered by stress, glucocorticoids, and mood stabilizers, indicating that they are critical in the etiology of anxiety disorders (for review, see [103]).

In particular, recent studies have demonstrated a physiological function amygdalar miR-34 in regulating stress responses.

Haramati and colleagues reported that acute stress upregulates miR-34 in the CeA of mice and that virus-mediated overexpression of miR-34 in this area prevents stress-induced anxiety and blocks the response of CRFR1 to its ligand CRF, suggesting that miR-34 regulates the molecular machinery of the response to stress [93].

Moreover, a recent study from our group showed that the miR-34 expression in the BLA controls the stress response and stress-induced anxiety [60], in which acute restraint stress upregulated miR-34 in the BLA (approximately 3.5-fold higher than in unstressed mice) [60]. Notably, genetic deletion of miR-34 in mice rendered them resilient to stress-induced anxiety and facilitated fear extinction. Moreover, no increase in BLA GABA release or stress-induced amygdalar dendritic remodeling was evident in miR-34 KO mice, implicating miR-34 in the regulation of amygdalar functions during the stress response [60].

Other miRs, such as miR-135a and miR-124, are modulated in the amygdala in mouse by stress [109]. Also, studies in rats have demonstrated a putative function of miRs in the amygdala in modulating the stress response. In a rat model of learned helplessness, in which rats were subjected to 2 h of immobilization per day and tail shocks for 3 consecutive days, miRs in the amygdala were substantially altered, leading to a global increase in the expression of many miRs, including miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463*, and miR-674* [110].

Among these species, amygdalar miR-19b is modulated by chronic social defeat. Mice that have been subjected to an aggressive mouse experience a significant rise in miR-19b in the BLA and greater freezing in the cue fear conditioning test; further, *in vitro* studies have shown a direct effect of miR-19b on adrenergic receptor beta 1 (Adrb1) mRNA levels by luciferase assay [111]. Notably, mice that were injected with miR-19b into the BLA had lower freezing times compared with control mice, concomitant with downregulation of Adrb1. Thus, the authors suggested that miR-19b has significant function in modulating behavioral responses to chronic stress through the control of adrenergic receptor-1 mRNA [111]. A relationship between miRs, amygdalar function, and stress has been also proposed in human studies.

DICER1 is an enzyme that generates mature miRs through genomewide differential gene expression. A survey of patients with PTSD and comorbid depression [112] reported that blood DICER1 expression is significantly lower than in healthy subjects and that this effect is associated with increased amygdalar activation that is induced by fearful stimuli [112].

5. Neuronal circuits in the stress response

The amygdala has emerged as a key region of the brain in the modulation of stress responses, thus having significant function in stress-induced psychopathologies, such as anxiety.

However, the amygdala orchestrates stress and anxiety responses, influencing many other brain areas by sending projections to such domains, as the pFC, that are involved in motor control and autonomic and neuroendocrine responses.

Many studies have implicated the prefrontal cortex-amygdala system in the stress response and stress-related disorders [113–115]. The mpFC modulates neuroendocrine function during stress and regulates peripheral responses to stress, including heart rate, blood pressure, and cortisol responses [116, 117].

The mpFC and amygdala have reciprocal anatomical interconnections [118–122], and the former appears to have regulatory function in amygdalar activation during the stress response.

Animal studies have demonstrated that activation of the mpFC increases the number of *c-Fos*-immunoreactive cells in intercalated amygdala neurons [123] and that electrical stimulation of the pFC inhibits central output neurons [124] and basolateral projection neurons [125] in the amygdala. Similarly, during stressful experiences, frontal cortical areas modulate emotional responsiveness through the inhibition of amygdalar function, and it has been hypothesized that stress-induced dysfunction of this mechanism underlies pathological emotional responses in patients with PTSD and, possibly, other anxiety disorders. Supporting this model, functional imaging studies in PTSD have reported amygdalar hyperactivation in response to threatening stimuli [126, 127] and decreased mPFC activation [128–130] compared with healthy controls. Moreover, functional analyses have revealed less connectivity between the amygdala and mpFC in PTSD [130]. Copious evidence demonstrates that 5-HT neurotransmission in the mpFC constitutes a potential mechanism through which the mpFC regulates amygdala-mediated arousal in response to emotional stimuli, such as stressful events. In a human study, Fisher and colleagues observed that the prefrontal 5-HT2A receptor density is associated with lower threat-related right amygdalar reactivity [131]. Studies on serotonin transporters (5-HTT) have also proposed 5-HT to function in mediating mpFC-amygdala interplay. Wellman and colleagues showed that the loss of 5-HTT function in mice compromises their ability to cope with environmental stress and effects morphological abnormalities in the BLA and mpFC—changes that were related to amygdalar hyperactivity and hypofunction in the pFC [132]. Further, regarding the function of the prefrontal 5-HT system in modulating the amygdalar stress response, we have demonstrated that bilateral selective 5-HT depletion in the mpFC in mice decreases the BLA GABA release that is induced by restraint stress and passive coping in the forced swimming test, implicating 5-HT and GABA transmission-mediated pFC/amygdala connectivity as a critical neural mechanism of stress-induced behavior [58, 59].

Overall, connections between the mpFC and amygdala normally allow individuals to adjust their behavior in response to several stimuli, including stress. A loss in prefrontal control of

the amygdala might underlie the inability to cope adequately with stressful situations, thus promoting the anxiety disorders that are related to stress exposure.

6. The amygdala and stress: evidence from human studies

Individuals can be exposed to various stressful conditions, such as childhood violence, divorce, physiological disease, international terrorism, economic insecurity, and job stress, which can lead to various diseases, including anxiety disorders, depression, and schizophrenia.

In humans and animals, the amygdala is activated by stressful stimuli [133], and over the past decade, interest in the human amygdala in stress-related psychiatric disorders has grown considerably, due to the progress in animal studies and the development of functional imaging techniques.

In human imaging studies, altered amygdalar responsiveness to negative stimuli has been shown to be associated with psychopathologies that are induced by stress [134–136].

Specifically, functional imaging studies have observed amygdalar hyperactivation [137–142] in response to threatening stimuli in anxiety disorders [143]. Moreover, amygdala alterations occur in other psychopathologies that are related to stressful conditions, such as depression. For instance, fMRI studies have reported that depressed patients develop an abnormally exaggerated amygdala in response to negative stimuli [144] and that antidepressant treatment normalizes this activity [145].

In humans, the patent link between stress, the amygdala, and anxiety disorders is evident in PTSD patients.

According to the 5th edition of the *Diagnostic and Statistical Manual of Mental Disorders* [146], anxiety and stress disorders are characterized by an excessive fear response or worry that interferes with normal functioning or causes significant distress. Fearful stimuli, such as fearful faces and fear-inducing images, have been found to activate the amygdala in several brain imaging studies using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) [147–149]. PTSD appears to combine aspects of severe stress responsiveness and enhanced conditioned fear and the inability to extinguish or inhibit conditioned fear. Accordingly, in PTSD patients, amygdalar activity is enhanced in response to trauma reminders and general negative stimuli [150]. For instance, amygdalar hyperresponsivity in PTSD occurs during the presentation of personalized traumatic narratives [151, 152], combat sounds, [153, 154] combat photographs, [155, 156], and trauma-related words [157].

Childhood maltreatment also increases one's susceptibility to PTSD and others anxiety disorders [158] and generally increases the sensitivity to stress in later life, of which amygdala hyperresponsiveness is an important aspect.

For instance, there is a strong association between childhood trauma questionnaire scores and amygdala responsiveness to sad—but not happy—facial expressions [159].

An fMRI study examined the emotional experiences and amygdalar responses of 50 healthy new recruits in the Israeli Defense Forces before they began their mandatory military service and after subsequent exposure to stressful events while deployed in combat units. Over time, some soldiers reported an increase in stress symptoms, an effect that correlated with greater amygdalar activation and hippocampal responsiveness to stress-related content [160]. Moreover, the authors noted that amygdalar reactivity before stress predicted the rise in stress symptoms [160].

The hypothesis that the amygdalar activity in response to negative stimuli predicts the individual vulnerability to stress is supported by several studies that have demonstrated that amygdalar responsiveness is strongly influenced by genotype. Genetic factors have been shown to govern amygdalar responsiveness to emotional stimuli and one of these is certainly represented by a polymorphism in serotonin transporter (5-HTT).

Studies reveal that polymorphisms in 5-HTT might be linked to the exaggerated responses of the amygdala on encountering environmental threats and to the risk for mood and anxiety disorders, especially in response to chronic or severe stress. Hariri and colleagues demonstrated that individuals with one or two copies of the short allele of the 5-HTT promoter polymorphism, which has been associated with reduced 5-HTT expression and function and increased fear and anxiety-related behaviors, exhibit greater amygdalar neuronal activity, as measured by BOLD functional magnetic resonance imaging, in response to fearful stimuli compared with long allele homozygotes [161]. Moreover, 5-HTT binding is suggested to correlate with threat-related amygdalar reactivity, up to 40% of the variability in threat-related amygdala reactivity predicted by 5-HTT binding levels [162]. Polymorphisms in genes that are linked to aminergic activity, such as catechol-O-methyltransferase (COMT), one of several enzymes that degraded catecholamines, might function in mediating the amygdalar activity in response to environmental threats.

In an fMRI study, healthy subjects who were genotyped for the COMT Val158Met polymorphism showed an increase predominantly in left-sided amygdalar activity in response to fearful and angry facial stimuli. This effect was observed online in the female subgroup, suggesting a gender-specific influence of COMT Val158Met on amygdalar activity in the processing of emotional stimuli [163].

7. Conclusion

The expression of anxiety disorders, including generalized anxiety disorder, specific phobias, social anxiety disorder, separation anxiety disorder, agoraphobia, panic disorder, and PTSD, is commonly caused by stress. Yet, little is known about the specific etiological pathways that lead from a triggering stressor to the development of a specific pathological phenotype.

Overwhelming data report alterations in amygdalar functions in anxiety and stress disorders. Animal and clinical studies support the critical function of the amygdala in stress and anxiety, characterized by general amygdalar hyperactivity that is associated with the anxiety symptoms and the response to threatening or stressful stimuli. This hyperactivation has evidenced by, for example, dendritic hypertrophy and reductions in the inhibitory neurotransmitter GABA following stress exposure.

Despite the clear involvement of amygdalar circuits in anxiety disorders, it remains unknown how this structure contributes to the specificity of various pathological anxiety disorders. Moreover, studies on different anxiety disorders have reported similar alterations with regard to neurotransmitter activity, neuroplastic changes, and alterations in amygdalar function, suggesting that these properties are common in anxiety disorders and that the phenotypic specificity is rooted in upstream mechanisms.

In this context, epigenetic mechanisms might be good targets. In particular, in the past decade, growing evidence has shown that miRs regulate amygdalar functions during stress response and anxiety-like behaviors.

MiRs control the expression of specific genes that are involved in neurobiological processes, including dendritic morphological changes and neurotransmitter homeostasis, and their function in mediating stress responses has recently been described. A systematic study of the relationships between specific stress-related disorders and alterations in epigenetic mechanisms, such as miR expression in the amygdala, might be a good strategy to identify upstream mechanisms and, eventually, selective therapeutic interventions for various anxiety disorders, given that in clinical practice, the choice of the appropriate pharmacological strategy is driven by symptoms release and lacks of specificity, is characterized by low response rate and high recurrence.

Acknowledgements

We are grateful to Professor Rossella Ventura for helpful suggestions. This research is supported by SIR, cod. "RBSI14G1HH", Italian Ministry of Education, Universities and Research.

Author details

Diego Andolina[1,2]* and Antonella Borreca[2,3]

*Address all correspondence to: diego.andolina@gmail.com; diego.andolina@uniroma1.it

1 Department of Psychology and Center "Daniel Bovet," Sapienza University, Rome, Italy

2 IRCCS Fondazione Santa Lucia, Rome, Italy

3 CNR—Institute of Cell Biology and Neurobiology, Rome, Italy

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Chapter 10

Amygdala and Emotional Modulation of Multiple Memory Systems

Jarid Goodman, Christa McIntyre and
Mark G. Packard

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.69109

Abstract

Stress and anxiety can either enhance or impair memory, and the direction of the effect partially depends on the type of memory being affected. Behavioral or pharmacological stressors typically impair cognitive memory mediated by the hippocampus, but enhance stimulus-response habit memory mediated by the dorsolateral striatum. Evidence also indicates that the effect of emotion on different kinds of memory critically depends on a modulatory role of the basolateral amygdala (BLA). BLA modulation of multiple memory systems may be achieved through its glutamatergic projections to other brain regions, which may enhance stress hormone activity, modulate competition between memory systems, and alter synaptic plasticity. The neurobiology underlying the emotional modulation of multiple memory systems may be relevant to understand the impact of emotional arousal on the development and expression of human psychopathologies characterized by maladaptive habitual behaviors (e.g., drug addiction and relapse).

Keywords: memory, stress, emotional modulation, basolateral amygdala, hippocampus, dorsolateral striatum, habit, post-traumatic stress disorder.

1. Introduction

The amygdala of the mammalian brain has been historically associated with emotional behavior (e.g., Ref. [1]), and studies conducted over the past several decades have indicated that this structure also modulates the storage of long-term memories (for review, see Ref. [2]). In particular, the amygdala confers the influence of emotional arousal on learning and memory processes occurring in other brain regions, such as the hippocampus and dorsal striatum [3, 4].

Within the context of a "multiple systems" approach to memory organization, the hippocampus is a critical part of a neural system mediating what are broadly defined as "cognitive" memories involving explicit, declarative information, such as facts and past experiences [5]. Cognitive memory function of the hippocampus also includes acquisition of spatial cognitive maps, i.e., learned mental representations of space which may be used for allocentric navigation [6]. In contrast, the dorsal striatum mediates associations between stimuli and responses and thus encodes memories broadly defined as procedural, stimulus-response (S-R), or habitual (i.e., perseverative responding following devaluation of the reinforcer) [7]. For instance, the dorsolateral striatum (DLS) mediates habitual lever pressing behavior in instrumental learning situations [8], as well as acquisition and expression of a consistently reinforced egocentric turning response in the plus-maze [9].

The hippocampus and dorsal striatum differ not only in terms of their anatomical loci and mnemonic functions, but also these brain regions are differentially influenced by parametric factors, including amount of training, reinforcement schedules, and stress/anxiety [10]. As noted above, the emotional modulation of memory systems is mediated by the amygdala, which becomes active during states of emotional arousal and sends glutamatergic projections to other structures to influence the consolidation of memory [2]. The impact of emotional arousal on memory conferred by the amygdala appears to influence hippocampus- and dorsal striatum-dependent memory differently. For instance, high levels of stress or anxiety typically impair learning and memory functions of the hippocampus and enhance learning and memory functions of the dorsolateral striatum [3, 4].

The present chapter provides an updated review of the emotional modulation of multiple memory systems with a focus on the role of the basolateral amygdala. The review begins by describing evidence for the existence of multiple memory systems in the mammalian brain, and this is followed by a review of the influence of stress and anxiety on hippocampus and dorsal striatum-dependent memory processes. Although emotional arousal potentially influences memory formation in several brain regions, we focus on the hippocampus and dorsal striatum. Potential mechanisms through which the amygdala modulates multiple memory systems are also discussed, including the role of memory system interactions, stress hormones, and synaptic plasticity. The relevance of these findings to understanding and treating some human psychopathologies is also considered.

2. Multiple memory systems in the mammalian brain

The mammalian brain is composed of anatomically distinct memory systems [11, 12]. An understanding of the relative contributions of these distinct systems to memory is based upon studies dissociating their involvement in specific memory tasks. Carefully designed behavioral tasks can be used to parse the unique roles of multiple memory systems by keeping sensory, motor, and motivational factors constant, but changing the learning and memory requirements from one task to another. When manipulations of one brain region affect task "A," but not task "B," and lesions of another brain region affect task "B," but not task "A," we can determine that these two brain systems play distinct and somewhat independent roles in memory processing [12].

The first studies demonstrating a double dissociation between hippocampus- and dorsal striatum-dependent memory systems employed an eight-arm radial maze [13, 14]. Two distinct versions of the eight-arm radial maze were used: a cognitive spatial version and an S-R habit version. In the spatial version (also called the win-shift task), a rat was given the opportunity to explore the maze and retrieve food contained at the end of each arm. Multiple extra-maze cues surrounded the maze, allowing rats to acquire a cognitive map of the learning environment, which they could use to seek the spatial locations containing food and avoid the spatial locations from which food was already retrieved. Rats given lesions to the hippocampal system made more re-entries into the unbaited arms, indicating an impairment in spatially guided memory performance, whereas rats given lesions to the dorsal striatum were not impaired [13, 14].

In the S-R habit version of the radial maze (also called the win-stay version), a light cue at each arm signaled whether food was available there. An error was recorded if the animal entered an arm which was not currently signaled with a light cue. This task involves S-R habit memory to the extent that successful performance requires animals to associate the light cue (S) with approach behavior (R) [15]. Lesion of the dorsal striatum impaired memory performance in this S-R habit version of the radial maze, whereas hippocampal lesions actually enhanced performance [13]. A later study indicated that lesions confined to the dorsolateral striatum (DLS) were sufficient to impair memory in the S-R radial maze [16]. Taken together, these findings indicate that the hippocampus but not the DLS mediates spatial memory, whereas the DLS but not the hippocampus mediates S-R habit memory. Considering that the spatial and S-R versions of the radial maze depend on similar motivational, motoric, and sensory requirements, the differential role of the hippocampus and DLS may be attributed to the distinct mnemonic requirements in each task. The observation that hippocampal lesions *enhanced* memory performance in the S-R habit task may be attributed to a competitive interaction between memory systems, which will be described later.

A plus-maze apparatus has also been extensively employed to examine multiple memory systems in rats. The plus-maze consists of four arms arranged in a cross-shaped (+) orientation. Three slightly different procedures can be carried out to dissociate multiple memory systems using the plus-maze. Rats trained in a dual-solution task were released from a consistent start arm (e.g., North arm) with a palatable food reinforcer located in a consistent goal arm (e.g., West arm). Two distinct learning strategies could lead to a food reward: 1) rats could learn the spatial location of the reinforcer and spatially guide behavior to the reinforced location or 2) rats could acquire a consistent body-turn response at the intersection of the maze (e.g., turn right), regardless of the spatial location of the reinforcer. The strategy was assayed using a subsequent probe trial in which the animal was released from the opposite starting position (e.g., South arm). If rats went to the same spatial location (i.e., making the opposite turn at the choice point), they were identified as place learners. If rats continued to make the same turn at the maze intersection as they did during training, they were identified as response learners.

One of the interesting features of the dual-solution plus-maze task is that early in training animals usually display place learning during the probe trial, whereas after extended training, animals predominantly display response learning, potentially indicating the gradual formation of a motor habit [9, 17, 18]. In addition to the training parameters, the expression of

place and response learning may also be influenced following temporary inactivation of the hippocampus or DLS. Early in training, when control animals displayed place learning, temporary inactivation of the hippocampus before the probe trial eliminated preferential use of either place or response learning, whereas DLS inactivation had no effect [9]. Later in training, when control animals mostly displayed response learning, temporary inactivation of the DLS before the probe trial shifted animals back to the predominant use of place learning, whereas hippocampal inactivation had no effect [9].

The role of the hippocampus in place learning and DLS in response learning has also been demonstrated using single-solution versions of the plus-maze. In a place learning version of the plus-maze, animals are released from alternating start arms (e.g., North and South), whereas the food reinforcer remains in a consistent spatial location (e.g., the West arm). Thus, in order for animals to be successful in this task, they presumably need to acquire a spatial cognitive map of the learning environment using extra-maze cues so that they may accurately guide behavior from different starting positions to the same spatial location. In the response learning version of the plus-maze, the animal is reinforced to make a consistent body-turn response (e.g., right turn) at the maze intersection, regardless of the animal's starting position. For instance, if the animal is started from the North arm, the food reward may be located in the West arm; if the animal is started from the South arm, the food reward is located in the East arm. In this particular example, regardless of the animal's starting position, a right body-turn will consistently lead to the reinforcer. Extensive evidence using these tasks indicates that learning and memory in the place version of the plus-maze requires hippocampal function, whereas learning and memory in the response version requires DLS function [19–22].

The radial maze and plus-maze experiments, as well as other experiments using a Morris water maze [23–25], have been critical in demonstrating the differential mnemonic functions of the hippocampus and DLS. These studies have provided strong converging evidence that the hippocampus mediates spatial cognitive memory, whereas the DLS mediates S-R habit memory. Additional studies have been conducted primarily using the plus-maze tasks to demonstrate that these different kinds of memory are differentially influenced by parametric factors, such as exposure to stress and anxiety [4, 10, 26].

3. Emotional modulation of memory systems in rodents

The influence of emotion on memory has been well known since ancient times. The *Rhetorica ad Herennium*, an ancient handbook on rhetoric written sometime in the late 80s BCE, emphasized the importance of associating to-be-remembered information with emotionally arousing images to facilitate later recall. Also, in medieval times, in order to commemorate important events, such as weddings, a child was thrown into a river, helping the child and spectators form a lifelong memory of the event. In addition to the well-known enhancing effects of emotion, there is evidence that high levels of emotional arousal may also cause memory impairments. Whether emotion enhances or impairs memory depends partly on the type of memory being affected.

Extensive experimental evidence from rodents and humans indicates that emotional arousal modulates hippocampus- and DLS-dependent forms of memory differently. In an initial demonstration of the emotional modulation of multiple memory systems, investigators employed two versions of the Morris water maze, a spatial version dependent on hippocampal function and a cued version dependent on DLS function. In the spatial version, animals were released into a large circular pool of water over the course of training from multiple starting positions. In order to escape the water, the animals could mount an invisible escape platform located in a consistent spatial location. Thus, learning to quickly find the platform over the course of training may require acquisition of a cognitive map of the learning environment. In the cued version of the Morris water maze, animals were also released from different starting positions, however the platform remained visible throughout training, enabling animals to form an S-R association between the visibly cued platform (S) and swimming approach behavior (R). Evidence indicates that spatial learning in the Morris water maze depends on hippocampal function [27], whereas cued learning depends on dorsal striatal function [24, 25, 28–30].

Consistent with the hypothesis that emotion differentially influences multiple memory systems, a stress regimen involving chronic restraint and tail shock impairs memory in the hippocampus-dependent spatial version of the Morris water maze, but enhances memory in the DLS-dependent cued version [31]. Similarly, in a dual-solution version of the plus-maze, a chronic variable stress regimen that includes chronic restraint and forced swim among other potent stressors leads to the preferential use of response learning over place learning during the probe trial [32]. This chronic variable stress regimen is also associated with increased changes in dendritic morphology in the DLS [32]. In a circular hole-board task that can also be solved adequately using either a place strategy or S-R strategy, chronic restraint stress also increases the use of an S-R strategy [33].

The effects of chronic stress regimens on memory systems may also be observed with acute behavioral stressors. One hour of restraint stress is sufficient to impair acquisition in a place learning version of the plus-maze and enhance acquisition in a response learning version of the plus-maze [34]. Acute restraint stress is also associated with an increase in the use of a response learning strategy in the circular hole-board task [35]. Similar effects may be observed with an ecologically valid stressor. Pre-training exposure to predator odor enhances acquisition of response learning in a plus-maze task and leads to the preferential use of response learning in a dual-solution version of the plus-maze [36]. Aside from acute or chronic behavioral stressors, hypertension and trait anxiety have also been associated with the use of a response learning strategy in dual-solution versions of the plus-maze and Morris water maze [37–40].

The majority of studies using chronic or acute behavioral stressors have employed procedures with an innate ability to induce emotional arousal (e.g., restraint stress, exposure to predator odor, etc.). However, some studies have also indicated that a previously neutral stimulus that has acquired the ability to induce emotional arousal can also modulate memory. In these studies, animals are initially trained in a standard fear conditioning paradigm, in which a tone is repeatedly paired with a footshock, encouraging the acquisition of a tone-shock association. Later, when the tone is played alone, the animal demonstrates freezing behavior, indicating emotional arousal or, more specifically, fear to the tone. Post-training exposure to

a tone previously paired with shock enhances consolidation of response learning in the plus-maze and produces a response learning bias in a dual-solution version of the task [41, 42]. In addition, exposure to a fear-conditioned tone is also associated with greater response learning over place learning in a dual-solution version of the Morris water maze [43].

The influence of behavioral stressors on multiple memory systems is mimicked by anxiogenic drugs. Systemic administration of anxiogenic α-2 adrenoreceptor antagonists yohimbine or RS 79948-197 produces a response learning bias in the dual-solution plus-maze [44, 45]. In addition, systemic administration of RS 79948-197 enhances memory in a response learning task and impairs memory in a place learning task [46, 47]. Administration of the stress hormone corticosterone also enhances response learning in the plus-maze and increases the use of response learning in a circular hole-board task [35, 48]; see also Ref. [34].

Thus, robust emotional arousal induced by behavioral stressors or anxiogenic drug injections appears to favor DLS-dependent S-R habit memory, while impairing hippocampus-dependent cognitive memory. It should be noted, however, that there is also evidence that emotional arousal may sometimes facilitate hippocampus-dependent memory (for review, see Ref. [2]). It is possible that the influence of emotional arousal on hippocampus-dependent memory follows an inverted U-shaped curve, in which high and low levels of emotional arousal impair and moderate levels enhance cognitive memory [49–51].

4. Emotional modulation of memory systems in humans

The majority of studies examining the influence of emotional arousal on multiple memory systems have employed lower animals, such as rats and mice. However, there is also evidence that emotional arousal may influence human memory in a similar manner. In one experiment, participants were instructed to select one of four cards, which were positioned face-down in a 3-D model of a room, with the goal of picking the "win card." During training, the win card remained in the same spatial location relative to the distal room cues and was also consistently located next to a proximal cue (i.e., a potted plant). During a subsequent probe trial, the plant was moved to a different spatial location. If participants selected the card next to the plant, they were considered to be using an S-R strategy. If participants selected the card from the original spatial location, they were considered to be using a place strategy. Pre-training psychosocial stress (i.e., public speaking) increased the use of an S-R strategy [52]. Likewise, in a 2-D version of the win-card task, chronic stress was associated with greater use of a response learning strategy over a place learning strategy [33].

