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Neuroplasticity
Insights of Neural Reorganization

Edited by Victor V. Chaban

NEUROPLASTICITY - INSIGHTS OF NEURAL REORGANIZATION

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http://dx.doi.org/10.5772/intechopen.68156
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First published in London, United Kingdom, 2018 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

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

Neuroplasticity - Insights of Neural Reorganization
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p. cm.

Print ISBN 978-1-78923-194-6

Online ISBN 978-1-78923-195-3

eBook (PDF) ISBN 978-1-83881-286-7

Meet the editor

Dr. Victor V. Chaban is a professor of Medicine with dual faculty appointment at Charles R. Drew University of Medicine and Science (CDU) and the University of California, Los Angeles (UCLA). Dr. Chaban completed his postdoctoral training in Neuroscience at the UCLA and graduate studies in Clinical Research at CDU. His area of expertise is signal transduction mechanisms of nociception associated with clinical presentations of visceral pain. Dr. Chaban is a member of the US National Institutes of Health and several international study sections. He also serves as an executive editor for the *Journal of Autacoids and Hormones* and editor in chief for the *International Journal of Research in Nursing*. He is an author of *Unraveling the Enigma of Visceral Pain* published by Nova Science Publishers, New York, 2016, and editor of *Irritable Bowel Syndrome: New Concepts for Research and Treatments* published by IntechOpen, 2017.

Contents

Preface

Neuroplasticity associated with a neuronal network capacity to change and the brain's ability to reorganize is a key process in human development and existence. The nervous system is chiefly responsible for concerted functioning of a logical sequence: prognosticate, plan and correlate widely divergent information by controlling bodily activities. This phenomenon is presumably a result of all neural circuits' activity; therefore, the degree of complexity in neural circuitry may determine the degree of functioning. The neuronal connectome forms the basis for communication; furthermore, its overall level may result in different levels of plasticity. For the last decade, the available information concerning mechanisms and significance of neuroplastic changes within the central and peripheral nervous systems has been significantly increased. Medical reports describe the extensive decline in the quality of life of patients suffering from neurodegenerative or neurodevelopmental syndromes that contribute to personal suffering, disability and high medical costs.

Neuroplasticity today is an important subject of neuroscience, medicine, bioinformatics, computer sciences, philosophy and other disciplines. It is theoretically impossible to give an overview of all aspects of this process; many specialized reviews dealing with the most common mechanisms of neuroplasticity have been published. In the present book we give readers cutting-edge reports on neuronal adaptations, brain dynamics, brain reorganization in early development along with late adulthood, the role of music in plastic changes, as well as brain changes associated with sleep and autoimmune disorders. These studies will provide a framework to seek a fundamental understanding of modulatory systems involved in neural reorganization and adaptation.

This book provides a comprehensive overview of the structural and functional changes associated with cortical remapping, sensory substitution, synaptic and non-synaptic compensatory plasticity due to brain damage, brain training, chronic pain, meditation, music, exercise and related states. This book also suggests possible strategies for therapeutic intervention and design of habilitative and special educational programs. Using systemic translational analysis of different pathways of neuronal reorganization, we will gain mechanistic insight into how nervous systems function and adapt to change. Continuing effort in this field will be the basis to unravel the enigma of neuroplasticity leading to the eventual goal of our research: to apply new knowledge to targeted therapy.

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Chapter 1

Neuroplasticity of Primary Sensory Neurons in Visceral Nociception

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73699

Abstract

Chronic visceral pain is the most common complication of many functional disorders that do not have a defined pathophysiological cause. Functional pain syndromes include common disorders, such as irritable bowel syndrome (gastroenterology), chronic pelvic pain (gynecology), interstitial cystitis/painful bladder syndrome (urology), fibromyalgia (rheumatology), across multiple medical disciplines. Patients suffering from functional diseases may progress to cognitive decline and depression through neuroplastic changes not only at the level of the central nervous system but also in the periphery. Moreover, most functional diseases are much more prevalent in women than men suggesting estrogen modulation of nociceptive pathways. Defining the etiology of functional diseases for possible therapeutic interventions will have a significant impact on our understanding of observed gender differences and on improving patient's quality of life.

Keywords: neuroplasticity, functional diseases, visceral pain, sensory neurons

1. Introduction

Despite considerable efforts made by the medical research community and pharmaceutical industry to develop effective therapeutical treatments aimed to treat chronic visceral pain resulted from functional disease, there is little progress to date. Functional syndromes are estimated to affect up to 15–20% of the population worldwide. Symptom description of interstitial cystitis/painful bladder syndrome (IC/PBS, urgency, frequency, and bladder pain generally relieved by voiding) is parallel to the description of irritable bowel syndrome-diarrhea (IBS-D) predominance (urgency, frequency and abdominal pain) relieved by defecation. IBS stands in contrast to a bowel's structural disorder: unlike ulcerative colitis and Crohn's

disease, which are forms of inflammatory bowel disease (IBD), it does not cause changes in bowel tissue. The cause of functional disorders is unknown. Chronic abdominal complaints are without a structural or biochemical cause. Bloating, mucous in stools, diarrhea, constipation or alternating diarrhea and constipation, depression, anxiety or stress are also common symptoms of IBS. Hypersensitivity of visceral primary afferent neurons could result from excessive production of different modulatory neurotransmitters in response to changes in their signal transduction pathways [1]. Nociceptors are small-to-medium size dorsal root ganglia (DRG) neurons, whose peripheral processes detect nociceptive physical and chemical stimuli. There is often no clear relationship between the severity of the chronic pelvic pain and pathology in the pelvic viscera, and there are also noticeable gender differences in the prevalence of functional diseases that affect more women than men. Most of studies in this area were focused on the central nervous system (CNS); however, our recent data that estrogen can gate primary afferent response to modulate nociception support the idea about the involvement of the peripheral nervous system (PNS) in the etiology of a wide range of the functional and inflammatory diseases [2]. This potentially could involve neuroplastic changes in primary sensory neurons and can be a novel target for therapeutic interventions for patients suffering from chronic visceral pain associated with functional diseases. Despite a successful reduction of pain using available analgesics, visceral pain relapses in most patients. Currently, it is a time for paradigm shift what we consider as visceral nociception, and in this report, author looks for possible new mechanisms of peripheral modulation of primary afferent sensory neurons in development of chronic pelvic pain.

2. Neuroplasticity in peripheral nervous system

Traditionally, the main mechanism involved in development of chronic visceral pain is thought to be neuroinflammation. This pathway effects peripheral and central nerve sensitization and/or dysfunction of inhibitory descending pathways [3]. However, in clinical studies, visceral nociception strongly affects negative sensations that difficult to correlate with visceral traumata. Most nociceptive systems involved in peripheral sensitization originate in free sensory nerve endings of target organs that send their signals toward primary afferent sensory neurons within the lumbar-sacral regions of dorsal root ganglia (L_1-S_3 DRG).

Visceral sensitization may develop as a result of interaction between the nervous and immune systems. All visceral afferents can be sensitized by proinflammatory mediators, such as serotonin, histamine, nitric oxide and ATP, leading to neuropathic hyperalgesia. During inflammation, mast cells and leukocytes secrete inflammatory mediators such as cytokines and prostaglandins that activate polymodal nociceptors triggering the response of normally silent mechano-insensitive receptors. Mast cell mediators can also activate vanilloid receptors (TRPV1), purinergic ($P2X_3$) and bradykinin (BK2) at PNS, causing hyperalgesia.