The finding that stress and anxiety enhance dorsal striatum-dependent, S-R habit memory has also been observed using an instrumental learning task. Subjects were first trained to perform two instrumental responses, each resulting in the delivery of a distinct food outcome. Later, one of the food outcomes was devalued by freely providing the participant with the food until satiety. Participants subjected to the socially evaluated cold pressor test, which promotes high levels of emotional arousal, continued to make the instrumental response for the devalued outcome, indicating habitual behavior, whereas control subjects decreased responding for the devalued outcome, indicating goal-directed behavior [53, 54].

Another task employed to examine the influence of emotional arousal on multiple memory systems is the probabilistic classification task, also known as the weather prediction task. This task involves presenting participants with a random series of cards covertly associated with distinct weather outcomes (rain or sunshine), and the participant is instructed to predict the weather based on the cards presented for a given trial. Over the course of training, participants are provided with trial-by-trial feedback indicating whether their prediction was correct, allowing participants to gradually learn what series of cards are most likely associated with rain or sunshine. Neuroimaging evidence suggests that either the hippocampus or dorsal striatum may be recruited for learning in this task [55–58], and the relative use of these systems may also be determined behaviorally using mathematical models [59–61]. Emotional arousal elicited using the socially evaluated cold pressor test biases subjects toward the use of a dorsal striatum-dependent strategy in the weather prediction task, whereas non-stressed control subjects show a preference for using a hippocampus-dependent strategy [61]. Moreover, functional magnetic residence imaging (fMRI) indicates that performance in this task is positively correlated with dorsal striatal activation in stressed subjects, whereas performance is positively correlated with hippocampal activation in non-stressed subjects [61].

Thus, converging evidence from humans and lower animals indicates that high levels of emotional arousal, in particular stress and anxiety, promote a shift from hippocampus-dependent cognitive strategies toward dorsal striatum-dependent habit strategies. The following sections consider some of the mechanisms that may be implicated, including a modulatory role of the amygdala, competition between memory systems, stress hormones, and plasticity.

5. Emotional modulation of memory systems: role of the amygdala

Early evidence indicated a clear role for the amygdala in learning and memory [1, 62, 63]; however, it took several decades of subsequent research before this brain region was considered one of the principal learning and memory structures of the brain (for review, see Ref. [2]). As mentioned above, the amygdala mediates emotional memories, such as stimulus-affect associations and emotionally charged CS-US associations underlying Pavlovian fear conditioning [64]. In addition to mediating emotional memories, the amygdala also has a prominent role in allowing emotional arousal to influence memory formation in other brain regions, such as the hippocampus and dorsal striatum. Presumably, during high levels of emotional arousal, the amygdala becomes active and modulates memory function of other brain regions either directly through glutamatergic projections to these brain regions or indirectly through activation of the hypothalamic-pituitary-adrenal (HPA) axis, which in turn leads to the release of adrenal stress hormones that directly stimulate memory structures of the brain [2].

Consistent with the view that the amygdala confers the effects of emotional arousal on memory, there is extensive evidence that the influence of stress and anxiety on hippocampus- and DLS-dependent memory depends on amygdala function. In one study, animals with amygdala lesions or sham lesions were subjected to chronic restraint/tail shock stress and were subsequently trained in a hippocampus-dependent version of the Morris water maze [31]. Stressed animals with sham lesions showed impaired hippocampus-dependent memory,

relative to non-stressed controls, whereas stressed animals with amygdala lesions were not impaired. Thus, the impairing effect of emotional arousal on hippocampal memory function depends on the integrity of the amygdala.

Whereas the study cited above employed permanent lesions of the amygdala, similar effects may be observed with temporary inactivation of the basolateral portion of the amygdala (BLA). In one study, animals were trained in either a hippocampus-dependent place learning or DLS-dependent response learning version of the water plus-maze and received post-training systemic infusions of the anxiogenic drug RS 79948-197 [46]. Post-training drug infusions target the consolidation phase of memory processing, i.e., when the short-term memory is consolidated into a long-term memory trace [65]. Post-training anxiogenic drug infusions impaired consolidation in the place learning task and enhanced consolidation in the response learning task, and both of these effects were blocked by temporary inactivation of the BLA with the sodium channel blocker bupivacaine [46]. Likewise, in the dual-solution plus-maze task, exposure to predator odor biased animals toward the use of response learning over place learning, whereas temporary inactivation of the BLA with bupivacaine eliminated the response learning bias produced by predator odor [36].

Additional evidence implicating a role for the amygdala in the emotional modulation of multiple memory systems comes from studies employing intra-BLA administration of anxiogenic drugs. Administration of the anxiogenic drugs RS 79948-197 or yohimbine directly into the BLA mimics the effects of systemic administrations. In a dual-solution plus-maze task, intra-BLA administration of RS 79948-197 or yohimbine leads to the preferential use of response learning over place learning [44, 45]. In addition, intra-BLA administration of RS 79948-197 enhances memory in the response learning version of the plus-maze and impairs memory in the place learning version of the task [66].

Finally, the critical role of the amygdala in the emotional modulation of multiple memory systems has also been demonstrated in humans. In one study, human participants were subjected to the socially evaluated cold pressor test, which stimulates emotional arousal, and subsequently performed the weather prediction task while undergoing fMRI [67]. Stressed subjects, relative to non-stressed controls, were more likely to use habit-based strategies over cognitive strategies, and this was accompanied by increased activation of the dorsal striatum and decreased activation of the hippocampus [67]. In addition, stressed subjects showed increased functional connectivity between the amygdala and dorsal striatum and decreased connectivity between the amygdala and hippocampus, relative to non-stressed control subjects [67]. Thus, the amygdala appears to be implicated in the emotional modulation of multiple memory systems in both lower animals and human subjects.

6. Mechanisms underlying amygdala modulation of multiple memory systems

The studies reviewed above clearly indicate that the amygdala, in particular the basolateral complex of the amygdala, is critically implicated in the emotional modulation of hippocampus

and DLS-dependent memory. However, the potential mechanisms through which the BLA modulates these systems have yet to be adequately addressed in this chapter. One possibility, as mentioned briefly above, is that the BLA modulates memory through direct glutamatergic projections to other brain regions. Indeed, the BLA strongly innervates the hippocampus [68], making it possible that a direct BLA-hippocampus pathway underlies the influence of emotional arousal on cognitive spatial memory. In contrast, the BLA does not innervate the dorsolateral region of the striatum implicated in habit memory. It is possible that the BLA could modulate DLS-dependent memory through its projections to the posteroventral region [69, 70], which may also be associated with habit memory function [29, 30]. In addition, the BLA projects to the ventromedial region of the striatum, which could possibly influence the DLS indirectly through an ascending spiral (see Ref. [71]). However, the potential roles of these amygdalostriatal projections in the emotional modulation of habit memory have yet to be explored.

Regarding the mechanisms that potentially underlie amygdala modulation of memory systems, evidence has indicated a role of stress hormones and competitive interactions between memory systems. There is also evidence that the amygdala may directly modulate synaptic plasticity in the hippocampus, which may be a neural mechanism underlying the emotional modulation of spatial memory.

6.1. Stress hormones

The influence of emotional arousal on memory formation has been attributed in part to stress hormones, including catecholamines (e.g., noradrenaline) and glucocorticoids (corticosterone in rodents; cortisol in humans). During an emotionally arousing event, stress hormones released via the HPA axis may influence memory by stimulating noradrenergic, glucocorticoid, or mineralocorticoid receptors in the brain [2, 72]. Following release from the periphery, corticosterone readily crosses the blood brain barrier and thus may modulate brain function directly. In contrast, adrenaline cannot cross the blood brain barrier and instead influences brain function by activating the vagus nerve, which enters the brain and innervates the nucleus of the solitary tract [73]. The nucleus of the solitary tract then projects to the locus coeruleus, which releases noradrenaline in multiple brain areas, including the amygdala [74].

Consistent with a potential role of stress hormones in the emotional modulation of memory, the effects of stress/anxiety on hippocampus- and DLS-dependent memory may be mimicked following systemic or intra-cerebral injections of drugs that increase stress hormone activity [44, 66, 75–78]. For instance, as mentioned above, systemic or intra-BLA injection of α_2-adrenoreceptor antagonist RS 79948-197, which increases noradrenaline release, leads to an enhancement of DLS-dependent habit memory and impairment of hippocampus-dependent spatial memory [44–47, 66]. In addition, systemic or intra-DLS glucocorticoid administration enhances habit memory [48, 77–82; but, see also Ref. 83], whereas systemic or intra-hippocampal glucocorticoids may either enhance or impair cognitive memory [75, 76, 84, 85].

The influence of emotional arousal on hippocampus- and DLS-dependent memory may be prevented via blockade of noradrenergic, glucocorticoid, or mineralocorticoid receptors [35, 67, 86–88]. For instance, the enhancement of DLS-dependent habit memory produced by

exposure to a fear conditioned stimulus may be blocked by systemic or intra-BLA administration of the β_2-adrenoreceptor antagonist propranolol [42].

Evidence suggests that glucocorticoid and noradrenergic mechanisms also interact to influence memory. The enhancing and impairing effects of intra-hippocampal glucocorticoids on cognitive memory depend on noradrenergic activity in the BLA [89, 90]. In addition, whereas systemic corticosterone administration enhances DLS-dependent habit memory in the water plus-maze, this enhancement is blocked by concurrent administration of propranolol [48].

It is possible that both glucocorticoid and noradrenergic mechanisms must be onboard in order for emotional arousal to influence memory. Whereas concurrent administration of hydrocortisone and α_2-adrenoreceptor antagonist yohimbine enhances DLS-dependent habit memory, administration of either drug alone has no effect [91]. This seems to contradict other evidence reviewed above suggesting that administration of either corticosterone or RS 79948-197 alone may enhance habit memory in the water plus-maze. However, it is possible that the aversive nature of the water maze may be sufficient to promote the endogenous release of glucocorticoids and noradrenaline in the brain, and since both stress hormones are onboard, subsequent administration of either corticosterone or RS 79948-197 alone may be capable of influencing memory. Indeed, previous evidence indicates that corticosterone administration may only be capable of modulating object recognition memory when the learning situation is sufficiently arousing and thus increases noradrenergic activity in the BLA [92].

6.2. Competition between memory systems

In addition to the role of stress hormones, there is also evidence that following BLA modulation of memory systems, the hippocampus and DLS potentially interact with each other in a competitive fashion to influence memory. Competition between two memory systems becomes evident when disrupting function of one memory system enhances function of the other system [57]. For instance, in some learning situations, lesions or temporary inactivation of the hippocampal system enhances memory in DLS-dependent habit memory tasks [13, 14, 93]. Likewise, disrupting dorsal striatal function facilitates memory in some hippocampus-dependent spatial memory tasks [94–96]. Moreover, consistent with a competitive interaction between memory systems, enhancing the function of one system through intra-cerebral injection of memory enhancing drugs sometimes impairs function of the other memory system. For instance, hippocampus-dependent spatial memory is impaired following intra-DLS administration of either glucose or a drug that increases CREB activity [28, 97].

Some investigators have suggested that the influence of emotional arousal on multiple memory systems may be partially explained by a competitive interaction between systems [10, 26]. As reviewed above, there is evidence that very high levels of emotional arousal impair hippocampus-dependent spatial memory. Consistent with a competitive interaction between memory systems, this stress-induced impairment of hippocampus-dependent memory may be similar to a hippocampal lesion, to the extent that it may indirectly enhance DLS-dependent habit memory. Indeed, the same anxiogenic drug doses that impair hippocampus-dependent place learning also enhance DLS-dependent response learning in the water plus-maze [34, 46, 66].

On the other hand, it is possible that stress or anxiety may indirectly impair hippocampus-dependent memory in part by increasing dorsal striatal function. As mentioned above, intra-DLS administration of corticosterone facilitates DLS-dependent habit memory. This suggests that stress hormones released following activation of the HPA axis may be able to directly enhance DLS-dependent habit memory through stimulation of dorsal striatal glucocorticoid receptors. Consistent with evidence that in some learning situations, augmenting dorsal striatal function indirectly impairs hippocampus-dependent spatial memory [28, 97], it is possible that an increase in dorsal striatal memory function is partially responsible for the impairment in hippocampus-dependent memory observed following stress or anxiety. This hypothesis has yet to be examined. Likewise, the precise neural mechanisms that mediate competition between memory systems have not been elucidated.

6.3. Synaptic plasticity

There is evidence to suggest that the emotional modulation of memory may be partially attributed to BLA efferents altering synaptic plasticity in multiple memory systems. Synaptic plasticity refers to experience-dependent changes in brain function and is often considered as a candidate neural substrate of learning and memory [98–101]. BLA stimulation may either enhance or impair long-term potentiation (LTP) in the hippocampus [102], a form of plasticity associated with spatial memory formation (for review, see Ref. [100]). Chronic restraint and tail shock have also been associated with impaired LTP in the hippocampus, and this impairment was blocked by amygdala lesions [31]. In the same study, the stress-induced impairment of hippocampal LTP mediated by the amygdala was also associated with impaired spatial memory and enhanced DLS-dependent memory in the water maze [31].

In addition to modulating hippocampal plasticity, BLA stimulation also facilitates induction of LTP in the ventral striatum [103]. Whether BLA facilitation of ventral striatal plasticity indirectly influences memory function of the dorsal striatum has not been examined.

7. Relevance to psychopathology

Amygdala modulation of multiple memory systems may be relevant to understanding the role of learning and memory processes in some human psychopathologies. Dorsal striatum-dependent memory processes have been linked to the habit-like behavioral symptoms in numerous psychiatric disorders, such as Tourette syndrome, post-traumatic stress disorder (PTSD), autism spectrum disorders, obsessive-compulsive disorder, drug addiction and relapse, and others [104–109]. Interestingly, stress and anxiety may have an important role in each of these disorders by promoting development, expression, or exacerbation of habit-like symptoms. For instance, one of the characteristic symptoms of PTSD involves avoidance behaviors, which are automatically evoked by trauma-related cues (e.g., jumping away from a loud noise). Some investigators have suggested that such symptoms may reflect heightened DLS-dependent habit memory due to the high levels of emotional arousal during and after the traumatic event

[3, 110, 111]. Indeed, neuroimaging evidence indicates differences in amygdala and dorsal striatal structure and function, as well as malformation of the hippocampus and impaired cognitive memory, in individuals with PTSD (for review, see Ref. [110]). Drug abuse may also be viewed as a manifestation of overactive habit memory, which may be exacerbated by stress [109]. In light of evidence in lower animals discussed above, it is likely that the stress-induced enhancement of habit-like symptoms in PTSD, drug addiction, and other disorders may be partially mediated by the amygdala and its connections to multiple memory systems.

8. Conclusion

Emotional arousal induced by stress and anxiety influences distinct memory systems in different ways. Behavioral and pharmacological stressors enhance S-R habit memory mediated by the DLS and impair cognitive spatial memory mediated by the hippocampus in both lower animals and humans. The BLA is the chief neural substrate implicated in the emotional modulation of hippocampus- and DLS-dependent memory. The BLA potentially influences spatial memory via its glutamatergic projections to the hippocampus and also alters synaptic plasticity in this brain region. Stress hormones (i.e., adrenaline and corticosterone/cortisol) are also involved in the emotional modulation of memory via activation of noradrenergic, glucocorticoid, and mineralocorticoid receptors across the BLA, hippocampus, and DLS. The BLA may also influence memory via modulating the competitive interaction between hippocampus- and DLS-dependent memory systems. These experimental findings may provide insight into the neural mechanisms underlying some clinical psychiatric disorders in which high levels of stress or anxiety are associated with impaired cognitive memory and enhanced habit-like symptoms.

Author details

Jarid Goodman[1,2], Christa McIntyre[2] and Mark G. Packard[1]*

*Address all correspondence to: markpackard@tamu.edu

1 Department of Psychology and Institute for Neuroscience, Texas A&M University, USA

2 School of Behavioral and Brain Sciences, University of Texas-Dallas, USA

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Chapter 11

Computational Models of the Amygdala in Acquisition and Extinction of Conditioned Fear

Guoshi Li

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67834

Abstract

The amygdala plays a central role in both acquisition and expression of conditioned fear associations and dysregulation of the amygdala leads to fear and anxiety disorders such as posttraumatic stress disorder (PTSD). Computational modeling has served as an important tool to understand the cellular and circuit mechanisms of fear acquisition and extinction. This review provides a critical appraisal of existing computational modeling studies of the amygdala and extended circuitry in acquisition and extinction of learned fear associations. It gives a broad overview of the computational techniques applied to amygdala modeling with an emphasis on how computational models could shed light on the neural mechanisms of fear learning, inform experimental design, and lead to specific, experimentally testable hypotheses. It covers different types of published models including rule-based models, connectionist type models, phenomenological spiking neuronal models, and detailed biophysical conductance-based models. Specific attention is given to the evolution of amygdala models from simple rule-based and connectionist type models to more sophisticated and biologically realistic models. Future direction on computational modeling of the amygdala and associated networks in emotional learning is also discussed.

Keywords: learning, plasticity, biophysical, neuron, network

1. Introduction

Anxiety and fear are normal human emotional states and the ability to efficiently learn about and appropriately respond to cues and contexts that predict or signal danger is critical for survival across species [1]. However, when fear becomes too generalized, this response mechanism might become very harmful [2]. Over-generalized fear could lead to anxiety disorders, especially disorders of fear regulation, including phobia, panic disorder, and posttraumatic stress disorder (PTSD). Posttraumatic stress disorder (PTSD), in particular, posts a great threat

to human health. Posttraumatic stress disorder (PTSD) is a serious psychiatric disorder that develops after exposure to a terrifying event or ordeal in which grave physical harm occurred or was threatened. People with PTSD may startle easily, become emotionally numb, more aggressive, or even violent [2]. In addition, they lose interest in life and have great difficulty in feeling affectionate. If not treated appropriately, PTSD may also lead to other mental complications such as depression, causing great suffering to the patients and their families. Posttraumatic stress disorder (PTSD) is the fifth most common psychiatric disorder with an occurrence rate of about 8% in the United States [3]. Overall, PTSD affects about 7.7 million American adults, but it can occur at any age, including childhood [4].

As the gateway to understand the pathophysiology of PTSD and other anxiety disorders, researchers used the fear conditioning paradigm, based on Pavlovian classical conditioning, which involves pairing an emotionally neutral conditioned stimulus (CS) with an innately aversive unconditioned stimulus (US). Animals appear to respond to the US with a constellation of physiological changes collectively known as the unconditioned response (UR). Following pairing of the CS in presentation with the US, the CS comes to elicit a conditional response (CR), which is generally similar to the UR [5]. Pavlov also noted that after successful conditioning, repeated presentation of the CS in the absence of US causes conditioned fear responses to rapidly diminish, a phenomenon termed fear extinction [6, 7]. Following extinction training, the animal would no longer respond to a CS that no longer predicts aversive stimuli [5]. The classical Pavlovian fear conditioning paradigm is the most valuable model to understand the pathological neural mechanism of PTSD and other anxiety disorders. Studies indicated that patients with PTSD demonstrate behavioral sensitization to stress and over-generation of the CS-US responses [8–10]. In addition, PTSD patients show delayed or impaired extinction learning as compared to controls [11, 12]. Thus, understanding the neurobiological mechanisms of fear conditioning and extinction is of great significance to understand the pathogenesis of PTSD and other fear-related mental disorders.

Taking advantage of the tractability of the fear conditioning paradigm, numerous studies have identified that the amygdaloid complex, an almond-shaped brain structure located within the medial temporal lobe, plays a central role in the acquisition and expression of learned fear associations [13, 14]. Indeed, damage of the amygdala impairs fear acquisition and expression, while electrical stimulation of the amygdala produces autonomic fear behaviors [15]. Anatomically, the amygdala receives sensory inputs from a wide range of cortical and subcortical areas including the thalamus, olfactory bulb and sensory cortex and polymodal sensory information from the prefrontal cortex (PFC), perirhinal cortex and hippocampus [16]. The projection of the amygdala is also widespread including cortical regions (especially PFC and the medial temporal lobe), striatum, hypothalamus, and brain stem areas [16, 17]. The amygdala could thus integrate a variety of sensory information and influence executive, motor, and memory functions via its divergent projections to downstream brain areas. The interactions between the amygdala, PFC and hippocampus are particularly important for the regulation and maintenance of fear memory [18, 19]. Indeed, subjects with PTSD show hyperactivation in the amygdala as well as reduced volume and activation of PFC and hippocampus [18, 20]; the reduced top-down control from the PFC and hippocampus may lead to hyper-responsive amygdala output to fearful stimuli [21].

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Due to the central role of amygdala in mediating fear acquisition and expression, computational modeling of signal processing within the amygdala circuit and its interactions with other brain areas such the PFC and hippocampus has been a subject of continuous interest. With improved understanding of the neurobiology of fear learning and rapid advance in computational power, computational models of the amygdala and extended circuits have evolved from the early simple rule-based models (e.g., [22]) to anatomically constrained connectionist type models (e.g., [23, 24]), to large-scale spiking neuron models (e.g., [25]), and more biophysically realistic conductance-based models [26–31]. These models addressed the various aspects of the functional roles of the amygdala in emotional learning including relative contribution of the thalamo-amygdala and cortical-amygdala pathways in fear conditioning [23, 32], contextual modulation of fear acquisition and extinction [25, 33], neural mechanisms of extinction [26], impact of infralimbic cortex in fear suppression [27, 34], and the role of competitive synaptic interactions in fear memory formation [28, 29]. These computational studies have significantly improved our understanding of the acquisition, maintenance, and regulation of learned fear associations. Below is a brief description of the amygdala circuitry critical for fear learning followed by a detailed review of the major computational studies of the amygdala in acquisition and extinction of conditioned fear.

2. The amygdala circuit

The amygdala consists of four major components that are critical for the acquisition and expression of conditioned fear including lateral amygdala (LA), basal amygdala (BA), central amygdala (Ce), and the intercalated (ITC) cell clusters (**Figure 1A**; [15, 35]). In auditory fear

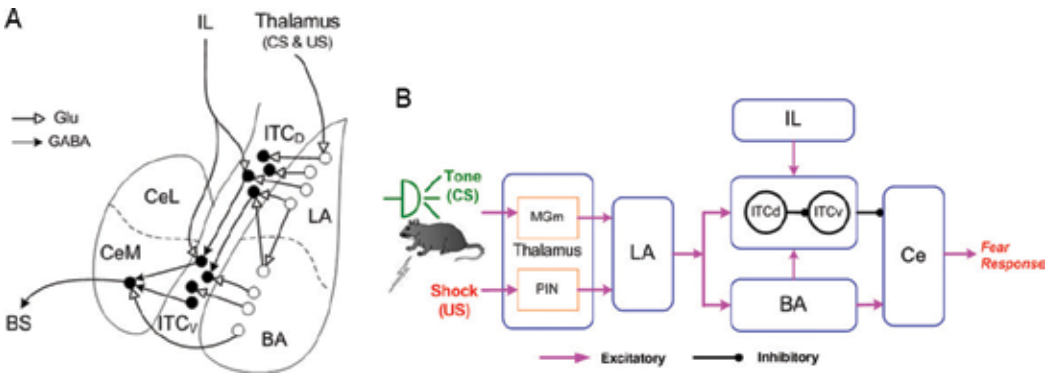

Figure 1. The amygdala circuitry for processing conditioned fear. (A) Scheme showing connectivity of the amygdala (adapted from Ref. [27]). The LA receives thalamic inputs conveying CS and US information. LA projects to the BA, and ITC neurons located dorsally (ITC_D), which in turn project to ITC cells located more ventrally (ITC_V). Intercalated cells located more ventrally (ITC_V) contribute GABAergic projections to CeM. The BA sends excitatory inputs to both ITC_D and ITC_V cells and to CeM. The infralimbic (IL) cortex also projects to both ITC_D and ITC_V cells. The CeM projects to brainstem structures mediating fear responses. (BS) Brainstem; (Glu) glutamate. (B) In auditory fear conditioning, convergence of tone (CS) and foot-shock (US) inputs in LA leads to potentiation of CS inputs, resulting in larger tone responses in LA. Increased LA responses are relayed to the Ce via the BA and the ITC cell clusters, eliciting fear responses via successive projections to brain stem and hypothalamic sites. As a result, rats learn to freeze to tones that predict foot shock. MGm: medial genicular body; PIN: posterior intralaminar nucleus.

conditioning, paring of the tone (CS) and foot-shock (US) inputs in LA potentiates the CS inputs, resulting in larger tone responses in LA (**Figure 1B**; [36–38]). Increased LA responses are relayed to the Ce via the BA [39] and the ITC cell clusters [35], eliciting fear responses via successive projections to brain stem and hypothalamic sites [13]. As a result, animals learn to freeze to tones that predict foot-shock. Those four nuclei (LA, BA, Ce, and ITC) have different physiological properties and serve distinct roles in fear conditioning and extinction, which are descried below.

2.1. The lateral amygdala (LA)

The LA is widely accepted to be a key site of synaptic events that contribute to fear learning [26, 35, 40]. It contains pyramidal-like glutamatergic projection neurons and local circuit γ-aminobutyric acid (GABA)-ergic interneurons [41]. In auditory fear conditioning, the tone (CS, auditory information) and foot-shock (US, somatosensory information) inputs are delivered to LA from the auditory cortex and auditory thalamus [37, 42]. Individual neurons within the LA respond to both auditory and somatosensory stimuli, suggesting convergence of CS and US inputs at the cellular level [40]. Conditioning significantly enhances the responses of LA neurons to CS input, which correlates tightly with the freezing behaviors of animals [36, 37]. Consistent with the anatomical data, lesioning or functionally inactivating the LA prior to training leads to deficits in fear conditioning [43, 44], suggesting that the LA is critically involved in the formation and storage of conditioned fear memories [13, 15, 40].

2.2. The basal amygdala (BA)

The BA plays an important role in contextual fear conditioning, fear extinction, and context-dependent fear renewal [44–46]. First, the BA constitutes a major route to relay CS and US information from LA to Ce, the output station of the amygdala that generates fear responses [39]. Consistently, posttraining BA lesions block the expression of conditioned fear responses [47]. Second, the BA receives contextual information from the hippocampus, a brain structure responsible for assembling contextual representations and transmitting these representations to the amygdala for association with US [15, 48]. Indeed, pharmacological inactivation of the BA prevents context-dependent fear renewal [45, 46]. Last, the BA also receives afferent inputs from the infralimbic (IL) cortex, a cortical region in the medial prefrontal cortex (mPFC) that is implicated in extinction of conditioned fear responses [49]. When BA is inactivated in a targeted and controlled manner, fear extinction is blocked completely, demonstrating that BA is necessary for the acquisition of extinction [46].

Interestingly, the differential functional roles of BA in fear acquisition and extinction are mediated by distinct neuronal circuits within the BA. Herry et al. identified that BA contains two distinct subpopulations of neurons (fear neurons and extinction neurons) whose activities correlate tightly with expression of high and low fear [46]. The fear neurons acquire CS responses as a result of fear conditioning but lose them following extinction training. By comparison, extinction cells remain unresponsive during fear conditioning, but become CS responsive during extinction training. The study demonstrated that a switch in the balance of the activity of fear and extinction neurons is essential to trigger behavioral transition

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between fear and extinction. It was further revealed that fear and extinction neurons are differentially connected with the hippocampus and mPFC, respectively, consistent with the well-documented roles of these two structures in contextual fear conditioning and extinction [50–52].

2.3. The central amygdala (Ce)

The Ce is the output station of the amygdala, which constitutes the interface to fear response systems [15]. Electrical stimulation of Ce generates fear behavioral responses [53], while lesions of the Ce impair both the acquisition and expression of conditioned fear [54, 55]. The Ce receives projections from both the BA and the ITC cells and sends dense projections to various brain stem structures involved in generating the behavioral and autonomic fear responses [56]. Anatomically, Ce can be divided into two subnuclei, the lateral sector of the Ce (CeL) and the medial sector of the Ce (CeM) (**Figure 1A**; [56]). Both the CeL and CeM contain GABAergic inhibitory neurons and CeL inhibits CeM [56].

2.4. The intercalated (ITC) cell cluster

Another component that is critical for conditioned fear responses is the intercalated (ITC) cell cluster that is located at the basolateral amygdala (LA & BA; BLA) and Ce border [57, 58]. Intercalated (ITC) cell clusters are GABAergic neurons and constitute an important alternate pathway (besides the BA) to relay CS/US information from the LA to Ce (**Figure 1B**; [35]). When ITC cells are damaged with pharmacological manipulation in rats, extinction memory is disrupted, mimicking the behaviors observed in anxiety disorders [59, 60]. Intercalated (ITC) cell clusters are ideally positioned to control Ce excitability because they receive glutamatergic inputs from principal LA and BA neurons and in turn generate feedforward inhibition in Ce cells (**Figure 1B**; [61, 62]). In addition, ITC neurons located dorsally (ITC_D) at the BLA-Ce border inhibit more ventral ones (ITC_V) [63], thereby allowing for a spatiotemporally differentiated gating of impulse traffic between BLA and Ce [62]. Moreover, ITC neurons receive massive projection from the IL cortex [64, 65] and stimulation of the IL substantially reduces conditioned fear responses [50, 66, 67], an inhibitory process believed to be mediated by ITC clusters [19, 35].

3. Computational models of the amygdala

3.1. Early computational models of fear conditioning

Early computational models of fear conditioning focused on learning theory or rules that describe the association between CS and US, i.e., associative learning theories (for an excellent review, see Ref. [68]). One representative early model of associative learning is the Rescorla-Wagner model, which proposed a learning rule based on prediction error [22]. Based on the Rescorla-Wagner rule, the change in associative strength of the individual components of a compound CS (e.g., AB) when paired with the US can be represented as:

$$\begin{aligned}\Delta V_{\mathrm{A}} &= \alpha_{\mathrm{A}}\beta(\lambda - V_{\mathrm{AB}}) \\ \Delta V_{\mathrm{B}} &= \alpha_{\mathrm{B}}\beta(\lambda - V_{\mathrm{AB}})\end{aligned} \tag{1}$$

where V_{AB} is the associative strength of the compound AB and must be specified in terms of the strengths of the individual components, e.g., $V_{\mathrm{AB}} = V_{\mathrm{A}} + V_{\mathrm{B}}$. The parameters α_{A} and α_{B} represent the stimulus salience, β is the learning rate, and λ represents the asymptotic value of associative strength for a particular US. According to Eq. (1), the associative change for the stimulus A after the compound AB is reinforced with the US is determined by the difference between the asymptotic value λ and the combined associative strength of A and B. Thus, the predictive error for associative strength change of a particular stimulus is governed by the combined associative strength of all stimuli present on a trial instead of that particular stimulus only. The introduction of such combined predictive error enables the model to explain the phenomena of blocking and conditioned inhibition [22].

Following the Rescorla-Wagner model, many other models of associative learning have been developed to extend or improve the Rescorla-Wagner rule [69–71]. For example, to account for the variations in the associability of stimuli with reinforcement, Mackintosh proposed a theory of selective attention by incorporating the notion of variable associability [69]. The learning rule is slightly modified from the Rescorla-Wagner rule:

$$\Delta V_{\mathrm{A}} = S\alpha_{\mathrm{A}}(\lambda - V_{\mathrm{A}}) \tag{2}$$

where S is the learning rate parameter. The main difference between the Mackintosh rule and the Rescorla-Wagner rule (Eq. (1)) is that the associability parameter α_{A} is modifiable dependent on the predictability of the stimulus A. If the stimulus A can predict the outcome better than other stimuli present on a particular trial, α_{A} will increase and decrease otherwise. With that modification, the Mackintosh model ensures that the associative change is not only dependent on a cue's associative strength, but also on the past relative predictive power of the cue. As each of those rule-based models attempts to best explain certain phenomena of the classical conditioning, they fail to offer a complete and satisfactory account of varying effects of associative history. To overcome this limitation and construct a "unified" theory of associative learning, Le Pelley proposed a hybrid model of associative learning that reconciles the effects of associative history [68].

Most of the early computational models of fear conditioning can be categorized as behavioral models in the sense that they attempted to reproduce many observed phenomena in classical conditioning such as generalization, blocking, and conditioned inhibition. Although these early models are useful in describing the associative process between the CS and US, they did not address how the CS and US information is processed within the amygdala circuit nor they took into account the neuroanatomical substrates underlying associative learning. Hence, these models provide little insights on the neuronal mechanisms of fear conditioning and extinction.

3.2. Connectionist models

As the neurobiological data of fear conditioning accumulated, connectionist or artificial neural network models of the amygdala were developed by researchers (e.g., [23, 24, 32]). Compared

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with early rule-based models, these connectionist models consist of multiple connected computational units (corresponding to a single neuron or a group of neurons) and take into consideration the anatomical structures of the amygdala circuit. There are two main features of connectionist-type models. First, the output of computational or neural units usually represents the firing rate or activation level of individual neurons, neural populations or a brain region. Second, the connection strength between computational units is usually modified based on Hebbian-type learning algorithm. For example, the activation levels of all neural units in a connectionist model of amygdala-hippocampal-prefrontal interaction [33] are computed as:

$$A_j(t) = f\left(\sum_{i=1}^{n} u_{ji}(t)x_i(t)\right) \tag{3}$$

where $u_{ji}(t)$ is the connection weight from unit i to unit j, n is the number of input units, and $x_i(t)$ is the input unit with binary value of either 0 (inactive) or 1 (active); f is the logistic sigmoid function

$$f = \frac{1}{1+e^{-x}} \tag{4}$$

Another way to capture amygdala activation is to use the classical mean-field formalism [72]:

$$\frac{dV_i}{dt} = \frac{F\left(\sum_j W_{ij}^{\text{Input}} * U_j^{\text{Input}}\right) - V_i}{\tau} \tag{5}$$

$$U_i = f_{\text{sigmoid}}(V_i) - \sum_k W_{ik}^{IN} * U_k^{IN} \tag{6}$$

where τ is the time constant, V_i and U_i represent membrane potential and firing rate of neuron i, U_j^{Input} and U_k^{IN} are the firing rates of input neuron j and inhibitory neuron k, respectively; W_{ij}^{Input} and W_{ik}^{IN} are the synaptic weights of the input and inhibitory connections; and F and f are nonlinear threshold and sigmoid functions, respectively. The synaptic weight is updated according to a Hebbian-type learning rule, which depends on the firing rates of pre and postsynaptic neurons [72]:

$$\frac{dW_{ij}}{dt} = ERR * \alpha U_j^{\text{Pre}} U_i^{\text{Post}} \tag{7}$$

where ERR is the prediction error (difference between the US value and amygdala output), α is the learning rate, U_j^{Pre} and U_i^{Post} are the firing rates of pre and postsynaptic neurons, respectively.

Early connectionist models of fear conditioning focused on the relative contribution of the thalamic versus the cortical pathway in fear conditioning. Armony et al. developed an anatomically constrained neural network model of fear conditioning based on known anatomical

and physiological observations [23]. The connectionist model focused on areas of convergence of CS and US pathways and specifically examined information processing via the two parallel sensory pathways to the amygdala from the auditory thalamus and the auditory cortex. The model considered tone input with a specific frequency associated with a mild foot-shock and was trained by a modified Hebbian-type learning rule. The model was able to reproduce frequency-specific changes of the receptive fields known to exist in the auditory thalamus and amygdala. In a following study [32], the model was used to simulate processing capacity of the thalamo-amygdala pathway by making lesions of the auditory cortex. The model predicted that lesions of the cortical pathway would not affect the specificity of the behavioral response to a range of frequencies centered on the training frequency and were consistent with experimental observations. However, in both studies [23, 32], extinction and other related phenomena were not included in the model.

Later connectionist models of fear conditioning aimed to replicate a wide range of conditioning phenomena. For example, Balkenius and Morén [24] proposed a model for emotional learning dependent on classical conditioning, which relied on crude representations and mathematically oriented circuits. The neural network model focused on the amygdala and the orbitofrontal cortex and their interactions. The amygdala was the locus of conditioning acquisition, and the orbitofrontal cortex was the site for extinction learning. Using two arbitrary subsystems (a base system and an auxiliary system) for reinforcement prediction and error tracking, respectively, the model simulated basic phenomena related to emotional conditioning including acquisition, extinction, blocking, and habituation. More recently, Burgos and Murillo-Rodríguez [73] used a neural-network model to simulate two context-dependent phenomena in Pavlovian conditioning: context specificity and renewal. Prior to that, the computational framework was used to simulate a wide range of conditioning phenomena such as reacquisition savings [74], reinforcement reevaluation [75], superstition [76], and latent inhibition [77]. Although these neural-network models were inspired by biological data of Pavlovian fear conditioning, there was no correspondence between the models and exact neural structures, and the amygdala circuit was not modeled explicitly.

Earlier connectionist models treated the amygdala as a "black box" or one homogeneous structure characterized by input-output function (e.g., [24]). With the advance of neurophysiology, we now know the amygdala can be divided into several functionally distinct nuclei in the processing of CS/US information (reviewed above). In keeping with new emerging neurobiological data, recent connectionist models of fear learning have started to model finer details of the amygdala circuitry and evolved from modeling only one or two brain structures to multiple regions and their interactions [33, 34, 72, 78]. For example, to understand the cognitive-emotional interaction mediating flexible behaviors, John et al. [34] developed an amygdala circuit model that consists of three subnetworks: (1) the BLA subnetwork; (2) the ITC subnetwork; and (3) the central output subnetwork. The BLA subnetwork contains LA and BA; the ITC subnetwork includes both the dorsal (ITC_D) and ventral groups (ITC_V), and the central network consists of the medial subnucleus of Ce (CeM). In the model, simultaneous presentation of the CS and US potentiated the cortical synapses on LA cells, LA synapses on BA, and LA synapses on ITC_D. This led to inhibition of ITC_V and excitation of CeM resulting in fear response. In contrast, presentation of the CS in the absence of US decreased the synaptic

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weight at LA-ITC_D synapses while potentiating the synaptic weight at BA-ITC_V synapses. As a result, CeM was inhibited and fear responses were suppressed. Besides normal fear acquisition and extinction, the model showed that cortical inputs from IL could bidirectionally modulate the circuit's behavior toward fear or extinction: stronger IL inputs to ITC_D could further disinhibit CeM promoting fear responses, while larger IL inputs to ITC_V enhanced the inhibition on CeM facilitating extinction. Moreover, model simulation indicated that if learning in ITC was faster than the BLA, the system could rapidly switch between the states of fear and extinction. Interestingly, cortical modulation from IL can be used to bias the system toward the "cautious" fear mode or the "rapid switch" mode.

In another study [78], the authors constructed a conceptual and computational neural model of fear conditioning (referred to as "FART") that included the structures and interactions of the amygdala, hippocampus, and PFC. Guided by a number of design targets based on known physiological data, the model was designed specifically to replicate many salient phenomena of fear conditioning including conditioning, extinction, secondary reinforcement, blocking, the immediate shock deficit, renewal, and a range of functional manipulation effects such as pre and posttraining inactivation of amygdala and hippocampus components. This model represents the first attempt to use one conceptual and computational model to simulate a wide range of empirically observed phenomena and effects of fear conditioning. One potential issue of such approach is that the model was designed specifically to account for those phenomena, which were not generated naturally from a biologically constrained model. It remains unclear whether the assumptions and parameters adopted by the model are biologically valid.

3.3. Phenomenological spiking neuron models

Though connectionist models are able to capture certain phenomenon of fear conditioning, such models have inherent limitation in that the output of computational units represents the firing rate or general activation, rather than the spiking activities of real neurons. Due to this limitation, connectionist models cannot be used to study specific spike patterns of individual neurons, nor the correlation in spike timing. On the other hand, although detailed conductance-based compartmental models can accurately reproduce the spiking dynamics of real neurons, these models are difficult to analyze because of intrinsic complexity. Phenomenological spiking neuron models have the advantage of emulating spiking behaviors while remaining analytically tractable and computationally feasible. As such, spiking neuron models are widely used to study neural coding, network dynamics, and learning and memory. Phenomenological spiking neuron models of fear conditioning are sparse with the exception of a large-scale spiking network model of the basal amygdala [25].

As mentioned earlier, the basal amygdala (BA) contains two types of neurons (fear neurons and extinction neurons) whose activation is correlated with the fear and extinction states, respectively [46]. However, the neural mechanisms underlying the differential activation of these two neuronal subpopulations remain unclear. To elucidate possible neural mechanisms involved in the encoding of fear and extinction memories in BA, Vlachos et al. [25] developed a large-scale spiking network model of the BA consisting of 3400 excitatory and 600 inhibitory neurons interconnected with both feedback and recurrent synapses. The excitatory neurons

were divided into two subpopulations, A and B, each receiving a different context input (CTX_A and CTX_B). In addition, all neurons in the network received CS-US input. The BA neurons were modeled with leaky-integrate-and-fire (LIF) scheme. Specifically, the subthreshold dynamics of the LIF neurons is described by the following differential equation:

$$\tau_m \frac{dV}{dt} = (E_0 - V) + g_{\text{exc}}(E_{\text{exc}} - V) + g_{\text{inh}}(E_{\text{inh}} - V) \tag{8}$$

where V is the membrane potential, τ_m is the membrane time constant, g_{exc} and g_{inh} are the excitatory and inhibitory conductance, and E_0, E_{exc}, and E_{inh} are resting membrane potential, excitatory, and inhibitory reversal potentials, respectively. When the membrane potential V crosses a static threshold θ in the upward direction, a spike is generated and the membrane potential is rest to a value E_k and clamped for 2 ms [25].

In simulation, conditioning was trained in context A while extinction was performed in context B. The strength of the CS and contextual inputs to excitatory neurons is modifiable according to a phenomenological rule, which specifies that the synapses are strengthened if the CS and contextual inputs overlap within a temporal window of ~100 ms. Based on this learning rule, the CS and CTX_A inputs to population A neurons were potentiated during fear conditioning leading to increased firing rates of population A cells. On the other hand, presentation of CS and CTX_B inputs to population B neurons during extinction potentiated those inputs resulting in activation of population B cells, which suppressed the activity of population A neurons through increased competitive inhibition. Since the behaviors of population A and B neurons resembled the fear and extinction neurons observed in [46], they were interpreted as fear and extinction neurons, respectively [25]. The model was also used to study renewal, extinction over-training, and extinction of contextual conditioning. In particularly, the model predicted that gamma oscillations will be generated if the connectivity between the excitatory and inhibitory neurons in BA is high. The main conclusion of this modeling study is that differential activation of fear and extinction neurons is a result of context specificity, i.e., fear and extinction neurons are innervated by different contextual inputs. Questions remain whether fear and extinction neurons would emerge differentially if both conditioning and extinction are trained in the same context. Also, the model assumes that both the CS and contextual inputs are potentiated if they are temporally coincided. This suggests that fear could be developed even without US inputs, which is not consistent with experimental observation.

3.4. Biophysically realistic models

Although phenomenological spiking neuron models (e.g., LIF models) are able to simulate neuronal spiking activities, they do not take into account the morphological and electrophysiological properties of actual neurons, thus neglecting the biophysical constraints on neural learning and computation. To accurately model the underlying processes responsible for fear learning, biophysical Hodgkin-Huxley type models are required. The first biophysically realistic model of fear conditioning was developed by Li et al. [26] to study the neural mechanisms of fear acquisition and extinction in LA neurons. In this pioneering study, conductance-based compartmental models of LA pyramidal cells and interneurons are first developed by incorporating detailed ionic channels and kinetics observed in LA neurons. The schematic

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representation of the two-compartment LA pyramidal cell and interneuron models is shown in **Figure 2A**, and the equivalent electrical circuit of two basic neural compartments is shown in **Figure 2B**. Each compartment has a membrane capacitance C_m, a fixed membrane resistance R_m, and an equilibrium potential E_m associated with the ohmic leakage current that flows across R_m. Based on the equivalent circuit, one can derive the current-balance equations for the two compartments:

$$\begin{aligned} c_m \frac{dV_s}{dt} &= -\frac{(V_s - E_m)}{R_m} - \sum_i G_{ki}(V_s - E_{ki}) - g_c(V_s - V_d) \\ c_m \frac{dV_d}{dt} &= -\frac{(V_d - E_m)}{R_m} - \sum_i G_{ki}(V_d - E_{ki}) - g_c(V_d - V_s) \end{aligned} \tag{9}$$

where V_s and V_d are the transmembrane potentials for the soma and dendrite compartments, respectively. G_{ki} and E_{ki} are the conductance and reversal potential for the channel i and g_c is

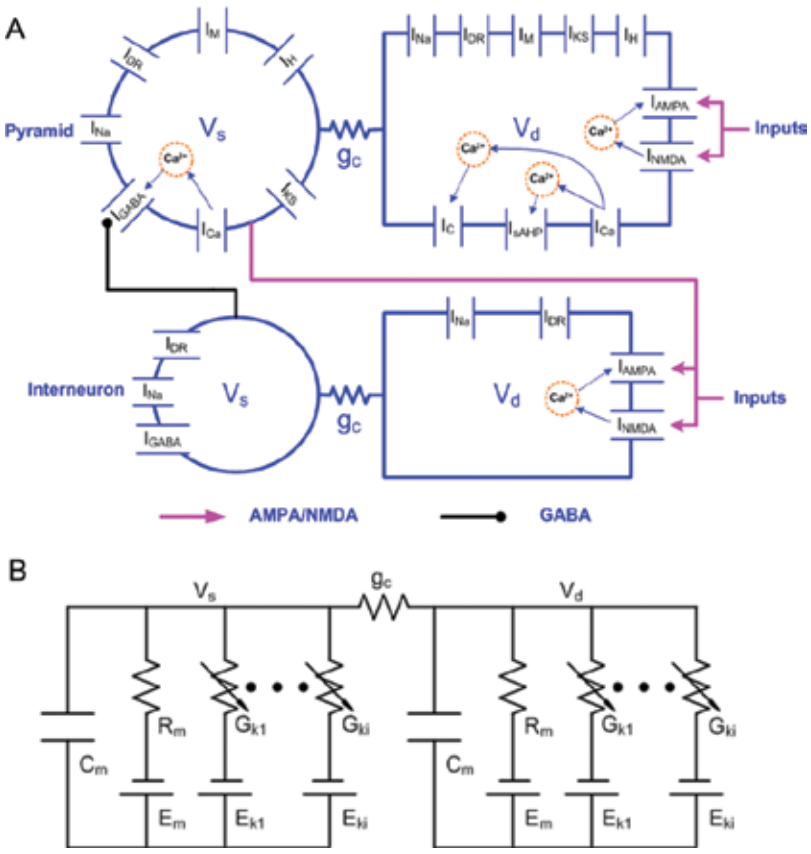

Figure 2. Conductance-based compartmental model of LA neurons. (A) Schematic representation of the LA pyramidal cell and interneuron models with distribution of active ionic conductances in each of its two compartments (adapted from Ref. [26]). (B) Equivalent electrical circuit of two interconnected neural compartments used to simulate LA cell excitability. C_m is the membrane capacitance, R_m is the membrane resistance, and E_m is the leakage reversal potential. Subscripts k1, k2, ⋯, ki denote i different active (variable) conductances and their associated reversal potentials (G_{Na}, G_{DR}, etc., with reversal potentials E_{Na}, E_K, etc.).

the coupling conductance between the soma and dendrite compartments. All ionic conductances in the LA model are modeled using the Hodgkin-Huxley kinetics [79]. Specifically, the conductance for channel *i*, G_{ki}, is modeled as:

$$G_{ki} = g_{ki} m^p h^q \tag{10}$$

where g_{ki} is its maximal conductance density, m its activation variable (with exponent p), and h its inactivation variable (with exponent q). The kinetic equation for the gating variable x (m *or* h) satisfies a first-order kinetic model,

$$\frac{dx}{dt} = \varnothing_x \frac{x_\infty(V) - x}{\tau_x(V)} \tag{11}$$

where ϕ_x is a temperature-dependent factor, $x_\infty(V)$ is the voltage-dependent steady state, and $\tau_x(V)$ is the voltage-dependent time constant. Equivalently, Eq. (11) can be written as:

$$\frac{dx}{dt} = \phi_x \left(\alpha_x(V)(1 - x) - \beta_x(V)x \right) \tag{12}$$

where $\alpha_x(V)$ and $\beta_x(V)$ are the voltage-dependent rate constants. The detailed kinetic parameters can be found in Ref. [26]. With careful parameterization, the LA neuronal models were able to accurately reproduce the firing properties of LA neurons as observed in experimental recording (**Figure 3**).

After successfully constructing single-cell models of LA neurons, Li et al. [26] developed a small network model consisting of eight pyramidal cells and two interneurons (**Figure 4A**). The network model was trained with a behavioral protocol including a sensitization, conditioning and two extinction phases (**Figure 4B**). In addition, the model implemented a biophysical learning rule termed "calcium control hypothesis" [81] to precisely model synaptic potentiation and depression during fear acquisition and extinction (**Figure 4C**). The biophysical realism enables the LA model to accurately replicate conditioning- and extinction-induced

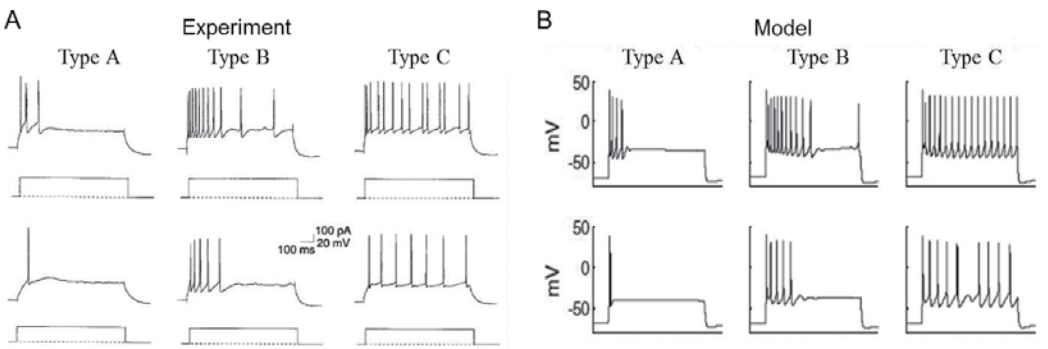

Figure 3. Biophysical LA neuronal models reproduce salient firing patterns of LA pyramidal cells. (A) Firing properties of three types of pyramidal cells (Type A, Type B, and Type C) recorded *in vitro* (adapted from Ref. [80]). (B) Responses of three types of LA model neurons to current injections (adapted from Ref. [26]).

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changes in tone responses of LA neurons in behaving rats during the classical auditory fear conditioning experiment [37] (**Figure 5**). By closely matching experimental data, the model has provided in-depth insights into the neural mechanisms of fear conditioning and extinction. First, the LA model demonstrates that both conditioning and extinction can be learned within the LA circuitry. This has significant implication as the LA, known to be a key site for fear acquisition [35, 40], can also encode extinction memory. Second, the LA model convincingly reconciles the two contrastive theories (unlearning versus inhibition) about the extinction mechanism. In the model, extinction not only causes depression in potentiated thalamic input

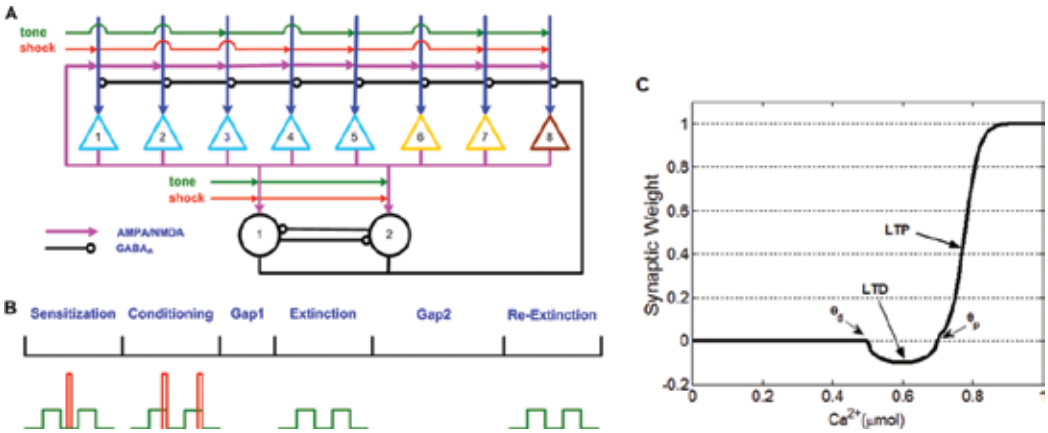

Figure 4. Architecture, training protocol and learning rule of the LA network model (adapted from Ref. [26]). (A) The LA network structure. Triangles represent pyramidal cells and circles representing interneurons. (B) Simulation schedule showing tone and shock inputs during sensitization, conditioning, and the two extinction phases. (C) Synaptic depression and potentiation as a function of the Ca^{2+} concentration. LTD: long-term depression; LTP: long-term potentiation.

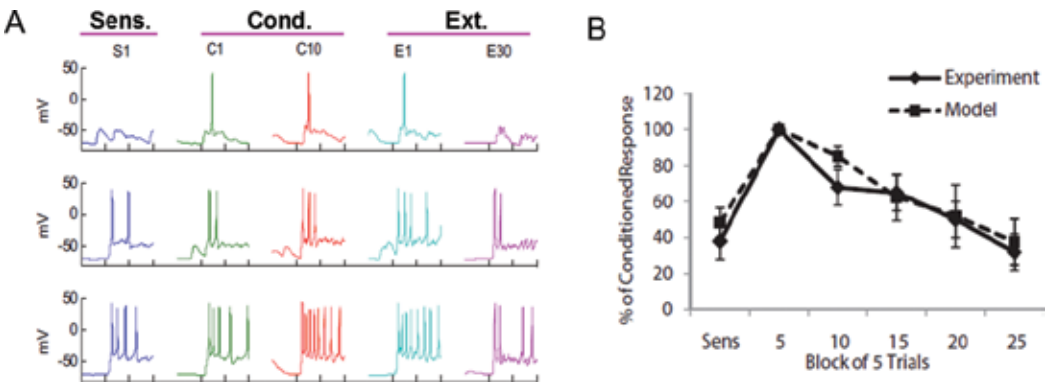

Figure 5. The LA network model reproduces conditioned tone response in behaving rats (adapted from Ref. [26]). (A) Early tone response (100 ms) of three representative pyramidal cells in the LA network during different phases of the training. S1: first tone in sensitization; C1: first tone in conditioning; C10: 10th tone in conditioning; E1: first tone in extinction; E30: 30th tone in extinction. (B) Comparison of the experimental data (**Figure 4** of Ref. [37]) and the model conditioned tone responses for the last block of five trials in sensitization and successive five-trial blocks during extinction.

synapses, but also potentiates inhibitory GABAergic synapses from local LA interneurons that inhibit conditioned responding in pyramidal cells. Therefore, both synaptic depression (unlearning) and potentiation of inhibition are required for a complete extinction of fear. Importantly, the model suggests that depotentiation induced by extinction is synapse-dependent in that the thalamus-to-LA pyramidal cell synapses will undergo stronger depotentiation than the LA pyramidal-to-pyramidal cell synapses. This finding agrees with an earlier experimental observation that a unique form of depotentiation during extinction reversed conditioning induced potentiation at thalamic input synapses onto the LA *ex vivo* [82]. Last, the LA model makes a number of important predictions that could guide experimental design. For example, the model makes specific predictions regarding the storage sites of fear and extinction memory within the LA circuitry. Also, the LA model suggests that while the low spontaneous firing rates of LA pyramidal cells serve to preserve the original fear memory, the relatively high spontaneous firing rates of interneurons lead to extinction decay and spontaneous fear recovery. The prediction that higher spontaneous firing rates result in faster decay of memory has been validated by experimental data *in vivo* [83]. Moreover, the model predicts that N-methyl-D-aspartic acid (NMDA) currents are required for extinction training, consistent with an experimental finding that depotentiation of conditioning-induced potentiation at thalamic input synapses onto the LA *ex vivo* requires GluN2B-containing NMDA receptors [84].

During conditioning, conditioned fear output in the LA is related to the Ce via both the BA and ITC cell clusters and generates fear response via successive projection to the brain stem and hypothalamic sites (**Figure 1B**). Thus, ITC cells play a critical role in regulating fear expression by controlling the impulse traffic between the LA and Ce. Also, brief stimulation of infralimbic cortex (IL) substantially reduces fear expression, an inhibitory process believed to be mediated by ITC cells [19, 35]. Thus, it is of great importance to understand how activation of ITC neurons by IL leads to fear suppression. However, ITC neurons are endowed with both unusual membrane characteristics (prolonged excitation or bistability [85]; **Figure 6A**) and synaptic properties (heterogeneous plasticity [86]; **Figure 6B**), and are embedded in complex neuronal circuit with both intercluster and within-cluster inhibition (**Figure 6C**). The functional roles of ITC cells in mediating fear extinction are precluded by such complicated cellular, synaptic, and circuit properties.

To address this critical issue, Li et al. [27] developed a biophysically realistic ITC neuronal model that precisely replicated the salient firing patterns and bistable properties of real ITC cells. By incorporating realistic heterogeneous short-term synaptic dynamics in a biophysical ITC network (**Figure 6C**), Li et al. [27] elucidated that: (1) ITC neurons could transform the transient fear signal arising in the LA/BA into a persistent pattern of activity; (2) over a wide range of stimulation frequencies and strengths, brief IL activation caused a marked increase in the firing rates of ITC neurons, resulting in a persistent decrease in Ce output, despite inter-ITC inhibition (**Figure 7**); (3) both intrinsic properties (i.e., bistability) and variations in the short-term synaptic dynamics of ITC neurons contributed to the effectiveness of IL stimulation; and (4) IL stimulation reduced Ce responses to conditioned stimulus in a temporally specific manner with the most effective inhibition given shortly after stimulus onset. All these important findings significantly improve our understanding of the functional roles of ITC cells in mediating fear conditioning and extinction. It offers the solid computational support that IL inputs are in a strategic position to control extinction of conditioned fear via the activation of

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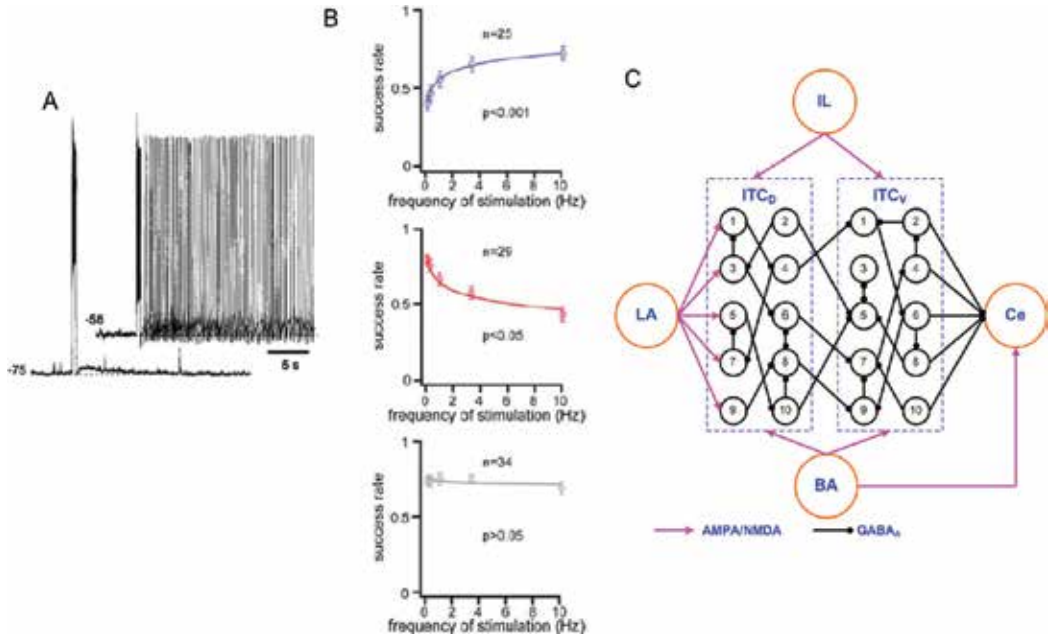

Figure 6. Intercalated (ITC) cell clusters neurons are endowed with both unusual membrane and synaptic properties, and embedded in complex neuronal circuit with both intercluster and within-cluster inhibition. (A) Bistable firing or prolonged excitation of ITC cells (adapted from Ref. [85]). Transient depolarization induces sustained firing. (B) The release probability of ITC synapses increases, decreases, or remains constant when the presynaptic stimulation frequency increases in three different types of synapses (adapted from Ref. [86]). (C) Structure of the ITC network model (adapted from Ref. [27]).

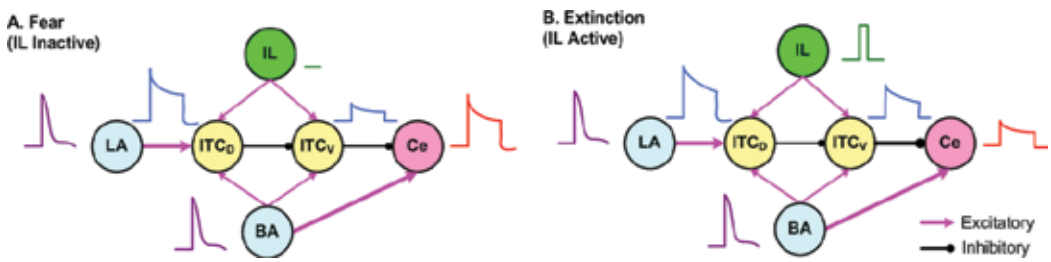

Figure 7. Effect of IL stimulation on amygdala network activity (adapted from Ref. [27]). (A) During fear conditioning, the LA-ITC_D synaptic strength is potentiated. Strongly adaptive LA inputs are transformed into sustained output by ITC_D neurons leading to persistent inhibition on ITC_V cells allowing for sustained Ce firing in the high fear state. (B) Brief IL stimulation increases the excitability of both ITC_D and ITC_V neurons, with a larger impact on ITC_V cells, which significantly reduces the Ce firing leading to a low fear state.

ITC neurons. Thus, targeting ITC neurons with IL stimulation or pharmacological interventions could potentially eliminate fear memories and reduce anxiety, offering new hope for the treatment of anxiety disorders such as PTSD.

As a pivot study, the network size of the LA model developed by Li et al. [26] was relatively small (eight pyramidal cells and two interneurons) and focused on the neural mechanisms of fear extinction. In a subsequent study, Kim et al. developed a large-scale biophysical model of the

dorsal portion of the LA (LAd) to study the mechanisms contributing to the induction and storage of Pavlovian fear memories [28]. The spatial LAd network model included 800 principal pyramidal cells and 200 interneurons placed in a horn-shaped 3D structure. In addition, the model network integrated spatially differentiated patterns of excitatory and inhibitory connections within the LA [87]. The model was able to replicate the behaviors of two types of LAd neurons (transient cells and long-term plastic cells) observed in experimental recording [88] as a result of differential intrinsic connectivity. Moreover, the model demonstrated that while the conditioning-induced increases in the CS responsiveness of thalamic/cortical neurons are required for fear memory formation, they are not necessary for long-term fear memory storage. Instead, the projecting synapses from thalamic/cortical neurons to LA pyramidal cells play a more important role in the storage of fear memory. In a following up study, the LAd network model was used to study an important question of how particular LA neurons are assigned to fear memory traces [29]. The model showed that LA neurons with higher intrinsic excitability have a larger chance of being recruited into the fear memory trace. Paradoxically, when the ratio of more excitably cells changed, the number of plastic cells remained relatively constant. Model analysis indicated that competitive synaptic interactions play a critical role in assigning the LA neurons to the memory trace. That is, a subset of pyramidal cells gain advantage in competition due to stronger excitatory interconnections and suppress the remaining pyramidal cells through the recruitment of inhibitory interneurons. Hence, assignment of LA neurons to a memory trace depends on a competitive process, consistent with experimental data [89]. The nature, specificity, and details of synaptic competition in fear memory trace formation are further examined in two subsequent biophysical modeling studies [31, 90].

Although the amygdala plays a central role in fear acquisition and extinction, the medial prefrontal cortex (mPFC) exerts strict top-down control over the amygdala on both the formation and expression of fear memory [52, 91]. Specifically, while prelimbic (PL) cortex increases fear expression, the infralimbic (IL) cortex reduces fear expression [67]. There is significant difference about the neural correlates of fear expression between the LA and PL. Specifically, the conditioned response in LA neurons is transient, lasting only a few hundred milliseconds after CS onset [36, 37, 88]. By comparison, the PL neurons have sustained conditioned response during the entire CS presentation which correlates closely with the fear expression [92]. This leads to the hypothesis that PL transforms transient fear signal in LA into sustained fear output in Ce via descending projections to the BA [92]. However, the neural and circuit mechanisms underlying such transformation are not clear. Using a biophysically realistic model of the BA-PL network consisting of 850 conductance-based compartmental model cells, Pendyam et al. [30] investigated three potential mechanisms involved in the LA-PL transformation including: (1) BA-PL network structure and connectivity; (2) dopaminergic and noradrenergic modulation; and (3) specific microcircuits within the BA-PL network. Model simulation indicated that BA-induced continuous release of dopamine and norepinephrine, rather than the BA-PL interconnections, plays a dominant role in sustaining PL conditioned responses. The model also predicted that specific microcircuit variations in the BA-PL network significantly modulate fear expression, which could possibly explain the individual heterogeneity in fear responses.

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4. Summary and future direction

Computational models of the role of the amygdala in fear conditioning and extinction have enjoyed a long history of success and greatly improved our understanding of the processes underlying emotional learning and memory. With the advance of neurophysiology and high performance computation, computational models of the amygdala have evolved from simple rule-based models to anatomically constrained connectionist models, and to large-scale biologically realistic network models. These different types of models have complementary utility, and the selection of models depends on the available computational resource and the nature of the problem being investigated. In particular, the development of biophysically realistic models of the amygdala and extended circuits has opened up new avenues to study the neural and circuit mechanisms of acquisition, storage, and regulation of fear memory in the brain. In the future, large-scale biophysical network models of the amygdala and associated circuits such as PFC and hippocampus are of particular interest in order to provide an integrated account of how multiple brain regions work in concert to regulate fear memory formation and expression.

While much modeling progress has been made, there is still a long way to go to model pathologies associated with the fear circuit (e.g., PTSD) and assist in the development of new treatments. To achieve this goal, a new class of translational models need to be developed that could simulate the systemic neural impairments with resulting symptoms observed in fear and anxiety disorders such as PTSD. In addition, such models should explore new treatment paradigms such as invasive deep brain stimulation (DBS) and noninvasive transcranial magnetic stimulation (TMS). This may lead to the development of hybrid type models that combine the system-level analysis and detailed cellular-level operation.

Acknowledgements

This work was supported by the National Institute on Deafness and Other Communication Disorders (NIDCD) of the National Institute of Health R03 Grant DC013872 to G Li.

The author declares no competing financial interests.

Author details

Guoshi Li

Address all correspondence to: guoshi_li@med.unc.edu

Department of Psychiatry, University of North Carolina at Chapel Hill, North Carolina, USA

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Chapter 12

The Human Amygdaloid Complex: Cellular Architecture and Dopaminergic Innervation

María García-Amado and Lucía Prensa

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.68391

Abstract

The human amygdaloid complex (AC) is associated with the perception of fear and consequent anxiety-related behaviors, apart from other functions ranging from attention to memory and emotion. The AC is composed of several regions with specific cytoarchitectures, chemistry, and connections that encode different aspects of fear. Detailed understanding of AC cell composition is basic to determining whether cell number alterations coincide with neurological and psychiatric pathologies associated to anxiety imbalances, as well as with changes in brain functionality during aging. Here, we describe quantitative data gathered applying stereological methods to human AC tissue; the amounts of neurons, glial and endothelial cells, as well as of various interneuron subsets that populate the AC regions were noted and compared with those collected in the AC of non-human primates and rodents. This chapter also addresses the dopaminergic innervation of the AC, which exerts a modulatory effect over the intrinsic AC network and is critical for reward-related learning and fear conditioning. This innervation is twice as abundant in the main output nuclei as in the principal entry nuclei of the human AC, and this irregularity may indicate functional variations between these entry and output amygdaloid territories.

Keywords: amygdala, human, dopamine, stereology, dopamine transporter, neurons, glia, endothelial cells

1. Introduction

The amygdaloid complex (AC) is a heterogeneous structure described for the first time by Burdach as an "almond-shaped" mass of grey substance located in the anterior part of the temporal lobe [1]. Since then, the various AC nuclei, which have diverse developmental features and functions, have been considered either as part of a single structural unit [2] or a collection of randomly aggregated structures [3]. There have also been many attempts to consistently

demarcate the various AC nuclear components and their subdivisions in the human and other non-human primates, but there is no consensus as yet [3].

The AC receives highly integrated sensory information of all modalities and is needed for the association between each sensory stimulus and its emotional and motivational significance [4, 5]. The AC also contributes to the visceral and somatic expression of endocrine response to these stimuli. Direct stimulation of the AC produces a subjective perception of fear and anxiety, as well as increased heart rate and blood pressure with pupil dilation [6]. A huge number of studies have related the AC with fear perception and the generation of appropriate affective responses to these types of stimuli [7–14]. Other investigations have, nevertheless, demonstrated that the activity of the AC also increases in response to positive emotional stimuli in humans [15–17] and non-human primates [5]. The AC is crucial to the acquisition, consolidation and extinction of fear memories, as well as to their retrieval after extinction. Therapies known as "exposition therapies" try to produce the extinction of those fear memories that trigger anxiety behaviors [12].

Bilateral lesion of the medial temporal lobes containing the AC produces a visual-limbic disconnection [18] or a sensory-affective dissociation [19]. Both alterations seem to be present in Rhesus monkeys affected by the Klüver-Bucy syndrome [20]. A variety of symptoms including aphasia, amnesia, or dementia have been observed in human patients with a bilateral temporal lobe lesion, and lesions specifically affecting the AC seem to be related to the difficulty in identifying the emotional significance of facial expressions [21].

The dopaminergic innervation received by the AC is intense in rats [22–26], non-human primates [27–29], and humans [30]. This particular neurochemical input is required to acquire, consolidate or extinguish fear-related memories, as well as to generate appropriate affective responses toward aversive stimuli [31–36]. A dysfunction of the dopaminergic system is related to psychiatric diseases such as schizophrenia [37, 38] and/or other stress-related disorders [39, 40]. In addition, the hyperdopaminergic phenotype of transgenic mice lacking the dopamine transporter (DAT) can show the positive symptoms observed in schizophrenic patients [41]. As discussed below, the dopamine in the AC affects projection neurons either directly or through various types of interneurons.

The present chapter will review data collected in recent years in the human AC related to the amount of neurons, glial and endothelial cells, as well as more specific quantitative data on two main AC interneuron populations, which modulate the activity of the projection neurons. The amount, distribution and specific neuronal targets, of the dopaminergic innervation of the human AC will also be addressed.

2. Anatomical delineation and nomenclature of the human amygdaloid complex

Numerous studies have addressed the anatomical nuclear division in the primate AC [2, 42–50], but the lack of well-defined anatomical limits between the various AC nuclei has complicated any consensus on the delineation of the AC nuclei and their subdivisions. **Table 1** shows the most relevant divisions and nomenclature used in the last years to define the AC nuclei in human and

Sims and Williams [49]	[1]Brady [52]	[2]Price et al. [48], Sorvari et al. [58]	Mai et al. [119]	García-Amado and Prensa [55]
		DEEP NUCLEI		**BASOLATERAL GROUP (BL)**
LATERAL	LATERAL	LATERAL	LATERAL	LATERAL (L)
•External		•Lateral	•Dorsolateral	•External (Lex)
•Dorsal		•Medial	•Dorsomedial	•Dorsal (Ld)
•Lateral			•Dorsal anterior	•Lateral (Ll)
•Medial			•Intermediate	•Medial (Lm)
			•Ventral	
BASAL	BASAL	BASAL	BASOLATERAL	BASAL (B)
•Lateral	•Magnocellular	•Magnocellular	•Dorsal	•Magnocellular (Bmc)
•Central	•Intermediate	•Intermediate	•Intermediate	•Intermediate (Bint)
•Medial	•Parvocellular	•Parvicellular	•Ventromedial	•Parvocellular (Bpc)
			•Ventrolateral	
			•Paralaminar	
ACCESSORY BASAL	ACCESSORY BASAL	ACCESSORY BASAL	BASOMEDIAL	ACCESSORY BASAL (AB)
•Dorsal	•Magnocellular	•Magnocellular	•Dorsomedial	•Dorsal (ABd)
•Ventral	•Parvocellular	•Parvicellular	•Centromedial	•Ventral (ABv)
		•Ventromedial	•Ventromedial	
			•Dorsolateral	
		PARALAMINAR		
		•Medial*		
		•Lateral*		
CORTICO-MEDIAL (CM) GROUP		**SUPERFICIAL NUCLEI AND/OR AREAS**		**CORTICO-MEDIAL (CM) GROUP**
CORTICAL	CORTICAL	CORTICAL	CORTICAL	CORTICAL (Co)
•Medial	•Ventral	•Anterior	•Anterior	•Medial (Com)
•Lateral	•Dorsal	•Posterior	-- Dorsal portion	•Lateral (Col)
			-- Ventral portion	
			•Posterior	
MEDIAL	MEDIAL	MEDIAL	MEDIAL	MEDIAL (Me)
		PERIAMIGDALOID CORTEX	•Anterior	
		LATERAL OLFATORY TRACT NUCLEUS		

Sims and Williams [49]	[1]Brady [52]	[2]Price et al. [48], Sorvari et al. [58]	Mai et al. [119]	García-Amado and Prensa [55]
		DEEP NUCLEI		**BASOLATERAL GROUP (BL)**
		REMAINING NUCLEI AND/OR AREAS		**CENTRAL GROUP (Ce)**
CENTRAL	CENTRAL	CENTRAL	CENTRAL	CENTRAL (Ce)
•Medial	•Medial	•Medial	•Medial	•Medial (Cem)
•Lateral	•Lateral	•Lateral	•Lateral	•Lateral (Cel)
•Dorsolateral				
•Ventrolateral				
•Interstitial				
CORTICO-AMIGDALOID TRANSITION AREA	CORTICAL TRANSITION AREA		PARAHIPOCAMPAL-AMIGDALOID	CORTICO-AMIGDALOID TRANSITION AREA (CTA)
			TRANSITION AREA	
ANTERIOR AMIGDALOID AREA	ANTERIOR AMIGDALOID AREA	ANTERIOR AMIGDALOID AREA	ANTERIOR AMIGDALOID AREA	PERIAMIGDALOID AREA (PA)
			PREAMIGDALOID CLAUSTRUM	
			PERIAMIGDALOID AREA	
INTERCALATED CELLULAR GROUPS	INTERCALATED NEURONS	INTERCALATED NUCLEI		
		AMYGDALO-HIPOCAMPAL AREA	AMYGDALO-HIPOCAMPAL AREA	

* Subdivisions added to the classification proposed by Price et al. [48]. The abbreviations used in this chapter are indicated in bracket.

[1] Classification developed in *Saimiri sciureus* and in humans.

[2] Classification developed in *Macaca fascicularis*. The other studies are referred to humans.

Table 1. Anatomical delineation of the human amygdaloid complex.

non-human primates. The correct and detailed division of the AC is important because its various nuclei have distinct developmental origins, specific connections, and codify different aspects of fear [7]. The proper delineation of the AC is also necessary to perform accurate quantitative studies such as stereological estimations of cell numbers or nuclear volumes, and the comparisons of such data collected in different studies. For instance, the basolateral group, especially the lateral nucleus, processes the emotional significance of every stimulus, allowing other structures access to this information, and it is involved in the suppression of fear responses and their

retrieval after extinction [7, 8, 11]. The central nucleus is activated by the basolateral group and can initiate key defense mechanisms against species-specific predators representing a danger to an individual given species [7, 8, 13, 51]. The corticomedial group and the lateral nucleus become activated after the presentation of faces expressing fear [52].

One of the first descriptions of the architectonic organization of the AC was made by Völsch [2, 42–50] in primates. Later, Brockhaus published a detailed report of the human AC architecture employing Nissl and myelin staining [47]. However, these classifications were rather complex and Crosby and Humphrey proposed a simpler nomenclature, which is still widely used and based on the one suggested by Johnston [53, 54]. Johnston grouped the amygdaloid nuclei into two groups based on developmental origin and age: the first group included the primitive or little-modified central, medial, cortical, and nucleus of the lateral olfactory tract; the second group included the more recently evolved basal and lateral nuclei formed by infolding or cell immigration. The basal and lateral nuclei together with the accessory basal nucleus conform the basolateral group of the AC, which has undergone a huge increase of volume in humans [2, 49].

In a more recent study, García-Amado and Prensa suggested a detailed nuclear division and nomenclature for the human AC based on the proposal by Sims and Williams [49] and Ledo-Varela et al. [56], and that also resembled those used by Schumann and Amaral [57] and Sorvari et al. [58] (**Table 1**; see [55] for further details). The García-Amado and Prensa study [55] was focused on providing accurate and objective limits of the entire AC and its various nuclear groups, nuclei, and nuclear subdivisions. The establishment of consistent anatomical limits is essential for making comparisons among quantitative data collected in different studies. To this end, the 2012 García-Amado and Prensa study did not outline as a single whole the structures with rather fuzzy boundaries like the anterior amygdaloid area described by Sims and Williams [49]. Instead, they only outlined the most lateral part of this region, the periamygdalar area, since this area can be objectively identified by its high content in acetylcholinesterase. Other relevant aspects of the study of García-Amado and Prensa are the consideration of the dorsal subdivision on the lateral nucleus, whose cells are much smaller and more packed than the ones that populate its three other subdivisions, and the inclusion of the paralaminar region within the parvocellular subdivision of the basal nucleus because the former has unclear limits.

3. Volume of the human AC

The volumes of the AC as a whole and some of its nuclei have been estimated in many studies dealing with psychiatric disorders as well as in others on Alzheimer's disease [59–64]. The range of AC volume for individuals without any neurological or psychiatric disease varied from 630 to 1380 mm^3 depending on the study [59–65]. The variability in the delineation of the AC nuclei and/or the different protocols used to process the human tissue could be responsible for the large range of AC volume reported in normal individuals in the different studies. Similar investigations performed in tissue obtained from schizophrenic and bipolar disorder patients report a decrease in the volume of several AC nuclei, such as the basal and lateral ones [63, 64].

The stereological study by García-Amado and Prensa [55] of the human AC from individuals without any neurological or psychiatric disease showed that the entire complex reached a volume of approximately 950 mm^3 with more than 80% of that volume corresponding to the basolateral group, 10% to the corticomedial group, and 6% to the central group (**Table 2**; [55]). Overall, these volumes fall within the ranges reported by other authors [59–64]. The volume of the total AC as estimated by García-Amado and Prensa [55] was smaller than the one reported earlier by Schumann and Amaral [62] due to the unclear limits of particular structures such as the nucleus of the lateral olfactory tract, the anterior amygdaloid area, the periamygdaloid cortex and the amygdalohippocampal area, some of which were not included in the AC by the former study. Nevertheless, the volume estimations for individual AC nuclei reported in these two studies were more alike except for certain minor differences in the lateral and basal nuclei. Chance et al. [61] and Berretta et al. [63] reported smaller volume estimations than García-Amado and Prensa [55], the disparities being most probably due to the difference in the methods used during tissue processing or to the variations in delineation of the nuclei (see [55] for details).

	Schumann and Amaral [62]			**Berreta et al. [63]**			**Kreczmanski et al. [64]**			**García-Amado and Prensa [55]**		
Region	**N**	**V**	**Nv**	**N**	**V**	**Nv**	**N**	**V**	**Nv**	**N**	**V**	**Nv**
L	4	452	8980	2.07	243	8598	4.5	400	11,000	5.48	376	14,608
B	3.24	343	9380	1.23	151	8173	–	220	–	5.02	259	19,510
AB	1.28	152	8600	0.59	69	8519	–	60	–	1.73	123	13,770
Co	–	–	–	0.41	44	9118	–	–	–	1.14	69	16,210
Ce	0.36	34	10,610	–	–	–	–	–	–	0.82	60	14,720
Total AC	12.21	1380	8870	–	–	–	–	–	–	15.39	956	16,088

N: number of neurons (× 10^6); V: regional volumen (mm^3); Nv: neuronal density (neurons/mm^3); –: no estimation reported. For abbreviations see **Table 1**.

Table 2. Estimations of the regional volume, and the neuronal number and density of the human AC.

4. Cellular architecture of the human AC

The AC contains three types of cell populations: neurons, glial, and endothelial cells. The morphology, number and density of the first two and the possible changes that they might present in pathologies such as schizophrenia or autism have been discussed in the last years [57, 60, 62–64, 66–68]. There are several morphological neuronal subtypes in the human AC [69]. Some 70% of neurons in the basolateral group show a pyramidal morphology and are thought to be projection neurons; the remaining 30% are interneurons. Cell morphology in the central and medial nuclei is quite variable, but pyramidal cells are not as common as in the basolateral group [70].

Data about the glial cells in the AC are scarce and mostly centered on astrocytes [70, 71]. Certain glial cell populations, such as oligodendrocytes, undergo quantitative changes in major depressive disorder [66, 67]. Further investigations are needed to determine whether other glial cell types (i.e. astrocytes and/or microglia) are involved in this and other psychiatric disorders [72]. The available data on endothelial cells are also very limited and mostly centered on determining the effects on microvasculature that are produced by the antipsychotic treatments or the schizophrenia [73, 74].

4.1. Number and density of neurons, glial, and endothelial cells

The human AC contains approximately 15 million neurons of which 80% are located inside the basolateral group, 10% in the corticomedial group and 5% in the central group [55]. The number of endothelial cells is quite similar to that of neurons, whereas the glial cell number is almost four times higher, and this proportion is maintained in most of the nuclear subdivisions of the AC. This glial/neuron ratio differs from that found in the cerebral cortex, where it ranges from 1.55 to 2.19 depending on the cortical area examined [75], or the one in subcortical structures, which varies from 14 in the mediodorsal thalamic nucleus and ventral pallidum to 3 in the nucleus accumbens [60]. The glial/neuron ratio in the AC increases across species, from rat to human [76], suggesting that the AC is more complex in primates than in rodents.

In terms of neuron number and density in the AC, the estimations of García-Amado and Prensa [55] (**Figure 1**) are comparable with, although slightly higher than, those reported in other studies performed in the lateral, basal, accessory basal, and central nuclei [62–64, 66]. Differences in neuron number between studies are due to variations in the delineation of the nuclei whereas differences in neuron density between studies might be the result of different nuclear volume estimations. Particular attention should be given to the work of Dall'Oglio et al. [70], which reported a neuronal and glial cell density in the medial nucleus that was almost 10 times higher than the one reported by García-Amado and Prensa [55]; this huge difference is probably explained by technical differences in the method used during tissue processing [77] and/or to a different delimitation of the nucleus.

Regarding neuronal density, the differences among the AC nuclei described in the various studies are consistent [55, 62–64, 66]. The neuronal density in the basal nucleus is considerably higher than that in the rest of the AC nuclei, and this is probably due to the extremely high neuronal density of its parvocellular subdivision [78–80]. The number of neurons in the different AC nuclei and nuclear subdivisions in several non-human primate species was analyzed by Carlo et al. [81]. Despite the fact that the values reported by these authors are markedly lower than those reported in the human brain by García-Amado and Prensa [55], the percentages of neurons between nuclei and their subdivisions are roughly similar. Consequently, what Carlo and his colleagues found indicates an increase in the number of neurons in every nucleus of the AC during the evolution of primate species. The percentage of increase in the central nucleus was markedly less than in the rest of AC nuclei.

The reported number and density of glial cells in the AC are consistent in the various studies [65, 66]. The high density of glial cells in the lateral nucleus may be related to the numerous projections from sensory associative cortical structures [82].

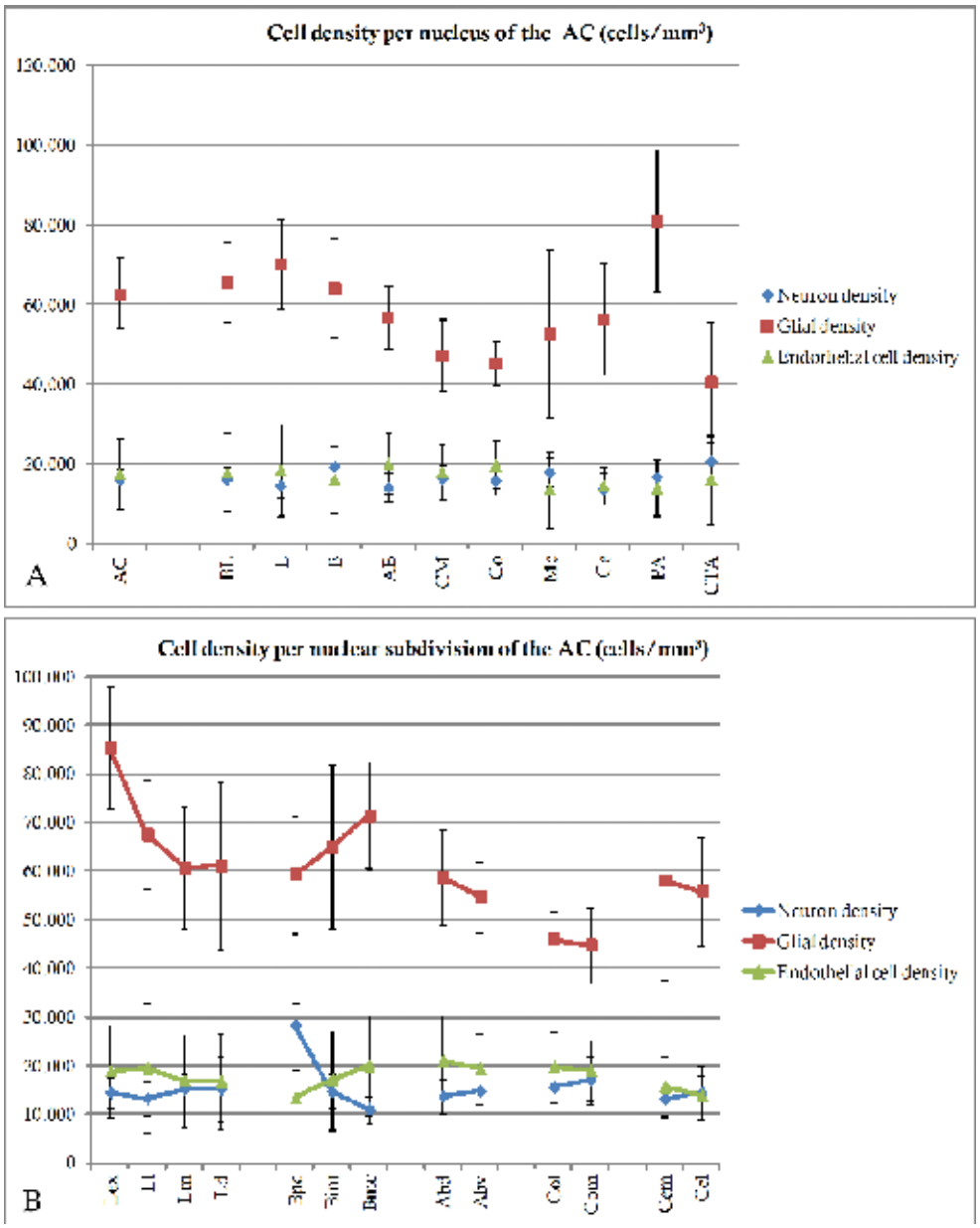

Figure 1. Density of neurons, glial and endothelial cells (cells/mm^3) in the human AC. (A) Mean and standard deviation of the density of neurons (rhombus), glia (squares) and endothelial cells (triangles) in the whole AC and their nuclear groups and nuclei. (B) Mean and standard deviation of the density of neurons (rhombus), glia (squares) and endothelial cells (triangles) in the nuclear subdivisions of the AC. For abbreviations see **Table 1**.

The density of neurons and endothelial cells in the AC tends to respectively decrease or increase with age, especially in the basolateral group [55]. In contrast, the number and density of glial cells in the grey matter of AC nuclei tend to increase moderately with age, an observation that could be interpreted as either a compensatory mechanism or a response by the glial cell

population to the neuronal loss occurring during aging. Both gliosis and fiber loss have been described as stages of age-dependent degeneration [83]. The parvocellular subdivision of the basal nucleus is the only AC region that did not show a decrease in the number of neurons over time [55]. The presence of a large number of immature neurons in the paralaminar territory of the basal nucleus in the adult brain might counteract the decrease in neuron number with aging [79, 80]. The increase in the number and density of the AC endothelial cells during aging is considered an adaptation of the brain to maintain the rate of oxygen delivery to this region when the blood flow decreases [84, 85].

4.2. Interneurons in the human AC

Various histochemically and electrophysiologically well-characterized subsets of interneurons exist in the AC (for review see Ref. [86]). Each of these subsets of AC interneurons is characterized by specific firing patterns, by their targets in discrete subcellular domains of projection neurons, and by their specific modulation by external sensory stimuli [87, 88]. From the functional view point, the AC interneurons exert an important inhibitory effect over the projection neurons of the basolateral nucleus and contribute to generating synchronous theta activity between the amygdala and the hippocampus during the acquisition of emotional memories [87]. The interneurons of the basolateral amygdala are activated by the hippocampal input with theta frequencies that reach the amygdala; this activation causes a transient feedforward inhibition of projection neurons that is followed by the increase of active excitatory synapses and the induction of long-term potentiation of these synapses during fear memory retrieval [89]. Alterations in the expression of calcium-binding proteins in some interneuron subsets of the AC [90] or of the cerebral cortex [91–95] have been described in disorders like anxiety in which the extinction of fear memories can be impaired.

As in rodents, four different subsets of interneurons have been determined in primates: (1) parvalbumin (PV) positive (+) interneurons (25% of these also contain calbindin (CB) [86, 96]; (2) CB+ interneurons (30–35% also contain PV) [86, 97, 98]; (3) somatostatin + interneurons [86, 99, 100]; and (4) calretinin (CR) + interneurons [86, 101]. Most of these data were obtained in non-human primates and the studies performed in humans did not precisely define either the number of AC interneuron subsets or the quantities and percentages of each interneuron population in the various AC nuclei. Nevertheless, PV+ and CR+ interneurons are the most abundant non-overlapping populations among all the calcium-binding protein-containing interneuron populations in the primate AC [86]. Furthermore, these two neurochemically well-defined interneuron populations are also distinguished by their electrophysiological properties; the PV+ interneurons are associated with "fast" and "burst" firing patterns, whereas the CR+ interneurons show a "regular" firing pattern [102]. PV+ interneurons can innervate the soma, proximal dendrites or the initial axon segment of pyramidal neurons [87, 88], and they receive excitatory inputs from axon collaterals of local pyramidal cells, which form a powerful inhibitory feedback [103].

In terms of their topographic distribution within the AC, the PV+ interneurons are restricted to the basolateral group, whereas CR+, as well as CB+, interneurons are homogeneously distributed through the AC. This means that PV+, CR+ and the CB+ subsets of interneurons exist in the basolateral group, but only CR+ and CB+ subsets of interneurons are present in the corticomedial and the central nuclear groups. CR+ interneurons are especially abundant

in the accessory basal nucleus, whereas the PV+ cells abound in the lateral nucleus and gradually diminish toward the more medial regions of the basolateral group [58, 96, 101, 104, 105].

Quantitative data regarding the relative proportion of the PV+ and the CR+ interneuron subtypes with respect to both the total interneuron population and the total neurons in the AC in rodents, primates and humans are already available in the literature. In the basolateral group, the PV+ interneurons represent 19–43% and the CR+ interneurons 17–20% of the total of all interneurons in rodents [106]. In the non-human primate basolateral group, the PV+ interneurons represent 28–37% of the GABAergic interneurons, while CR+ interneurons represent 23–27% [86]. In rats, PV+ interneurons make up 6% of the total AC neuron population whereas CR+ interneurons are 4% [26]. In the human AC, however, the proportion of PV+ interneurons is lower than that of CR+ interneurons with respect to total AC neurons; PV+ interneurons do not reach 1% in any AC territory whereas CR+ interneurons range from 4 to 23% depending on the AC area studied [105]. Taken together, these data show that the amount of PV+ and CR+ interneurons in the AC decreases and increases, respectively, over the phylogenetic scale, a finding that is in agreement with previous reports made in the striatum, in comparisons of humans with the squirrel monkey and the rat [107].

5. The dopaminergic innervation of the human AC

The AC receives a substantial dopaminergic innervation originating mainly from the A8, A9 and A10 ventral mesencephalic groups [29] and dopamine is a key neurotransmitter in the AC that modulates the entry of information through the basolateral group. Furthermore, this dopaminergic innervation is required for the acquisition, consolidation and extinction of fear memories as well as for generating appropriate affective responses [31–36] and, as mentioned earlier, dysfunctions of this dopaminergic system have been proposed as pathogenic mechanisms in psychiatric diseases such as schizophrenia [37, 38] and stress-related disorders [39, 40]. Accurate quantitative data regarding the amount of dopaminergic axons and their distribution in the AC from human donors who had not been diagnosed with neurological or psychiatric diseases before their death was collected by García-Amado and Prensa [108] using DAT immunoreactivity as a marker for the dopaminergic fibers and stereological approaches. Since intrinsic dopamine instability prevents its immunodetection in brain tissue that has not been rapidly fixed by perfusion after the donor's death, previous studies that were focused on analyzing the dopaminergic innervation of the human AC had used the TH protein to detect dopaminergic profiles. However, TH protein also labels noradrenergic and adrenergic fibers in the AC [109, 110]. Since the AC consists of several nuclear groups with a vast array of interconnections with the cerebral cortex, hippocampal formation, basal ganglia, thalamus, hypothalamus, and brainstem (for review see Refs. [48, 50]), information on the content of dopaminergic axons in each of the nuclear groups is needed to better understand the internal functional organization of this complex.

The human AC is targeted by widespread DAT-positive fibers, which are dense and unevenly distributed in every subdivision of this nuclear complex [108] (**Figures 2** and **3**). Furthermore, their study has yielded accurate information regarding the quantity of DAT-ir fibers per neuron in each amygdaloid territory. As shown by these authors, the amount of DAT-ir axons in

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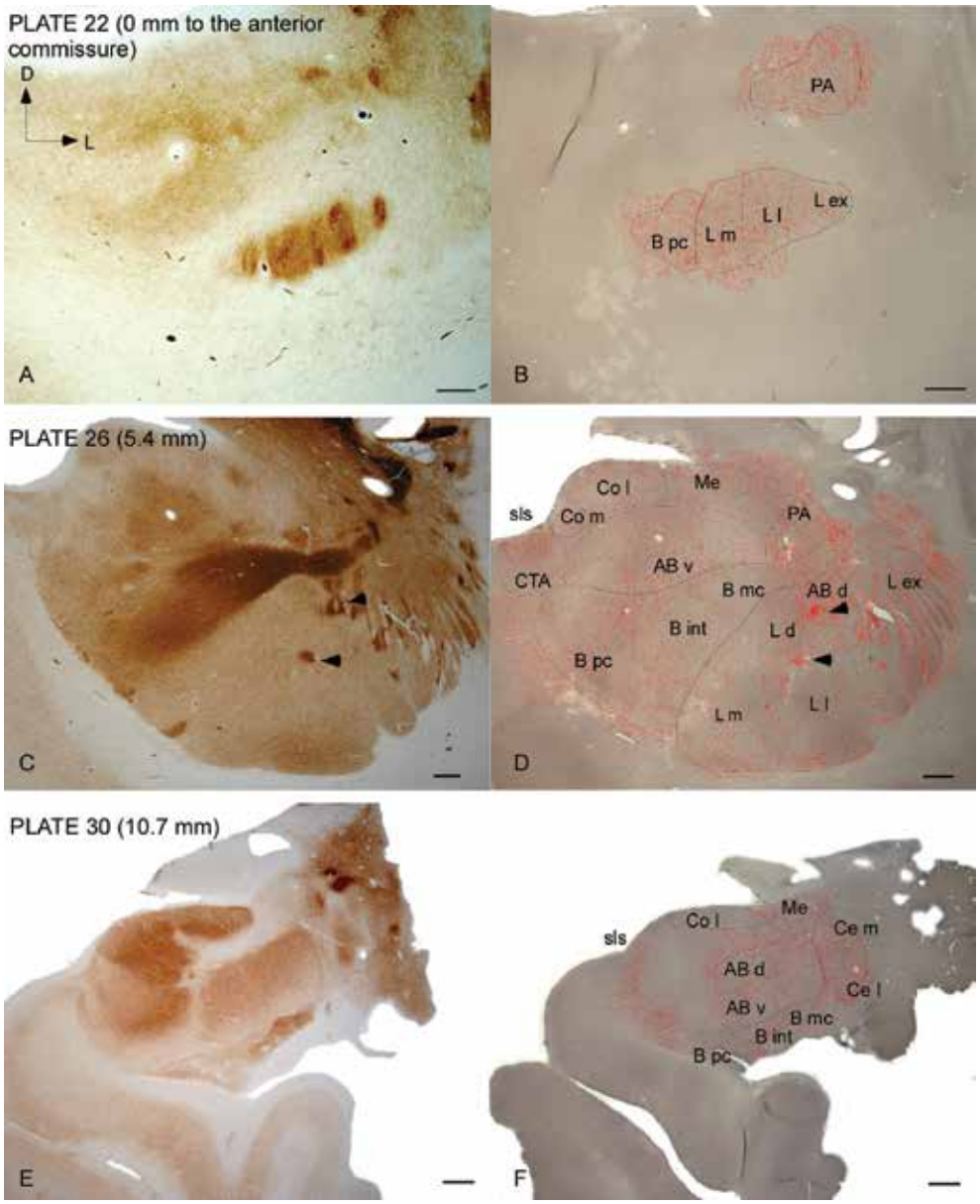

Figure 2. Distribution of DAT-positive fibers in the human AC. Series of two adjacent coronal sections stained for acetylcholinesterase (AChE) (A, C, E) and DAT (B, D, F) at three anteroposterior levels of the AC, with the corresponding plates from Ref. [119]. The stippling in B, D and F represents the DAT-positive axons drawn with camera lucida at 20× and superimposed over the same micrographs stained for DAT. Arrowheads in C and D indicate patches with either AChE or DAT enriched staining, respectively. For abbreviations see **Table 1**. Scale bar: 1 mm.

the human AC varies among the several nuclei of the AC and also varies considerably in the various subdivisions of a given AC nucleus (**Figure 2**), indicating functional variations among these territories.

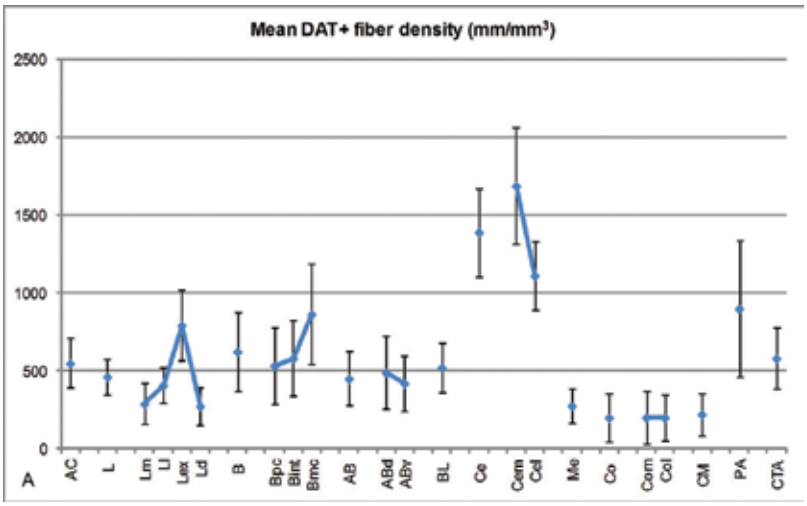

Figure 3. Length density of DAT-positive fibers in the human AC. Mean DAT-positive fiber length density for every nuclear group, nucleus and nuclear subdivision of the AC. The error bars represent standard deviation. For abbreviations see **Table 1**. Modified from García-Amado and Prensa [108].

One of the most striking gradients in the amount of the DAT-ir fibers occurred along the mediolateral axis of the lateral nucleus: the total length of DAT-ir axons range from nearly 300 mm/mm^3 in its medial subdivision to nearly 800 mm/mm^3 at its external (most lateral) subdivision (**Figures 2** and **3**). This large variation in the amount of DAT-ir fibers between the medial and lateral sectors of the lateral nucleus might be related to their differentiated extrinsic and intrinsic connections. Thus, the lateral nucleus would be the main target of sensory information from the external world, and it sends heavy projections to the other amygdaloid nuclei [111]. The external subdivision of the lateral nucleus receives most of these sensory projections (**Figures 2** and **3**), and the information flows toward the medial side of the nucleus [111, 112]; in addition, this AC region has the shortest latency of conditioned responses elicited by sensory stimuli associated with adverse events in emotional learning tasks [113]. On the other hand, the medial subdivision of the lateral nucleus receives information from higher-order cortical processing areas [114–117]. In the hippocampus, DAT-positive axons were present only in the outer two-thirds of the molecular layer of the dentate gyrus, where the perforant pathway ends [118], indicating that dopamine may potently and selectively regulate the input from the entorhinal cortex and thus the early stages of hippocampus processing, as might be the case for the sensory information entering the AC lateral nucleus.

The central nucleus receives information from the rest of the AC nuclei and is one of the main output nuclei of the AC [111]. Descending projections from the central nucleus terminate in a wide mediolateral region of mesencephalic dopamine cells [120]. In turn, this nucleus receives the heaviest DAT-positive dopaminergic innervation of all the AC nuclei, however its innervation is not uniformly distributed and markedly decreases along a mediolateral gradient, a finding that agrees with the distribution pattern of TH-ir fibers [27]. In the basal and accessory basal nuclei of the AC, the content in DAT-ir fibers decreases from dorsal to ventral

sectors, though this gradient is much less marked in the latter than in the former nucleus (see **Figure 2A** and **B**) [27–29, 49, 58, 121].

The regulation of extracellular dopamine levels is controlled by distinct mechanisms in different brain areas and is probably related to DAT content. Thus, whereas the dorsal striatum and the nucleus accumbens show an "uptake-dominated" regulation (i.e. one in which dopamine is quickly recaptured from the extracellular space to end its action), the medial prefrontal cortex and the AC show a "release-dominated" regulation (i.e. dopamine is maintained in the extracellular space more time) [122]; these findings agree with the observation that there is more DAT in the striatum than in the other two structures [27–29, 49, 58, 121].

The AC is a main target for mesencephalon projections made up of cells from the substantia nigra pars compacta (A9 dopaminergic group), the ventral tegmental area (A10 dopaminergic group) and the retrorubral field (A8 dopaminergic group) [4, 123–125]. In the human mesencephalon, DAT abounds in neurons located in the lateral ventral tegmental area and in the substantia nigra pars compacta and is largely absent from the medial ventral tegmental area [30]. DAT mRNA is more abundant in the A9 ventral tier than in the dorsal tier [125]. The human AC nuclei that contain the most DAT-ir fibers correspond to those that receive strong projections from the ventral mesencephalon, as also observed in primates [29]. There are, nevertheless, other AC regions showing a high density of DAT-positive fibers, such as the lateral subdivision of the central nucleus, that do not seem to receive innervation from any part of the ventral midbrain [29]. There are other possible sources of AC dopamine that lie outside the ventral midbrain, but whether they contribute to the DAT-ir fibers encountered in the AC or not, is not yet clear. The parabrachial nucleus projects to the central and medial nuclei of the AC [29, 123, 124] and it contains putatively dopaminergic neurons that do not carry DAT [126]. Moreover, the neurons of the parabrachial nucleus that project to the AC also lack tyrosine hydroxylase (TH) [29, 123, 124]. The periaqueductal gray substance is another source of input to the AC and it contains dopaminergic neurons (i.e. A11 group) that contain DAT [126] and project to the central and medial AC nuclei [4, 123, 124, 127–129]. This dopaminergic connection is relevant as it specifically targets the lateral subdivision of the central nucleus, a region that sends efferent projections to the medial subdivision of the central nucleus, which in turn projects back to the periaqueductal gray substance handling "*freezing*" behavior in animals exposed to a potentially dangerous stimulus [12]. There are also TH+ cells in the dorsal raphe nucleus that project to the central AC nucleus [129], but the DAT content of these cells has not been yet determined.

The ultrastructural localization of DAT in the primate AC is unknown at present. In the cerebral cortex, most of the DAT-labeled profiles correspond to thin unmyelinated axons that rarely form synapses, whereas TH-labeled profiles vary more in their diameter and TH-ir varicosities contain abundant vesicles and frequently form synapses [118]. Consequently, Lewis et al. believe that DAT is likely to be restricted to the intervaricose segments [118]. The specific postsynaptic targets of the dopaminergic fibers that reach the human AC are not known. Several studies in rodents have demonstrated that these fibers make synapses with both projection neurons [23, 24, 130] and interneurons [25, 26, 130]. Although projection neurons receive the majority of dopaminergic synapses [130], the CR+ and PV+ interneuron

subsets are also innervated by these fibers, especially the ones containing PV [26]. The CR+ interneurons receive only 6% of the dopaminergic synapses, whereas the PV+ cells receive 40% [26]. In the central and basal nuclei, as well as in the paracapsular intercalated groups, the dopaminergic terminals form symmetric synapses more frequently than asymmetric ones [23, 24, 26, 130].

Dopaminergic fibers in the AC form perineuronal nets around the soma of the projection neurons and the PV+ interneurons, and 72% of the contacts that these nets establish with the PV+ interneurons are synaptic [25, 26, 130]. These nets are abundant in some 10–15% of all PV+ interneurons and they appear to avoid other interneuron subsets. These nets are functionally related with the strong inhibition observed in the activity of the projection neurons of the basolateral group after dopamine release [131, 132]. The dopaminergic innervation of the various interneuron populations of the AC could contribute to the induction of long-term potentiation mechanisms involved in conditioned fear acquisition, which requires suppression of GABAergic interneuron inhibition of projection neurons [35]. Dopamine inhibits the "fast firing" interneurons, which coincide with the PV+ interneurons [102], and reduces the inhibition of projection neurons in the lateral amygdaloid nucleus. More recently, Chu et al. have demonstrated that dopamine blocks GABA release from PV+ interneurons to projection neurons acting on type D2 presynaptic receptors but it does not affect the release of GABA to other interneuron types from this interneuron population [133]. The blockade of both D1 and D2 receptors in the basolateral group prevents fear conditioned acquisition [134–136].

6. Concluding remarks

The AC is a heterogeneous structure formed by numerous nuclei with diverse morphological and functional features. Numerous neurological or psychiatric diseases are linked to alterations in specific cell populations, as well as neurotransmission systems in the human AC. Understanding how AC dysfunction may be related to the pathogenesis of human disorders or accompany behavioral impairments requires a profound knowledge of the normal anatomy of the human AC. In this sense, several studies performed in the last 5 years have provided accurate quantitative data related to the cellular composition and dopaminergic innervation of the various nuclear complexes and their subdivisions that make up the human amygdala. Data from these studies have revealed, for instance, that the human AC contains approximately 15 million neurons with nearly 80% residing in the basolateral group, 10% in the corticomedial group and 5% in the central group. The number of endothelial cells is similar to the number of neurons whereas the number of glia is approximately four times that of neurons. Most amygdaloid neurons are glutamatergic or GABAergic neurons that project their axons outside the AC. The activity of the AC principal neurons is tightly modulated by local circuit interneurons and this modulation is required for the acquisition of fear memories. The AC interneurons are cataloged into different subsets based on their firing properties, their synaptic inputs and their expression in proteins such as calcium-binding proteins. Among all the interneurons containing calcium-binding proteins in the primate AC, the PV+ and the CR+ interneurons are the most abundant, representing 1% and 6–24% of the total neuron

population of the human AC, respectively. PV+ interneurons exert robust perisomatic inhibition of principal neurons, but their activity is likely to be mostly concentrated in the basolateral complex, since almost none of these neurons populate other AC territories. In contrast, the CR+ interneurons are, basically, homogeneously distributed through the entire AC. The human AC receives a heterogeneous dopaminergic innervation that mostly originates in the midbrain areas and regulates the activity of the various subsets of AC neurons. Dysfunctions of this dopaminergic system have been described in schizophrenia and stress-related disorders. Stressful events enhance dopamine release in the AC and this facilitates the formation of fear memories as well as appropriate affective responses. A recent study has demonstrated that the dopaminergic innervation of the human AC is heterogeneous and that the main output nucleus of the AC (i.e. the central nucleus) receives the highest density of dopaminergic axons containing the dopamine transporter, with almost double the density of these fibers compared to the density in the main entrance nucleus of the AC (the basolateral group). The postsynaptic targets of the dopaminergic fibers in the human AC remain unknown, but these fibers make synapses with both projection neurons and interneurons in rodents. The CR+ and the PV+ interneurons of the AC are important targets of the dopaminergic synapses in rodents, but further studies are needed to determine what the main neuronal targets of this neurotransmitter are in the human AC and the role that this innervation has in emotional learning.

Acknowledgements

The project was supported by grants from the *Fundación Eugenio Rodríguez Pascual*.

Author details

María García-Amado* and Lucía Prensa

*Address all correspondence to: maria.garciaamado@uam.es

Department of Anatomy, Histology and Neuroscience, School of Medicine, Autónoma de Madrid University, Madrid, Spain

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Chapter 13

The Key Amygdala-Hippocampal Dialogue for *Adaptive* Fear Memory

Aline Desmedt

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67582

Abstract

For centuries, philosophical and clinical studies have emphasized a fundamental dichotomy between emotion and cognition, for instance between implicit/emotional memory and explicit/representative memory. However, in the last few decades, cognitive neuroscience has highlighted data indicating that emotion and cognition are in fact in close interaction and that reciprocal amygdalar-hippocampal influences underlie their mutual regulation. While supporting this view, the present chapter discusses experimental data indicating that the hippocampal and amygdalar systems not only regulate each other and their functional outcomes but also qualify specific emotional memory representations through specific activations and interactions. Specifically, we review consistent data unveiling a direct contribution of both the amygdala and septo-hippocampal system to the identification of the predictor of a threat in different situations of fear conditioning. Our suggestion is that these two brain systems and their interplay determine the selection of relevant emotional stimuli, thereby contributing to the adaptive value of emotional memory. Hence, beyond the mutual *quantitative* regulation of these two brain systems described so far, we propose that different configurations of the hippocampal-amygdalar network qualitatively impact the formation of memory representations, thereby producing either adaptive or maladaptive (e.g., PTSD-like) fear memories.

Keywords: memory representation, cognition, emotion, amygdala, hippocampus

1. From dissociation to interaction of emotion and cognition

From the antiquity until the last 50 years, it was commonly held that emotion, and more generally, all somatic (body-related)/physiological processes disrupted cognition, seen as "pure representation" and more recently as "information processing" whose investigation was supposed to avoid all "interfering" body-related processes [1, 2]. This view was particularly

emphasized by the cognitive revolution, which was inspired by the computer metaphor and thus considered that analyses of cognition had to be expurgated of all emotional/physiological, potentially disruptive, processes. As Damasio wrote 20 years ago, we all grew up with the wide common view "that emotions and reason did not mix any more than oil and water (…) that the mechanisms of reason existed in a separate province of the mind, where emotion should not be allowed to intrude," and when thinking about the brain behind that mind, we "envisioned separate neural systems for reason and emotion" [3].

In the field of memory research, the essential dissociation between sensory/emotional memory and representation-based memory can for instance be found in Aristote (De memoria et reminiscentia, see in Ref. [4]) in the form of "simple memory" that would be directly related to sensory perception and shared by all species, *vs*. "voluntary memory retrieval" that would be unique to humans. In the XIXth century, Maine de Biran was the first philosopher who explicitly referred to different forms of memory as a function of the information processing involved. An automatic "mechanical memory" involved in the acquisition of habits and a "sensory memory" would both operate at an unconscious level and differ from a "representative memory" allowing the "conscious recollection of ideas and events" [5]. Finally, it is Henri Bergson who offered the most detailed discussion regarding this dissociation between a behavioral/procedural/implicit memory *vs*. a representative/explicit memory in his monography entitled "Matter and memory" [6]. According to Bergson, "The past survives under two distinct forms: first, in motor mechanisms; second, in independent recollections." Regarding the first, "Habit rather than memory, it acts our past experience but does not call up its image." "The other is the true memory, (…) leaving to each fact its place and consequently marking its date, truly moving in the past and not, like the first, in an ever renewed present." Strikingly, this description directly echos a distinction between nondeclarative and declarative memory systems referring to knowing how and knowing what, respectively, and proposed almost a century later by Cohen and Squire [7].

In support of this dissociation are the observations of patients with specific brain lesions who display a deficit for one type of memory leaving intact the other one. For instance, a clear double dissociation was found between fear conditioning and declarative memories, which were shown to be respectively dependent on the amygdala and the hippocampus [8]. Patients with bilateral lesion to the amygdala, an almond-shape brain structure adjacent to the hippocampus in the medial temporal lobe, could not acquire a conditioned fear response (measured as a change in skin conductance) to a conditional stimulus (CS+) previously paired with an aversive event (an unconditional stimulus, US), whereas they could acquire the explicit knowledge about the contingency between the CS+ and the aversive US. Thus, although these patients knew and could declare what they previously learned, they could not display any physiological expression of conditioned fear. In contrast, patients with selective bilateral lesion of the hippocampus showed an opposite pattern of results, that is, a normal conditioned fear response to the CS+ but no explicit knowledge of the past CS+/US association. This study first supports previous data obtained in nonhuman animals indicating a critical role for the amygdala in emotional learning [9–13]; second, it indicates that the hippocampus may underlie a factual representation of emotionally laden memory; and third, it points out the existence of two different neural substrates underlying representative and emotional/sensory memories that may not overlap.

http://dx.doi.org/10.5772/67582

This fundamental dichotomy between emotion and cognition, and more specifically between behavioral/emotional memory and explicit/representative memory, has been recurrent for several centuries both in philosophical and neuropsychological studies more recently. However, the last few decades have highlighted clinical and experimental data indicating that emotion and cognition, as well as their underlying neural networks, are in close interaction. Overall, these findings even suggest that the understanding of natural cognition requires the consideration of emotion.

First, on the basis of several consistent observations of brain-lesioned patients who combined normal intellect but profound deficit in decision making together with compromised ability to express emotion and to experience feelings, Damasio [3] developed an elegant theory supporting the idea that "reason may not be as pure as most of us think it is or wish it were, that emotions and feelings may not be intruders in the bastion of reason at all (…)," but on the contrary "indispensable for rationality." The somatic marker hypothesis he developed [3, 14] is an alternative to the pure reasoning, which cannot explain by itself the normal decision making that generally takes a few minutes or seconds. In brief, this hypothesis suggests that when confronted with a situation that requires an effective choice, biological markers, which relate to emotion-related physiological body-states or representation of them, automatically qualify or connote, positively or negatively, some particular options or images (as a function of previous experiences of such a situation). This emotional connotation leads thereby individuals to immediately reject some negative outcome-related options and thus to choose a particular action among the remaining more positive alternatives. In this view, emotions, and more generally body-related physiological mechanisms, directly contribute to normal cognition, which guides adaptive behaviors.

Second, in the specific field of memory research, a growing body of evidence consistently shows that emotion contributes to the enhancement of episodic/representative/declarative memory. Thus, patients with amygdala damage do not show the normal enhancement of episodic memory for emotionally laden events when compared to memory for neutral events [15–17]. It is classically assumed that such memory enhancement may be due to amygdala's influence on the encoding and/or consolidation of hippocampal-dependent memories [13, 18–22]. In accordance with the last assumption, manipulation of the amygdala after encoding alters memory enhancement classically observed with physiological arousal [23].

Although most of studies examining hippocampal-amygdalar interactions focused on the amygdalar influence on episodic memory, several observations indicate that hippocampal-dependent representative memory can also reciprocally influence amygdala-dependent emotional memory. For instance, in the paradigm called "instructed fear," the actual CS+/aversive US conditioned association is replaced by an explicit oral communication about the CS-US contingency. This learning thus requires the hippocampus for acquisition of an episodic representation of the emotional significance of the fearful stimulus. Interestingly, it has been shown that such paradigm results in robust physiological fear responses to the CS+ that are correlated to the activation of the left amygdala [24] and specifically dependent on this brain structure [25]. These results indicate that a hippocampus-dependent instructed representation of the emotional significance of a stimulus can lead to the amygdala-dependent expression of

conditioned fear in situations in which fear is anticipated but the aversive event never actually experienced [26]. Another example of this hippocampal influence comes from studies devoted to emotion regulation. These studies emphasize the impact of cognition on emotion by showing that reasoning and conscious strategies of reappraisal of emotional scenes can diminish both experience of fear and related amygdalar activation [27, 28].

Altogether, these cognitive neuroscience studies first indicate that contrary to what has been claimed for centuries, emotion can serve cognition, as exemplified by its critical contribution to decision making or to the enhancement and persistence of episodic memory. Second, they also reveal that, reciprocally, cognitive processes as reasoning, conscious appraisal, or explicit representation of events can modulate emotional responses, either promoting or reducing fear. Third, they emphasize the idea that reciprocal amygdalar-hippocampal influences might underlie such mutual regulation of emotion and cognition. While supporting this view, the present chapter discusses experimental data, obtained in rodents, indicating that the hippocampal and amygdalar systems not only regulate each other and their functional outcomes but also qualify specific emotional memory representations through specific activations and interactions. Hence, beyond the mutual quantitative regulation of these two brain systems described so far, we propose that different activations of the hippocampus and amygdala leading to specific neural configurations qualitatively impact the formation of emotional memory, thereby producing either adaptive or maladaptive fear memories.

2. Adaptive versus maladaptive fear memories

Classical fear conditioning is one of the most used paradigms to study emotional memory. Depending on the CS-US contingency, the predictive CS can be either the discrete (i.e., phasic) salient CS or the surrounding background context (i.e., tonic contextual cues). If the discrete CS is systematically *paired* with the aversive US, it becomes the right predictor of the US and overshadows tonic contextual cues which are thus consigned in the background. In contrast, if the discrete/salient CS is presented but explicitly unpaired with the US (pseudo-random distribution of the discrete CS and US), then, although salient, it is not predictive of the footshock. Consequently, contextual cues that are continuously present during the aversive experience become the primary stimuli that enter into association with the footshock and thus constitute the right predictor of the US [12, 29–32].

The two fear conditioning procedures described above allows the assessment of adaptive *vs*. maladaptive conditioned fear responses. Typically, animals submitted to the CS-US pairing procedure (predicting-discrete CS situation) will normally express more conditioned freezing when re-exposed to the discrete CS (e.g., tone) than animals submitted to the CS-US unpairing procedure (predicting-context situation). In contrast, when the same animals are re-exposed to the conditioning context, those previously submitted to the predicting-context situation express more conditioned freezing than animals submitted to the predicting-discrete CS situation. These results indicate that with the pairing procedure, animals normally form a preferential discrete CS-US association, identifying the tone CS as the right predictor of the threat

http://dx.doi.org/10.5772/67582

to the expense of the background context. In contrast, under the unpairing procedure, the context is identified as the right predictor, and this is attested by the expression of an adaptive high fear response to contextual cues but not to the discrete CS.

Compared to these adaptive fear responses, a significant fear response to the discrete salient CS instead of the context in a predicting-context situation or an increased fear response to the conditioning context to the expense of the discrete CS in a predicting-discrete CS situation is the behavioral expression of maladaptive fear memories. In both cases, animals take the wrong predictor of the threat.

3. The amygdala directly contributes to the formation of *adaptive* fear memory

In the traditional model describing the amygdala function in emotional learning [12], the lateral (LA) amygdaloid nucleus is required for the acquisition of discrete CS-US associations, while the basolateral (BLA) nucleus, which receives projections from the hippocampus [33], is thought to mediate context–US association. Using reversible inactivation of either LA or BLA, one of our studies first confirmed the critical role of the LA in the formation of discrete CS-US association and also revealed that the BLA is specifically required for conditioning to contextual cues but not to a discrete tone CS [34]. These data, together with consistent electrophysiological findings showing that thalamic but not polymodal cortical stimulation induces LTP in LA, whereas polymodal cortical but not thalamic stimulation induces LTP in BLA strongly suggest that LA activation is a sensory interface underlying simple, unimodal CS/US association, whereas the BLA may serve as an amygdaloid sensory interface for more complex information, thus underlying associations between the US and configural/contextual cues. As this complexity dimension is observed both for input and output pathways of the amygdala, it would constitute a common organizing factor in the functional anatomy of this brain structure [35]. Second, our study also revealed that the LA enhances or reduces BLA-mediated conditioning to the context depending on whether the context (CS-US unpairing) or the discrete CS (CS-US pairing) best predicts the footshock US. Thus, refining the classical model of amygdala function in fear learning, these findings indicate that depending on the CS-US contingency, LA-BLA interaction (through a competing or synergistic mode) promotes the selection of the best predictor of the aversive US, leading thereby to adaptive fear responses [34].

Now, to what extent hippocampal-dependent representative memory can be dissociated from amygdala-based emotional memory? Several studies from our laboratory obtained in mice provide evidence for a dissociation between the behavioral expression (freezing behavior) of fear conditioning and hippocampal neurophysiological coding (changes in hippocampal–septal synaptic transmission) involved in this emotional learning [29, 31]. In one of these studies, we showed that while lesions of the amygdala dramatically reduced the behavioral expression of fear conditioning, they did not prevent the hippocampal neurophysiological changes associated with different types of fear conditioning previously experienced but alter their direction. Thus, despite the absence of conditioned freezing, different changes in hippocam-

pal–septal excitability were specifically associated with a predicting-context and predicting-discrete CS learning situation [29]. Our suggestion is that these neurophysiological changes contribute to a form of knowledge about the conditioning situation encountered by subject the day before that would be dissociated from the behavioral expression of this aversive experience. Nevertheless, while sparing specific hippocampal neurophysiological changes as a function of the type of learning, amygdala lesions also clearly interfered with these synaptic changes, as they produced opposite changes with respect to nonlesioned controls. Strikingly, this observation strongly suggests that the amygdala-dependent and hippocampal-dependent forms of knowledge involved are not independent but interactive. Specifically, the content of hippocampal-dependent representations about relations among various sensory exteroceptive stimuli [36, 37] might be altered if fear conditioning is prevented by amygdala lesions.

In another series of studies, it was shown that the amygdala can indeed modulate synaptic plasticity in the hippocampus [38, 39]. Priming the amygdala just prior to an attempt to induce long-term potentiation (LTP) in the hippocampus (e.g., by stimulating the BLA 1 hour before high-frequency stimulation to the perforant path) modulates the level of plasticity in a region-specific manner [40–42], much like the effects of exposure to stress [38, 43]. It was later found that this modulation was specifically mediated by the basal amygdala [44]. Furthermore, stress and amygdala modulation of hippocampal plasticity were shown to be dependent on the exact way the amygdala was activated [45, 46], indicating that such modulation involves a dynamic interaction between these two structures, as predicted before [47].

Hence, from an initial emphasis on the dissociation between representative memory and emotional/behavioral memory, several findings come to indicate that the underlying neural systems of these two types of memory are interactive. It turns out that the hippocampal system is modulated by the amygdala, and that such modulation is even dependent on the type of learning situation [29]. This last observation led us to propose that the amygdalar influence of the hippocampal system might contribute to the selection of relevant emotional information and thus to the formation of adaptive fear memory.

4. The septo-hippocampal system is critically required for adaptive fear memory

Beyond the classical “restricted” role of the hippocampus in fear memory: a direct contribution to the selection of predictor of threat.

Lesion and electrophysiological studies indicate that the hippocampus is involved in relational memory. This theory postulates that the hippocampus is engaged in the encoding of events, episodes as sequences of events, and importantly, in linking these episodes by common features into relational representations in declarative memory. These hippocampus-dependent relational representations are required for the subject to compare different events and contribute to memory flexibility [48–50].

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On these bases, studies dedicated to identify the role of the hippocampus in fear memory, and more specifically in fear conditioning, overall indicate a requirement of this brain structure in contextual fear conditioning [51, 52]. Indeed, lesion of the hippocampus was shown to disrupt conditioned fear responses to the context in which fear conditioning occurs. The current neurobiological model of fear conditioning postulates that the hippocampus is required for forming an integrated representation of contextual cues. Then, this representation is subsequently associated with the US within the amygdala. Experiments from our laboratory contributed to confirm the current view of the role of the hippocampus in contextual conditioning. Nevertheless, they also unveiled a more extended role for the hippocampus in fear conditioning. Indeed, our data repeatedly and consistently showed that the hippocampus is differentially engaged in fear conditioning depending on the predictive value of CSs and contributes to the selection of the right predictor of threat [29, 30, 34, 53–55]. As written above, subjects adaptively select among environmental stimuli those that best predict an aversive event, either a discrete CS when it is paired with, and thus predictive of the aversive US or the context when a CS-US unpairing procedure is used.

Comparing these two conditioning procedures (CS-US pairing *vs*. unpairing), one of our studies revealed that a long-lasting strengthening of CA1 synaptic efficacy is specifically observed when the context, but not the discrete CS, is the right predictor of the US [56]. At the molecular level, a biphasic pattern of ERK1/2 CREB activation in the CA1 is specifically associated with a predicting-context situation, whereas only early monophasic activation is observed in a predicting-discrete CS situation [56]. Moreover, blockade of ERK1/2 activation with intra-hippocampal infusion of MEK inhibitor severely disrupted conditioned fear to the context, when the context, but not the discrete CS, was the right predictor of the US [56]. Altogether, these findings show that the hippocampus is differentially engaged in fear conditioning depending on the relative predictive value of the discrete CS *vs.* context for the occurrence of an aversive event. Specifically, they also indicate that hippocampal activation is critically required for contextual conditioning when the context, but not the discrete CS, is the right predictor of a threat.

In another set of experiments, we found that changes in the hippocampal-septal glutamatergic neurotransmission directly contribute to the identification of either the discrete CS or the context as predictor of the footshock US. First, a decrease in the hippocampal-lateral septal (HPC-LS) synaptic transmission was found to be associated with a predicting-context situation, whereas no change or even an increase in this neurotransmission was observed in predicting-discrete CS situations [29, 31, 57]. On the basis of these data, we have assessed the behavioral consequences of pharmacological manipulation of this glutamatergic neurotransmission. In accordance with previous electrophysiological data, we showed that pretraining infusion of glutamic acid into the lateral septum, which mimics an increase in the HPC-LS neurotransmission, promotes the selection of a discrete tone CS to the detriment of the context as predictor of a footshock US. In contrast, infusion of glutamatergic antagonist (Kynurenate), which inhibits this neurotransmission, promotes the selection of the context as major predictor of the US, while blocking the identification of the discrete tone CS as predictor [55]. In full accordance with these data, we have previously shown that pretraining infusion of arginine vasopressine (AVP) or its antagonist into the lateral septum, which is known to increase and

decrease the HPC-LS glutamatergic neurotransmission, respectively, could also promote the selection of the discrete tone CS or the context, respectively, as predictor of the US [30].

Altogether, these findings indicate that the role of the hippocampal system is not restricted to the processing of contextual information in fear conditioning. Beyond this classic role, consistent data unveil a direct contribution of the hippocampal-septal system to the acquisition of adaptive fear responses as a function of the learning situation. Our suggestion is that this system plays a key role in the selection of relevant stimuli, which are the right predictors of threat (i.e., discrete CS *vs*. contextual cues), thereby contributing to the adaptive value of emotional memory.

How can changes in the HPC-LS glutamatergic neurotransmission favor the selection of either a discrete tone CS or contextual cues as predictor of a threat? Previous experiments have emphasized the functional importance of changes in HPC-LS excitability for feedback regulation of hippocampal activity [58]. Based on these findings, we postulate that glutamatergic receptors localized into the LS would exert, *via* an increase in GABAergic cells excitability, an inhibitory effect on cholinergic cells in the medial septum [59, 60], which projects to the hippocampus. This means that in a predicting-discrete CS situation (CS-US pairing), the observed increase in HPC-LS glutamatergic neurotransmission would result in a GABA-mediated decrease in the activity of cholinergic neurons in the medial septum. This negative feedback would thus down regulate the hippocampal cholinergic activity, reducing thereby the hippocampal-dependent processing of contextual cues to the benefit of the discrete CS. By an opposite mechanism, in a predicting-context situation (CS-US unpairing), the observed decrease in the HPC-LS glutamatergic neurotransmission would result in a stronger hippocampal cholinergic activity, thereby enhancing the hippocampal-dependent processing of contextual cues, which would finally be selected as predictor of the footshock US to the detriment of the discrete CS [30].

The hippocampal processing is thought to be powerfully modulated by cholinergic projections originating in the medial septum/vertical limb nucleus of the diagonal band of Broca (MS/VDB). The hippocampal cholinergic neurotransmission has been previously involved in various CSs processing [61–66] and is thought to play a critical role in the coordination between different memory systems leading to the selection of appropriate behavioral strategies [67–69]. Specifically, increasing cholinergic activity in the hippocampus increases the selection of a place strategy to the detriment of cue-based strategy [70, 71]. We thus reasoned that changes in this cholinergic neurotransmission might also contribute to the adaptive selection of amygdala-mediated CS-US or context-US associations as a function of the type of procedure used. First, we showed that the magnitude of the hippocampal cholinergic release is dependent on the conditioning procedure used. In a predicting-context situation, that is, when the context is the right predictor of the US, Acetylcholine (Ach) release is stronger than in a predicting-discrete tone CS situation. Second, increasing the hippocampal cholinergic transmission with pretraining intra-hippocampal infusion of physostigmine (acetylcholinesterase inhibitor) results in the selection of the context as best predictor of US occurrence at the expense of the discrete tone CS. Conversely, decreasing the hippocampal cholinergic transmission with intra-hippocampal infusion of muscarinic antagonist scopolamine results in the selection of the tone CS instead of the context as main predictor

of the US, thus mimicking tone fear conditioning to the detriment of contextual conditioning. These results demonstrate that level of hippocampal cholinergic transmission determines the selection among the context and the discrete CS that one that best predicts the aversive US [53]. Interestingly, the pharmacologically induced increase (physostigmine) and decrease (scopolamine) in hippocampal cholinergic neurotransmission is respectively associated, at the molecular level, with increase and decrease in ERK1/2 activation in both CA1 and Dentate Gyrus. Although previous studies have suggested that decreasing hippocampal cholinergic neurotransmission may prevent animals from forming an integrated representation of the context Gale [62], our findings reveal a more extended role for this neurotransmission in fear conditioning. It turns out that hippocampal cholinergic neurotransmission regulates the hippocampal recruitment in fear conditioning as a function of the procedure used (CS-US pairing *vs*. unpairing), thereby contributing to the selection of relevant emotional information (predicting- discrete CS or context) and thus to the expression of adaptive fear responses [53].

Overall, our findings have led us to propose an additional function of the septo-hippocampal system in fear conditioning. Beyond to the classical role of this system [49], in the processing of CSs as configural representation, we assume that the hippocampal engagement would result in a relational representation of the learning situation including the relative predictive value of all CSs (discrete CS and tonic context). When the context is the main predictor of the occurrence of an aversive event, the recruitment of the septo-hippocampal system would be stronger when a discrete cue best predicts the occurrence of the aversive US. Importantly, this differential engagement of the hippocampal system may causally contribute to the attribution process of different predictive values to the various emotionally laden CSs (i.e., phasic/discrete CS *vs*. tonic context).

5. A hippocampal-amygdalar dialogue *qualifies* emotional memory representation

The current neurobiological model of fear conditioning postulates that the only way the hippocampus can influence the amygdalar function is by conveying the representation of the context where conditioning has occurred to the amygdala. Then, this hippocampus-dependent representation of the context is supposed to be associated with the US in the amygdala. According to this model, the hippocampus has almost no critical role in conditioning to a discrete tone CS, while the amygdala is critically required for forming the discrete CS-US association.

According to our proposition, the hippocampus is engaged in the processing of a relational representation of CSs and directly contributes to the formation of adaptive fear memories. It would thus be involved in both auditory and contextual fear conditioning. Its differential engagement as a function of the conditioning procedure would causally contribute to the selection of one or another CS as predictor of the threat. As it was previously shown that the amygdala also directly contributes to adaptive fear memory, our findings suggest a more complex

relationship between the hippocampus and the amygdala in fear conditioning. In order to specify the hippocampal-amygdalar relationship, we reasoned that because changes in the hippocampal cholinergic neurotransmission could determine the selection of either the discrete CS or the context as predictor of an aversive US, these changes should also qualitatively constrain the amygdalar activation. As described above, decreasing the hippocampal cholinergic transmission by intra-hippocampal infusions of scopolamine prior to fear conditioning promotes the selection of the discrete tone CS as predictor of the US at the expense of the context. As expected, our study further showed that this hippocampal manipulation not only *mimics* tone fear conditioning but also produces a pattern of phosphorylated-ERK (p-ERK1/2) expression within the lateral (LA) and basolateral (BLA) amygdala similar to the one observed in control mice for which the discrete tone CS is objectively the right predictor. Conversely, increasing the hippocampal cholinergic transmission with intra-hippocampal infusion of physostigmine results in the selection of the context as predictor of US. In that case, and in a very consistent manner, physostigmine infusion produces a pattern of p-ERK1/2 expression in the LA/BLA similar to that one observed in mice for which the context objectively best predicts the US [53]. These findings reveal that the hippocampal cholinergic neurotransmission constrains the amygdala function: depending on its level, it produces two different patterns of amygdalar activation, which specifically underlie either context or discrete CS-US association, thereby leading to predominant conditioned fear responses either to the context or to the discrete CS. Hence, depending on the objective predictive value of the CSs for the occurrence of an aversive event, changes in the hippocampal cholinergic neurotransmission determine the relative engagement of the LA and BLA nuclei in bringing about the adaptive selection of either the discrete CS or the context as valid predictor. These findings are in accordance with the view supporting a role for Ach in regulating the relative contribution of different neural systems for learning [67, 68, 72]. These findings are also congruent with previous clinical studies indicating that having a hippocampal dependent episodic representation of the predictive value of CSs influences amygdala-based memory [24, 27, 28].

Altogether, these findings have led us to propose a modified version of the neurobiological model of fear conditioning. When the discrete tone CS is the main predictor of US, an increased HPC-LS glutamatergic neurotransmission would result in a GABA-mediated decrease in the activity of cholinergic neurons located in the medial septum, which projects to the hippocampus. Under this predicting-discrete CS situation, the low hippocampal cholinergic activity constrains the LA/BLA functioning in such a way (competition) that the discrete CS, and not the context, is ultimately selected as valid predictor. Conversely, when the context is the best predictor, a decrease in the glutamatergic hippocampal-septal neurotransmission would result in an increased release of acetylcholine in the hippocampus. This high level of Ach release contributes to a synergistic functioning of LA and BLA nuclei necessary for the selection of the context as the main predictor of the threat [34, 53]. Finally, different but specific engagements of the septo-hippocampal-amygdalar network appear to underlie the selection of relevant information, thereby contributing to the formation of adaptive emotional memories. The hippocampal and amygdalar systems not only regulate each other and their functional outcomes but also *qualify* specific emotional memory representations through specific activations and interactions [73].

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6. Dysfunction in the hippocampal-amygdalar dialogue might contribute to PTSD-related memory

Experimental studies just described above indicate that direct [53] or indirect manipulation [30, 55] of the hippocampal cholinergic neurotransmission can result in maladaptive fear memories. The identification of the discrete CS instead of the context in a predicting-context situation, or conversely, the selection of the context instead of the discrete CS in a predicting-tone CS situation reflects the formation of false memory that leads to the expression of inappropriate fear responses to the wrong predictor of a threat.

Specifically, these studies consistently show that all manipulations that aim at blocking or decreasing the hippocampal cholinergic transmission lead to the selection of an irrelevant but salient discrete CS instead of the background contextual cues as predictor of the footshock US. This striking formation of a prevalent discrete CS-US association to the detriment of the context-US association in a situation in which the discrete CS does not yet predict the occurrence of the threat is reminiscent of some critical aspects of traumatic memory as observed in posttraumatic stress disorder (PTSD). Indeed, one of the cardinal features of PTSD-related memory is a paradoxical qualitative alteration of memory including both memory intensification for the core traumatic event and a memory deficit for the traumatic environment. In other words, *hypermnesia* for some salient trauma-related cues that received the full attention of the subject during the traumatic event would co-exist with *amnesia* for peri-traumatic contextual stimuli that were too briefly apprehended to receive enough conscious attention [74–78]. Of particular relevance with the PTSD-related memory profile are the studies indicating that an increase in emotional arousal can promote the use of cue-based memory, but that such bias could result from an impairing effect of emotion on hippocampus-dependent cognitive memory [79].

Most of animal models of PTSD-related memory have exclusively focused on the quantitative alteration of memory, that is, the persistence of a strong fear memory, neglecting the qualitative alteration of traumatic memory. Yet, for over a century, clinical studies have consistently described the underrepresentation of the trauma in the context-based hippocampal-dependent memory system in favor of its overrepresentation in a cue-based sensory/emotional/implicit amygdala-dependent memory system [74, 76, 77]. In full accordance with the data presented above, these studies strongly suggest that a hippocampal-dependent deficit in contextual processing of stressful situations might contribute to the development of PTSD-related paradoxical memory.

In order to explain this hypermnesia/amnesia paradoxical profile, Layton and Krikorian [77] proposed an interesting neurobiological model in which PTSD-related memory would be the result of an increasing amygdalar inhibition of the hippocampus along with situations of increasing stress intensity. In low-to-mild stressful situations, the amygdala, weakly activated, would stimulate the hippocampus, promoting thereby the consolidation of hippocampal-dependent declarative information that would culminate in the formation of long-term "flashbulb" memory. As discussed above, this hypothesis is supported by considerable data indicating that the

amygdala modulates hippocampal activity and positively contributes to the consolidation of autobiographical information that are emotionally connoted [26, 29, 80]. In contrast, in intense or extreme stressful situations, the amygdala would be more and more recruited and would increasingly inhibit the hippocampus. As a consequence, trauma-related stimuli could not be consolidated anymore in the declarative memory system and thus would be susceptible to amnesia. Nevertheless, because the amygdala would directly contribute to the consolidation of the trauma, this one could be retrieved. The memory of the trauma, however, would be mostly implicit and would only correspond to what the amygdala can encode and store, that is the core of the trauma as well as certain details like the most salient simple cues and associated emotional feelings. This hypothesis is extremely congruent with the idea developed here that hippocampal disruption and dysfunction in hippocampal-amygdalar interaction can produce a switch from normal to abnormal fear memory-like PTSD-related paradoxical memory.

In line with this idea, we recently demonstrated a key role for the hippocampus in PTSD-like fear memory in mice under high-stressful situation. Because corticosterone (CORT), the main stress hormone in rodents, was shown to enhance the consolidation of adaptive fear memory in some stressful situations [26, 81–84], but also to impair the hippocampal function and disrupt context-based memory in others [85–87], we hypothesized that injection of CORT into the hippocampus, its main brain target, immediately after fear conditioning might either promote adaptive or produce PTSD-like fear memory as a function of the intensity of the stressful event. In order to observe a putative experimental bias toward a cue-based memory at the expense of a hippocampal-dependent context-based memory of the trauma, we used a predicting-context condition in which a discrete (tone) CS is present but irrelevant (not predictive of the US). In that case, the erroneous selection of the discrete CS as predictor of the footshock US reflects the expression of a maladaptive (PTSD-like) fear memory. As expected, we first showed that in a predicting-context situation using low footshock intensity, CORT injection in the hippocampus enhances adaptive conditioned fear to the context. In contrast, after a high-stress condition, the same CORT injection produces PTSD-like memory with the induction of a fear response to the most salient but irrelevant cue (a discrete tone) together with a decreased fear response to the right predictor (the conditioning context). Second, as in humans [88–91], compared to normal fear memory, PTSD-like memory induced in mice was found to be associated with hyperactivation of the right amygdala together with hypoactivation of the hippocampus [92].

Altogether these studies unveil a key role for the hippocampal-amygdalar network in the appraisal of emotional information and the formation of adaptive fear memories. As attested by behavioral and brain imaging outcomes of experimental manipulations of this network, dysfunction in this neural circuit, especially mediated by disruption of the hippocampal activation, turns out to be at the core of the development of abnormal/maladaptive fear memory, as observed in stress-related disorders like PTSD.

7. Conclusion: co-determined emotion and cognition

Although emotion can impair cognitive processes in certain extreme circumstances leading to maladaptive fear memory like in PTSD, it can also serve cognition. A growing body of

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evidence indicates that in contrast to artificial cognition, our natural cognition implies emotional experience with all its underlying somatic/physiological mechanisms. The fact that emotion can serve cognition, as illustrated by its critical contribution to decision making and to the enhancement of episodic memory, has been associated for a few decades to this other fact that, reciprocally, cognitive processes regulate emotional states. In addition, numerous data now indicate that this mutual regulation is mediated, at least in part, by a reciprocal modulation of the amygdalar and hippocampal systems.

Beyond the mutual quantitative regulation of these two brain systems described so far, the present chapter developed the idea that different recruitments of the hippocampus and the amygdala lead to specific neural configurations that qualitatively impact the formation of emotional memory, thereby producing different memory representations of an aversive experience. First, showing that the amygdala differentially modulates the hippocampal-septal excitability depending on whether a discrete CS or a complex background context is identified as predictor of a threat (i) indicates that a brain structure traditionally involved in the attribution of an affective value to neutral stimuli interact with another brain system that is supposed to underlie the "cognitive" processing of factual information and (ii) suggests that the hippocampal-dependent appraisal of such information may thus be dependent on the amygdala-dependent emotional experience encountered. Second, showing reciprocally that manipulating the hippocampal system during the acquisition of an aversive experience dramatically alters, and in fact can even lead to the switch from adaptive to maladaptive conditioned fear responses, indicates that a brain system known to underlie factual/representative memory directly contributes to the formation of specific emotional memory representations. Third, the systematic comparison of two different fear-conditioning situations, that is, predicting-context *vs*. predicting-discrete CS situation, revealed that the hippocampal system and amygdala both contribute to each learning situation. Importantly, their contribution to these two different learnings implies different patterns of activation and thus different recruitments of the hippocampal-amygdalar network as a function of the predictor of the threat. Hence, the main implication of this observation is that two neural systems known to underlie two well-known dissociable forms of memory turn out to be closely interactive when normal individuals form adaptive emotional memory.

While this chapter started with the idea of a fundamental dissociation between behavioral/emotional memory *vs*. representative memory, it comes to the idea that, except in some extent in pathological states, any of these two forms of memory can be conceived as isolated from the other. As proposed by Varela et al. 20 years ago [2], while all formal representations or cognitive processes are fundamentally embodied and "emerge from recurrent sensory-motor patterns," reciprocally all behavioral expressions of a past experience are necessarily constraint and guided by the cognitive structure of the individual concerned. In other words, our cognition would be "a creative form of enacting significance on the basis of the animal's embodied history."

In line with both the somatic markers hypothesis proposed by Damasio [3, 14] and the concept of "enaction" proposed by Varela et al. [2], the studies reviewed here support the general idea that "cognitive representation" and "emotional experience" should be conceived as co-determined entities, while factual representation takes its roots in somato-visceral

experiences, reciprocally, emotional experience depends on a more or less sophisticated guiding factual representation, such mutual dependency serves the expression of adaptive behaviors.

Acknowledgements

This work was supported by Institut National de la Santé et de la Recherche Médicale, Fédération pour la Recherche sur le Cerveau, Ministère de l'Enseignement supérieur et de la Recherche, Bordeaux University, and ANR (ANR-14-CE13-0026-01).

Financial disclosures

The authors report no financial interests or potential conflicts of interest.

Author details

Aline Desmedt

Address all correspondence to: aline.desmedt@inserm.fr

Neurocentre Magendie, Bordeaux University, Bordeaux, France

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Chapter 14

The Contribution of the Amygdala to Reward-Related Learning and Extinction

Rose Chesworth and Laura Corbit

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67831

Abstract

There has been substantial research into the role of the amygdala in fear conditioning and extinction of conditioned fear. The role of the amygdala in appetitive conditioning is relatively less explored. Here, we will review research into the role of the amygdala in reward-related learning. Research to date suggests that the basolateral and central amygdala are responsible for learning about distinct aspects of a reinforcing event. For example, the basolateral amygdala is essential for distinguishing and choosing between specific rewards based on the specific-sensory properties of those rewards as well as updating the relative value of specific rewarding events. In contrast, the central amygdala is involved in encoding reinforcement more generally and for regulating motivational influences on responding. We will also review what is known about the role of the amygdala in extinction of reward-related behaviours and highlight areas for future research.

Keywords: reward, Pavlovian conditioning, instrumental learning, reinforcement, basolateral amygdala, central amygdala

1. Introduction

While less is known about the role of the amygdala in reward-related learning compared to its role in fear conditioning where detailed circuitry has been mapped out, research to date nonetheless points to a very interesting and important function for the amygdala and distinct roles for sub-nuclei of this structure. In this review chapter, we will focus on rodent studies (using rats and mice) examining the role of the basolateral (BLA) or central (CeA) amygdala in the formation and expression of both Pavlovian and instrumental associations, the effects of changes in reward magnitude or value on responding, and to changes in reward contingencies

to define the role of the amygdala and subnuclei of this structure in learning about reward and control of reward-seeking behaviours.

2. Basolateral nucleus of the amygdala

2.1. Involvement of BLA in encoding reward expectation in Pavlovian tasks

Neuronal firing in the BLA is elevated in response to reward-predictive CS's, occurring prior to reward delivery. Such activity is thought to drive reward-seeking behaviours. For example, in an odour discrimination paradigm, BLA firing differs following presentation of stimuli that predict a positive outcome (sucrose) versus a negative outcome (quinine), suggesting the BLA is involved in learning about expectancy for consequences of a response [1]. Importantly, this discriminative neural activity precedes reliable behavioural discrimination, suggesting that the neural activity may support learning and behavioural change and is consistent with a role for the BLA in encoding of information about a reward predictive CS. Data regarding a causal role for this activity in Pavlovian learning are, however, more mixed.

A number of studies have found no evidence of any effect of BLA lesions on the acquisition of a Pavlovian response, with lesioned rats demonstrating food cup approach in the presence of a CS+ similar to that of sham rats ([2–4] see also [5]). The acquisition of Pavlovian autoshaping responses was also similar to that of controls [6]. However, other studies indicate BLA lesions or inactivation impairs acquisition of Pavlovian associations. Lesions of the BLA reduce rats' preference for a flavoured solution paired with sucrose compared to sham controls, with no effect on consumption of CS+ and CS− solutions when these were not paired with sucrose, suggesting involvement of the BLA in Pavlovian associations between the flavour of the liquid and the sucrose reward [7]. BLA lesions impair taste-potentiated odour aversion, and infusions of the GABA-A agonist muscimol into the BLA indicate the BLA mediates the acquisition, but not the expression of taste-potentiated odour aversion ([8], see also Ref. [9]). Also, lesions of the BLA impair the acquisition, but not expression of magazine approach in a task where discrete cues signal the location of a sucrose reward [10]. BLA lesions impair second-order conditioning [3, 11, 12]; but this deficit is secondary to the role of the BLA in the assignment of motivational value to the first-order CS+ without which motivational significance cannot then be transferred to the second-order CS+ to produce conditioned responding [12]. Inactivation of the BLA with the NMDA antagonists AP-5 or D,L-2-2-amino-5-phosphonovalerate (D-APV) impairs acquisition, but not expression of Pavlovian conditioned approach for sucrose or taste potentiated odour aversion [13, 14], while inhibition of dopamine D_1 receptor activity with SCH-23390 impairs acquisition of Pavlovian discriminative stimulus responding (i.e. approaching the food cup in the presence of a CS+, but not a CS−) [15]. D_1 antagonism has no effect on responding when animals are trained further and tested drug free, suggesting a specific role for BLA D_1 receptors in the performance of Pavlovian discriminative stimulus approach [15]. It appears acquisition of Pavlovian associations rely on a BLA to nucleus accumbens (NAcc) pathway, as optogenetic inactivation of the BLA to NAcc pathway using halorhodopsin impairs acquisition of licking behaviour for sucrose in response to sucrose

http://dx.doi.org/10.5772/67831

predictive cues ([16], see also Ref. [17] for related function of this pathway). When optical stimulation was removed, licking returned to non-stimulation levels, suggesting no long lasting effects of BLA to NAcc inhibition on subsequent task acquisition [16]. Thus, despite some inconsistencies in the literature, it appears that the BLA is required for acquisition, but not for expression of Pavlovian stimulus-reward associations.

There is strong evidence implicating the BLA in the acquisition and expression of conditioned place preference (CPP) for a food reward. Electrolytic and neurotoxic lesions of the lateral amygdala (LA) impair acquisition of CPP for a food reward [18], while BLA lesions or inactivation performed after acquisition of CPP for a food reward impair expression of CPP [19, 20], suggesting that BLA is implicated in both the acquisition and expression of Pavlovian place learning. Furthermore, muscarinic receptors in the BLA are required for the consolidation of food CPP, as intra-BLA scopolamine infusions following conditioning sessions impairs consolidation of food CPP [21]. Disconnection of the BLA from the NAcc also impairs expression of sucrose CPP, implicating this pathway in the expression of context-food associations [19]. One study has demonstrated no effect of BLA lesions on place conditioning [10]; however, in this study, discrete cues were used to signal the presence of reward within a *y*-maze, providing an alternative strategy by which the animals could solve the task and as discussed above, there are a number of studies demonstrating no effect of BLA lesions on Pavlovian learning using a discrete cue. As such, the majority of research suggests the BLA, and its projections to the NAcc are required for the acquisition and expression of CPP.

2.2. Involvement of BLA in instrumental learning

The involvement of the BLA in the acquisition and expression of instrumental appetitive learning has been extensively examined, with somewhat mixed results. For example, infusion of the NMDA antagonist AP-5 [22] or the D_1 antagonist SCH23390 [23] into the BLA prior to training has been reported to impair acquisition of lever pressing for sucrose pellets but once learning has occurred, BLA inactivation via AP-5 or SCH-23390 has no effect on the expression of action-outcome contingencies, suggesting involvement of the BLA in task acquisition, but not expression [22, 23]. It is important to note that in these studies, performance of the lever-press result produced not only primary reward but also a range of visual cues, e.g. offset of the houselight and onset of a stimulus light followed by sucrose at a 3 second delay. The presence of these stimuli as well as the delay in reward delivery makes it unclear whether the BLA is involved in acquisition of the instrumental response-outcome contingency or these other aspects of the task. Indeed, other studies using a more pure instrumental design where the instrumental response produces reward without the presence of any stimuli or secondary reinforcers report no effect of BLA lesions on acquisition of instrumental responding for a single action-outcome contingency (e.g. lever press delivers food pellets) ([2, 24], but see [25]). Furthermore, lesions of the BLA do not impair acquisition of instrumental responding when two action-outcome contingencies earning distinct outcomes are trained (e.g. lever press delivers sucrose solution, chain pull delivers food pellets) [4, 26, 27]. In more complex discriminative stimulus tasks, where rats are required to initiate the correct action following stimulus presentation, BLA lesions or inactivation using the combined GABA A (muscimol)

and B (baclofen) agonists [28, 29] or selective serotonin lesions of the BLA [30] do not impair task acquisition, suggesting that the BLA is not essential for stimulus-response learning despite its role in stimulus-reward learning. In contrast, BLA inactivation with muscimol or baclofen impairs expression of previously trained discriminative stimulus responding, suggesting that the BLA may contribute to task expression in a discriminative stimulus task but that when the BLA is inactivated during acquisition, the rats can solve the task, perhaps by using a different strategy [17].

2.3. Involvement of BLA in detecting changes in reward-predictive nature of an action

In accordance with a role for the BLA in predicting reward, the BLA is also involved in detecting changes between an action and a rewarding outcome. This has been demonstrated through contingency degradation paradigms, in which the association between an action and its expected outcome is reduced through non-contingent presentations of the reward. Lesions of the BLA performed prior to behavioural training impair contingency degradation, under conditions of extinction (when there is no opportunity to update action-outcome contingencies) and also under conditions of partial reinforcement [26]. BLA lesions also impair contingency degradation when lesions are performed after acquisition of action-outcome contingencies, ruling out any potential learning impairment which could impact the contingency degradation [31]. These studies suggest BLA involvement in detecting changes in the association between an action and expected reward.

2.4. Involvement of BLA in updating the value of an outcome

The BLA appears critical for generating internal representations of a reinforcer to guide choice. For example, BLA neurons are sensitive to reward magnitude. Rats in an eight arm radial maze exhibit differential BLA firing to rewards of high and low magnitude [32]. Also, BLA activity is altered in response to changes in expected reward magnitude (i.e. upshift or downshift in reward magnitude) [33]. However, once this contingency is learnt, BLA activity decreases, suggesting involvement of the BLA in the acquisition, but not expression, of reward magnitude changes [33]. Noradrenaline (NOR) is released in the BLA following an increase in the number of sucrose pellets delivered in an instrumental task, suggesting NOR in the BLA contributes to signalling changes in reward value [34].

In addition to changes in magnitude, the value of rewarding outcomes can change based on changes in the animal's motivational state. Involvement of the BLA in updating the value of an outcome is demonstrated through incentive-learning tasks. In these tasks, the value of a reinforcer is updated based on changes in internal states (e.g. hunger, satiety) and this information used to control goal-directed responding. In an incentive learning paradigm, the experience of food consumption in a deprived state subsequently drives responding for that food in a future state of deprivation [35, 36]. Importantly, without the opportunity for direct contact with the reward in a novel motivational state, instrumental responding for that reward is not altered even when animals experience motivational state changes at test, e.g., responding is not increased despite an increase in hunger until the animal experiences that food while hungry. Consolidation and reconsolidation of this form of learning are blocked by intra-BLA

infusions of the protein synthesis inhibitor anisomycin [37]. Infusions of the non-selective opioid antagonist naloxone into the BLA also impair acquisition but not retrieval of incentive learning [36]. This effect appears dependent on μ-opioid receptors, as infusion of a μ-receptor antagonist blocks acquisition of positive incentive learning (i.e. under conditions which enhance reinforcer value), and infusion of a μ-receptor agonist impairs negative incentive learning (i.e. under conditions which reduce reinforcer value) [38]. K and δ antagonists have no effect on positive or negative incentive learning, suggesting a specific role for μ-receptors in the BLA in incentive motivational processes [38].

Related results have been found in outcome devaluation studies. In this task, hungry rats are, for example, trained to perform two distinct instrumental responses each earning a unique food outcome. Rats are then pre-fed one of these outcomes to satiety prior to a choice test where the two responses are available but no outcomes are delivered. BLA lesions or inactivation impairs sensitivity to outcome devaluation [4, 6, 26, 31, 39, 40]. This effect is observed when lesions are conducted both prior to instrumental learning, and after acquisition of instrumental learning [26, 31], indicating any potential effects of lesions on action-outcome learning do not contribute to the loss of sensitivity to outcome devaluation. Also, BLA inactivation prior to the pre-feeding treatment, but not after pre-feeding but before the lever test impairs outcome devaluation, suggesting the BLA updates reinforcer value to guide choice, but that once reinforcer value has been updated, the BLA is no longer needed ([41], see also Ref. [6]). Such results have been taken as evidence that the BLA associates the specific sensory features of stimuli with motivational significance and updates this association as needed. This information can then be used to guide choice in outcome devaluation and related paradigms [26].

There are some reports of BLA lesions having no effect on outcome devaluation; however, it appears these results are due to differences in experimental parameters. When rats are trained in a Pavlovian magazine approach paradigm with a single reinforcer and devalued using lithium chloride (LiCl), BLA lesions do not impair outcome devaluation [3, 42]. There are a number of variables which could contribute to this apparent discrepancy, namely the method of training (Pavlovian or instrumental), the number of reinforcers used (one or two reinforcers) and the method of devaluation (LiCl or specific satiety). Johnson and colleagues [39] assessed the contribution of the method of training and method of devaluation to establish how these factors may help understand what aspect of learning requires BLA involvement. All rats were lesioned following training, to isolate the effect of the lesion to devaluation. All rats were trained with two reinforcers. Four groups of rats were used: rats were either given Pavlovian or instrumental training, and devalued via LiCl or specific satiety, creating the following four groups: Pavlovian-LiCl, Pavlovian-specific satiety, instrumental-LiCl and instrumental-specific satiety. BLA lesions impaired outcome devaluation in all four groups. As the only variable which was not manipulated was the number of reinforcers trained (in this case, two), this led the authors to suggest the number of reinforcers used may mediate whether the BLA is required for outcome devaluation. Indeed, if the BLA encodes sensory representations of a stimulus and associations of these with value, then successful devaluation performance may depend on the ability to generate sufficiently detailed outcome representations so that performance specifically related to the currently devalued outcome, but not other possible outcomes, being specifically affected. Thus, in the case of two reinforcers, the BLA is required

to generate this specific representation so that animals can then directly respond to the two reinforcers based on specific sensory features in order to guide action selection as the motivational significance is largely overlapping. However, when only one reinforcer is present, no discrimination between reinforcers nor any association of value with the sensory features of the outcome is required, which may leave outcome devaluation intact when the BLA is offline ([39], for discussion, see Refs. [31, 43]).

2.5. The BLA, reward prediction and stimulus influences on responding

Despite some inconsistencies in the effects of BLA lesions or inactivation in the acquisition of instrumental responding, incentive learning and outcome devaluation tasks suggest that the BLA is important for assigning motivational significance to outcomes based on their specific sensory features. There is strong evidence to suggest that the BLA is important for assigning reward value to actions and external stimuli more generally. Early reports demonstrate that in an eight arm radial maze, BLA neurons exhibit enhanced firing to an anticipated reward encounter, implicating BLA activity in predicting rewarding events [32]. In operant tasks requiring rats to nose poke for sucrose, BLA firing is enhanced during reward expectation, but decreases when animals no longer anticipate reward delivery following their actions under behavioural extinction [44]. Also, LA neurons respond to reward predictive cues, and activity in LA neurons is associated with task efficiency and accuracy, as well as increased synaptic strength [45]. Finally, neurons activated in response to a discriminative stimulus fire in the BLA prior to the NAcc, suggesting the BLA drives NAcc neuronal responses to reward predictive cues to promote reward-seeking behaviour [17].

Following detection of reward-predictive stimuli, it appears glutamate transients in the BLA are involved in initiating reward-seeking action. Glutamate transients in the BLA are enhanced during a seeking-taking chain task for sucrose pellets, and glutamate transients tend to precede lever responses on both the distal lever (i.e. lever responses which gave access to the proximal lever) and proximal lever (i.e. lever responses on which are rewarded with sucrose) [46]. Furthermore, in a simple instrumental task, BLA glutamate transients are more likely to be associated with initiating the pressing bout, than with reward or non-reward earning lever presses [47]. These data suggest glutamate signalling is critical for driving actions which lead to reward.

Experiments using outcome devaluation indicate involvement of the BLA in encoding the sensory specific properties of reinforcers. Further support for this notion is derived from Pavlovian-instrumental transfer (PIT) experiments, where presentation of a CS previously paired with a reinforcer drives instrumental responding for the same reinforcer, despite the CS and instrumental response having never been trained together before. Importantly, when rats are trained with two reinforcers (i.e. two CSs are paired with two distinct rewards and two instrumental responses earn those same two rewards), responding during PIT can be identified as 'specific', with increased instrumental responding on the lever that, in training, delivered the same outcome as that predicted by the stimulus. In contrast, 'general' PIT is an elevation in responding that does not rely on a common outcome in instrumental and Pavlovian training phases (for a discussion, see [4, 48] #321). When rats are trained with mul-

tiple rewards, BLA lesions impair specific, but not general PIT [4, 27], suggesting the BLA is required for distinct stimuli to direct instrumental responding. Furthermore, blocking AMPA, but not NMDA receptors in the BLA inhibits PIT [47], and BLA glutamate transient frequency correlates with instrumental responding during the CS, which was trained with the same outcome, but not the different outcome [47]. Importantly, BLA glutamate transient frequency is enhanced during initiation of lever pressing, suggesting BLA engagement following detection of reward-predictive stimuli which initiates goal-directed responding [47]. It appears the involvement of the BLA in PIT is dependent on the number of reinforcers (stimuli and responses) trained, because when rats are trained with only one reinforcer, BLA lesions have no effect on PIT [2, 49]. When two reinforcers are trained, specific sensory properties need to be utilised to permit discrimination; this requires the BLA and is impaired following BLA lesions or inhibition. However, when only one reinforcer is trained, it is not necessary to distinguish between reinforcers via their sensory properties to direct responding; thus, this behaviour does not require the BLA and is therefore unimpaired following BLA lesions.

Consistent with PIT studies, BLA lesions abolish outcome-guided responding in an outcome-specific reinstatement task, in which outcome presentation selectively increases performance of a response previously associated with the same, but not a different outcome as that which was just presented [31], supporting the involvement of the BLA in the representation of sensory-specific properties of stimuli and integration of those stimulus properties with motivational significance to direct choice behaviour.

2.6. The BLA mediates risky and effortful decision making

Experiments using delay discounting paradigms indicate the BLA guides choice towards high effort/high reward options. When rats are required to choose between high effort/high reward versus low effort/low reward options in a T-maze, BLA lesions reduce choice for the high effort/high reward option [50, 51]. Similarly, when a high reward choice requires a longer delay, as in delay discounting paradigms, BLA lesions also bias choice to smaller, immediate reward [52, 53]. There can be some recovery from bias towards low effort/low reward options, suggesting the BLA is involved in the acquisition of the value of a reward in an effortful task [51]. Disconnection of the BLA from the medial prefrontal cortex or anterior cingulate cortex (ACC) also biases choice to smaller, immediate rewards ([50, 52] but see Ref. [54]), consistent with a role for these structures in effort-based decision making [55].

Paradigms involving risky decision making indicate the BLA also guides choice towards high risk/high reward options. BLA inactivation with baclofen or muscimol induces a risk-averse pattern of choice and, in a similar paradigm, reduces high effort choice, irrespective of the delay to reward [56]. It is possible that the BLA directs responding toward risk when loss is involved, as rats with BLA lesions bias their behaviour away from risk when loss was a consequence of a high risk choice, but do not alter their behaviour when potential gains are available [57]. BLA lesions or inactivation does not alter choice when two rewards are equal, or there is no risk involved [56–58], suggesting a particular role for the BLA in biasing choice in the face of aversive consequences. It appears a BLA-NAcc connection mediates BLA-induced biasing of choice, as contralateral lesions of these structures biases choice toward a less risky option [54].

Some studies demonstrate BLA lesions enhance, rather than decrease risky decision making in a rodent gambling task [59], or when foot shock is used as punishment (instead of reward omission [58]). However, a recent study indicates individual differences may help explain these results. BLA inactivation can affect animals differently at an individual level—BLA inactivation increases effortful choice in rats which, at baseline, chose low effort/low reward options and BLA inactivation decreases effortful choice in rats which, at baseline, chose high effort/high reward options [60]. Furthermore, this biasing of choice appears dependent on BLA dopamine receptors. In risk-averse rats, D_1 agonist infusions into the BLA increase risky choice, whereas in risk-prone rats, enhancing D_1 activity reduces risky choice [61]. Also, infusions of the D_2 agonist quinpirole reduce risky choice in risk-prone rats [61]. It is possible dopamine receptors in the BLA mediate the interaction between costs and benefits in a task to generate subjective value which could differ between individuals or across experiments. Approaching the BLA as a mediator of decision making based on a cost/benefit analysis may explain why some studies report an increase in risky decision making following BLA inactivation—the effects of inactivation of this structure on behaviour may be dependent on task parameters which can bias decision making in a certain direction.

2.7. No consistent involvement of the BLA in reversal learning

Several studies have examined the role of the BLA in reversal learning; however, the results at present are inconsistent. One study demonstrates inactivation of the BLA with muscimol impairs reversal learning in an odour discrimination task [52]; however, another study demonstrates no effect of BLA lesions on reversal learning in a go/no-go odour task [62]. Interestingly, in this study BLA lesions ameliorated impairments in reversal learning induced by orbitofrontal cortex lesions, suggesting projections between these two regions may control reversal learning [62]. In an operant nose-poking discriminative stimulus task, BLA lesions have been shown to facilitate reversal learning, and limit the number of mistakes made following feedback on an incorrect trial [63]. However, in a similar nose-poking discriminative stimulus paradigm, serotonin depletion in the BLA had no effect on reversal learning [30]. It appears the involvement of the BLA in reversal learning is not dependent on the task employed, as similar tasks (e.g. odour discrimination, operant nose poking) report inconsistent effects of BLA inactivation on reversal learning. Further research in this field is required to more conclusively determine the role of the BLA in reversal learning.

2.8. Involvement of the BLA in the appetitive extinction learning

A number of studies demonstrate that the BLA is critical for the acquisition of appetitive extinction learning. Excitotoxic lesions of the BLA enhance resistance to extinction learning when a magazine light and sucrose reinforcer are omitted, indicating BLA lesions impair extinction learning [28]. However, in this study, the use of excitotoxic lesions did not permit analysis of whether BLA lesions impair encoding, consolidation or retrieval of extinction learning [28]. Inactivation of the caudal BLA with bupivacaine (a sodium channel blocker) impairs acquisition, but not retrieval of extinction of instrumental responding, demonstrating

BLA involvement of the acquisition of extinction learning [64]. In apparent contrast, intra-BLA infusions of the NMDA partial agonist DCS, which should increase rather than decrease activation of BLA neurons, prior to extinction learning in an odour discrimination task has been reported to impair extinction and enhance responding at a retention session [65]. While a number of studies demonstrate DCS-enhanced extinction learning (reviewed in Refs. [66, 67]), it appears the timing of DCS administration is critical in determining whether it enhances or impedes extinction learning (for a discussion, see Ref. [65]). Nonetheless, this study [65] demonstrates that NMDA receptors in the BLA contribute to extinction of appetitive learning. Finally, a subset of BLA neurons respond specifically during extinction of operant nose poking for sucrose; this subset does not respond during task acquisition and activity of these neurons is inversely correlated with responding during extinction [68]. These studies demonstrate a critical role for the BLA in detecting the absence of an expected reinforcer during instrumental appetitive extinction and are in agreement with the role of the BLA in detecting changes in reward value.

There is also some evidence to support a role for BLA signalling in the extinction of Pavlovian appetitive learning. For example, when rats are trained to lick for sucrose in the presence of a combined tone/light CS+, the firing of BLA neurons during extinction correlates strongly with extinction behaviour [69]. Furthermore, a subset of BLA neurons which responded during extinction also respond during reinstatement, suggesting the BLA is a site of plasticity mediating responding for motivationally significant stimuli [69]. These studies suggest that the BLA mediates aspects of Pavlovian appetitive extinction; however, further research in this field is required to determine the precise role of the BLA in appetitive extinction.

2.9. The BLA as part of a broader circuit involved in reward-related learning

It is important to recognise that the BLA does not operate in isolation to control learning and performance. Below we highlight some example of interactions of the BLA with other structures. This section is not meant to comprehensive but to provide examples of how the BLA interacts with other brain areas. The BLA has dense projections to the posterior dorsomedial striatum (pDMS), insular cortex (IC) and NAcc [70], which, following detection of a change in reinforcer value in the BLA, mediate aspects of goal-directed responding, such as knowledge of and engagement in action-outcome contingencies. A BLA-IC connection is required to encode and retrieve changes in reinforcer value [71]. BLA inactivation using the NR2B NMDA antagonist ifenprodil prior to specific satiety, but not prior to a choice test impairs outcome devaluation, suggesting BLA involvement in encoding changes in reinforcer value. However, ifenprodil infusions into IC prior to specific satiety or a choice test impair devaluation, suggesting that the IC mediates expression of devaluation. Finally, ifenprodil infused unilaterally into BLA prior to specific satiety and into the IC prior to a choice test blocks expression of devaluation, but ifenprodil infused into the IC prior to specific satiety and then into BLA prior to choice has no effect on expression of devaluation. This suggests the BLA updates and encodes information about reinforcer value during specific satiety, sending information to the IC prior to choice, and at test, the IC retrieves this information to guide choice between actions [71].

Also, connections between the BLA and posterior dorsomedial striatum (pDMS) are required to direct action-outcome responding following a change in reinforcer value. The pDMS is critical for updating action-outcome contingencies, as changes in response-outcome associations are impaired following pDMS lesions [72, 73]. It appears the pDMS is required to retrieve action-outcome associations following a change in reinforcer value, as unilateral lesions of the BLA coupled with inactivation of contralateral pDMS prior to a choice test impairs expression of outcome devaluation [73]. This suggests information from the BLA regarding the specific value of outcomes is transferred to the pDMS, which retrieves action-outcome associations to guide instrumental performance [73].

Finally, a connection between the BLA and NAcc shell is necessary for action selection following reinforcer devaluation. Disconnection of the BLA and NAcc via contralateral excitotoxic lesions impairs outcome devaluation, without reducing overall responding [74]. It is possible the BLA conveys sensory-specific outcome information and/or changes in reinforcer value to the NAcc, where it is used to direct outcome-appropriate instrumental responding [74]. Previous reports demonstrate that NAcc shell lesions impair the ability for action-outcome cues to bias action selection in Pavlovian-instrumental transfer [75], supporting the NAcc shell being a limbic-motor interface structure [76]. Thus, it appears sensory-specific information from the BLA is used to drive action selection in the NAcc shell, which can direct actions through motor output structures such as the ventral pallidum and medial dorsal thalamus.

3. Central nucleus of the amygdala

3.1. The CeA in Pavlovian learning

The CeA is involved in conditioning with both appetitive and aversive reinforcement [77], and one proposed role for the CeA is determining the valence of reinforcing events. For example, *c-fos* immunoreactivity is increased in the medial CeA following exposure to a CS+ signalling food delivery, compared to a CS which did not signal food delivery [78]. CeA *c-fos* immunoreactivity is also increased following exposure to a CS+ signalling foot shock, particularly in ventral regions of the structure, suggesting sub-regions of the CeA may detect the valence of a CS [78]. We focus here on data related to appetitive learning.

When a visual or auditory CS is paired with food, rodents can acquire distinctive behaviours to CS presentation; they may orient themselves to the CS, either by approaching or rearing to a light or startling in response to a tone (orienting responses) or approach the site of food delivery, usually a food cup or magazine (conditioned approach). There is considerable evidence to support a role for the CeA in conditioned orienting responses to a Pavlovian CS+, but not for conditioned approach. Additionally, CeA lesions do not impair second-order Pavlovian conditioned approach [3]. Lesions of the CeA prior to training impair the acquisition of conditioned orienting responses (e.g. rearing to a light), but leave conditioned approach intact [79–81]. Similarly, inactivation of the CeA with the AMPA antagonist NBQX impairs acquisition of orienting responses [80]. CeA lesions or inactivation after Pavlovian training have no effect on the expression of Pavlovian orienting responses or food cup approach, suggesting a role for the

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CeA in the acquisition, rather than expression of orienting responses [80]. While some studies report no effect of CeA lesions on Pavlovian learning, these studies have only assessed conditioned approach behaviour ([2, 82] see also [7]), supporting a dissociation between conditioned approach and conditioned orienting responses in the CeA.

The CeA may be involved in conditioned approach behaviour when rats are trained to approach the magazine following the presentation of a CS+, but not a CS−, and a discrimination score is created which depicts their approach following one CS presentation over another CS. In Pavlovian approach paradigms, each CS+ is associated with a reinforcer, but not with the absence of reinforcement. CeA lesions or intra-CeA inactivation of D_1 or D_3 receptors reduces conditioned approach behaviour [15, 83, 84]. If the CeA is involved in discriminating positive or negative reward value (discussed below), CeA inactivation may impair this discrimination, leading to a lower discrimination score. Supporting this interpretation, Andrzejewski and colleagues reported equal nose poking rates between the CS+ and CS−, rather than an abolition of nose poking [15], which would support lack of discrimination between the two CSs but not an inhibition of nose poking following CeA inactivation. It is also possible that these effects relate specifically to dopamine function within the CeA.

3.2. Circuitry mediating conditioned orienting responses

In rats injected with fluorogold, a retrograde tracer, into the substantia nigra pars compacta (SNc) there was a greater number of *c-fos* positive/fluorogold positive cells in the CeA following food-tone pairings than unpaired food and tone presentations, implicating this pathway in conditioning [85]. Furthermore, contralateral lesions to disconnect the CeA and SNc impair orienting responses but not food cup approach, compared to an ipsilateral lesion control group [85]. Considering that the CeA has a substantial projection to the SNc that provides dopaminergic innervation to the dorsolateral straitum (DLS) [86, 87], it is possible that a CeA-SNc-DLS pathway mediates orienting responses to Pavlovian food CS's. Evidence for this comes from the demonstration that unilateral lesions of the CeA coupled with dopamine depletion in the DLS in the opposing hemisphere impairs conditioned orienting responses for food pellets, while leaving food cup approach behaviour intact [88]. Similar results were obtained when the DLS was reversibly inactivated with lidocaine [88]. Recovery of conditioned orienting responses occurred on drug free days in rats previously treated with intra-DLS lidocaine, suggesting no long lasting effects of CeA-DLS inactivation on acquisition of conditioned orienting [88]. Together, these results suggest orienting responses to a Pavlovian cue are mediated by indirect connections between the CeA and the DLS likely via the SNc.

3.3. The CeA in instrumental learning

The CeA does not appear to be critical for the acquisition of instrumental action-outcome contingencies. Lesions of the CeA do not impair instrumental learning when there is a single action-outcome contingency (i.e. one lever, one reinforcer) [2, 82] or two action-outcome contingencies (i.e. two levers, two reinforcers) [4]. There is some evidence that the CeA is involved in updating action-outcome contingencies. The omission of an expected reward at test enhances *c-fos* immunoreactivity in the CeA, suggesting CeA detects the lack of reward [89]. Furthermore, fluorogold

injections in the SNc demonstrate these *c-fos* positive CeA cells project to the SNc [89], implicating a CeA-SNc pathway in the detection of changes in reward contingencies. Lesions of the CeA produce a mild impairment in performance when an expected reward of small magnitude is omitted [90]; however, lesions of the entire CeA and BLA combined substantially reduce sensitivity to omission and so the specific contribution of the CeA is somewhat unclear [91].

CeA involvement in the detection of changes in reward value appears to depend on the paradigm used to assess this change. In studies using the outcome devaluation task, where an outcome is devalued either with selective satiety or LiCl-induced sickness, CeA lesions do not impair behavioural sensitivity to changes in reward value [3, 82], indicating no role in this evaluative process and further substantiating intact action-outcome learning necessary for performance in this task. Of interest, CeA lesions prevent loss of sensitivity to outcome devaluation that typically occurs with over-training, suggesting a role for the CeA in habitual behaviour [82]. Similar effects are observed following disconnection of the CeA from the DLS, produced by contralateral lesions of these structures, suggesting that the CeA sends a reinforcement signal to the DLS to strengthen the stimulus-response (S-R) association that is thought to underlie habit learning [82].

While the CeA is not necessary for normal sensitivity to devaluation, it is involved in learning about changes in the magnitude of reward. When rats are trained to run in a straight alley maze task for a large food reward, a downward shift in the magnitude of the food reward increases the latency of intact rats to reach the smaller reward [92]. Post-shift lidocaine infusions into the amygdala, which were mostly aimed at the CeA, reduce the latency to reach a smaller reward, suggesting reduced sensitivity to the change in reward magnitude [92]. Similarly, pre-training CeA lesions slow learning about a downward shift in reward magnitude in a straight alley maze, supporting a role for the CeA in detecting reward magnitude changes [93, 94]. More recently, optogenetic stimulation of CeA with channelrhodopsin was shown to enhance lever pressing for a sucrose pellet when both the delivery and consumption of this pellet were paired with laser stimulation, compared to delivery of sucrose pellets alone, suggesting CeA stimulation may enhance the perceived magnitude of a reward [95]. Finally, the μ-opioid agonist DAMGO administered within the CeA enhances sniffing and nibbling at a food cup or reward predictive lever, suggesting enhanced reward value attributed to these stimuli following CeA μ-opioid stimulation [96]. Collectively, these studies implicate the CeA in processing changes in reward magnitude. Performance may be spared where tasks rely on discrimination and choice between rewards based their relative value and distinguished using specific sensory properties. Such tasks rely instead on the BLA as described above. Together these findings are consistent with the idea that whereas the BLA is responsible for assigning and updating the value of specific outcomes based on their sensory properties, the CeA is responsible for a less specific reinforcement signal, accounting for its role in both habit learning and adjusting performance following changes in reward magnitude [43, 82].

3.4. CeA involvement in stimulus influences on instrumental responding

The CeA plays a role in signalling the general motivational information carried by stimuli but consistent with the studies above, not in detailed representation of the specific features of distinct rewards or their representation. Evidence for this comes from Pavlovian-instrumental

http://dx.doi.org/10.5772/67831

transfer (PIT) tasks, which assess control of instrumental responding by Pavlovian cues, despite the two types of training being conducted separately. PIT occurs when presentations of a CS (previously paired with a US) drives instrumental responding which was previously trained to obtain the same US. The involvement of the CeA in PIT is dependent on the type of PIT being examined. When rats are trained on one instrumental action-outcome contingency and undergo Pavlovian training which involves one CS-US association, CeA lesions impair PIT [2, 48, 82], while intra-CeA infusion of the μ-opioid agonist DAMGO enhance PIT [97]. However, when rats are trained on two instrumental action-outcome contingencies and two Pavlovian CS-US pairings, lesions of the CeA have no effect the outcome-specific PIT that is generated by this type of training [4]. Importantly, in an experimental design where a third excitatory CS+ is introduced in the Pavlovian training phase and paired with a third reward not earned by either instrumental response, CeA lesions impair responding to the third CS, but leave outcome-specific PIT intact, suggesting the CeA is involved in general appetitive arousal rather than directing outcome-specific responding [4]. This suggestion also accounts for the experiments described above which use only one action-outcome contingency and one CS-US pairing; when there is no choice between CS driven responses, a reduction in general appetitive motivation reduces responding in general. These findings suggest the CeA encodes a reinforcement signal which is devoid of specific details about an outcome.

3.5. Some involvement of the CeA extinction of appetitive learning

Several electrophysiological studies implicate the CeA in the extinction of appetitive learning. For example, Toyomitsu et al. [69] recorded from neurons in the BLA, LA and CeA during extinction of Pavlovian licking for sucrose reward. CeA firing during extinction correlated with extinction of licking; however, this correlation was not as strong as BLA firing [69]. Furthermore, while there were changes in the firing rate of CeA neurons between extinction and reinstatement, this was not as pronounced as in BLA neurons [69]. Calu et al. [98] recorded from the CeA during an over-expectation task where initially multiple stimuli are trained as independent predictors of reward (e.g. individual stimuli such as a tone, light, etc., each predict a food pellet). The critical manipulation comes when two or more of these stimuli are then presented together, as a compound. Animals typically increase responding to such a compound indicating that they expect more reward based on the multiple predictors (e.g. since tone and light alone previously predicted one pellet, the two stimuli together should predict two pellets). However, if this compound is followed only by the original reward (one pellet) behavioural responding decreases across trials, as does activity of the CeA [98]. Together with data from extinction paradigms, this suggests that the CeA may signal reward reduction in general, not just reward omission. A recent study by Iordanova et al. [99] examined this possibility by exploring the role of the CeA in updating reward expectancies following a reduction in reward achieved either through extinction, where reward was omitted entirely, or through generating over-expectation where, due to the presence of multiple predictors, a large reward is expected but not received. In both paradigms, the majority of recorded cells showed an increase in firing during the period where food reward was delivered and also during the preceding stimulus presentations reflecting reward expectancy. Neural firing to the extinction stimulus was reduced across trials compared to a control stimulus. When a combination of previously rewarded stimuli was introduced to generate over-expectation, neural firing to this

compound was increased relative to a control compound in early trials but then equivalent in later trials, presumably as animals came to expect the reduced reward that was received. A subpopulation of the reward-responsive cells showed a reduction in firing to both the extinction and over expectation trials, suggesting a common role in signalling reduced reward expectation. Importantly, this change in neural activity preceded and predicted the decline in behavioural responding observed under both extinction and over-expectation conditions. Because these conditions involved the delivery of different amounts of reward (no reward in extinction whereas reward was still delivered in over-expectation, albeit less than initially expected based on the stimuli) the similar changes in neural activity are unlikely to reflect absolute reward magnitude, but rather may signal the reduction in reward expectancy. It is possible that this reduction in reward creates an aversive motivational state, in which case these findings could be consistent with a more general role for the CeA in emotional learning [99].

4. Conclusion

While less is known about the role of the amygdala in reward-related learning compared to its role in fear conditioning where detailed circuitry has been mapped out, research to date nonetheless points to a very interesting and important function for the amygdala. For example, the basolateral amygdala is involved in associating sensory-specific aspects of different outcomes with the rewarding effects of that outcome, a function critical for choice between alternatives and behavioural control more generally. Further, the amygdala appears to be involved in updating representations of value both when the value of the outcome is changed, for example, following devaluation, or when the relationship between predictors and outcome delivery is changed, as in extinction. Thus, the amygdala plays an important role in reward-related learning. With the advent of tools such as optogenetics, researchers can now go on to explore how these functions are achieved within the complex circuitry of the amygdala and associated structures.

Author details

Rose Chesworth[1,2] and Laura Corbit[1]*

*Address all correspondence to: laura.corbit@sydney.edu.au

1 School of Psychology, University of Sydney, Sydney, Australia

2 School of Medicine, Western Sydney University, Sydney, Australia

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Edited by Barbara Ferry

The amygdala is a central component of the limbic system, which is known to play a critical role in emotional processing of learning and memory. Over these last 20 years, major advances in techniques for examining brain activity greatly helped the scientific community to determine the nature of the contribution of the amygdala to these fundamental aspects of cognition. Combined with new conceptual breakthroughs, research data obtained in animals and humans have also provided major insights into our understanding of the processes by which amygdala dysfunction contributes to various brain disorders, such as autism or Alzheimer's disease. Although the primary goal of this book is to inform experts and newcomers of some of the latest data in the field of brain structures involved in the mechanisms underlying emotional learning and memory, we hope it will also help stimulate discussion on the functional role of the amygdala and connected brain structures in these mechanisms.

ISBN 978-953-51-4757-2

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