The serotonergic pathway, one of the main inhibitory mechanisms in the CNS, may also be functionally important in facilitating peripherally mediated visceral pain [4]. Most antidepressants gradually increase serotonin level in the brain. Indeed, depression has been reported to be associated with immunosuppression. Serotonin plays a major role in the gut-brain axis by

modulating intestinal movement and the perception of visceral pain. An imbalance of serotonin in the gut, an improper reaction of the digestive system to serotonin, or a faulty serotoninergic network between the gut and the brain may be a cause of depression associated with functional diseases. Enteric nervous systems that innervate gastrointestinal tract include differentiated (visceral) primary afferent neurons that innervate intrinsic and extrinsic pathways implicated in the pathology of many inflammatory as well as functional diseases. There is a noticeable correlation between inflammation induced by gut infection and symptom occurrence of functional disorders such as IBS [5]. New data changed the previous paradigm that each primary afferent neuron innervates only one viscus. The concept of viscero-visceral cross-sensitization is well accepted and has been documented clinically [6]. Inflammation in one organ can induce peripheral (in addition to central) sensitization affecting another viscera. Therefore, nociceptive mechanisms involved in the progression of functional diseases are complicated by comorbid disorders. Both components of pain—discriminative and affective—concomitantly affect motor and cognitive systems. These systems can be gated by estrogen to modulate perception of pain, pain threshold and tolerance.

3. Gender differences in visceral pain

The chronic pelvic pain (CPP) from pelvic structures is more prevalent in female subjects compared to males. In clinical studies, this sexual dimorphism is well recognized: the incidence of functional disorders is 2–3 times higher in women with IBS and even greater with IC/PBS. A large body of literature supports that concept indicating estrogen modulation of different nociceptive pathways [2]. In our previous studies, we found that estrogen receptors (ERα and ERβ) are present in small-to-medium size DRG neurons (presumably nociceptors) and ATP-sensitive DRG neurons respond to 17β-estradiol (E_2) [7], which correlated well with the idea that visceral afferents are E_2 sensitive. Our data clearly suggest that in addition to CNS actions, E_2 can act in the periphery to modulate nociception [1]. Specifically, E_2 acts in DRG neurons to modulate L-type VGCC and through group II metabotropic glutamate receptors [8]. One prominent way E_2 modulates neuronal excitability is through the interaction with antinociceptive opioids such as enkephalins, β-endorphin and pronociceptive nociceptin/orphanin. Furthermore, our hypothesis is that increased nociceptive input from an inflamed organ (i.e. uterus) sensitizes neurons that receive convergent input from an unaffected organ (i.e. colon). In summary, our data suggest that potential site of visceral cross-sensitivity is the dorsal root ganglion [9]. DRG neurons could be responsible for changes observed in the perception of pain during the etiology of different functional syndromes associated with pain.

Several lines of evidence indicate that E_2 directly influences the functions of primary afferent neurons. Both estrogen receptors (ERα and ERβ) are present in DRG and visceral pain is affected by hormonal level in cycling females [1, 2]. Even a large body of literature supports the idea that E_2 modulates nociceptive responses in pelvic pain syndromes, the exact mechanism remains unresolved. Within the context of our hypothesis, E2 modulation of nociceptive response depends on the type of pain, its durations and the involvement of other nociceptive-mediated mechanisms.

4. Primary afferent nociceptors as target in modulation of nociception

Visceral nociceptive signal transduction depends on type of pain (type of sensory fiber), severity, duration and effects of other endogenous nociceptive-mediated molecules. Extrinsic primary afferent fibers can be directly modulated by activation of different chemosensitive or mechanosensitive receptors in target organs. There are 31 pairs of polymodal nerves carrying sensory motor and autonomic signal transduction in human spinal cord. This information is further transferred to the CNS by the spinothalamic ascending pathways to the primary sensory motor cortex for integration and analysis. In DRG neurons, afferent and efferent processes function as a single axon-proximal and distal part connected to somata as an offshoot. DRG first synapses with a dorsal horn neuron through the contralateral spinothalamic tract. These primary sensory neurons have been studied intensively in pain sensory physiology. Small-to-medium size DRG neurons express a variety of receptors involved in pain perception such as ATP-sensitive P2X3, capsaicin-sensitive TRPV1 or acid-sensitive ASIC channels. Since DRG neurons are responsive to estrogen [10] through ERα type [11] and show sensitization, it makes them a suitable model to study gender differences in nociception. Interestingly, TRPV1 and P2X3 transduction is significantly altered during inflammatory response. DRG is also an important site for primary afferent fiber convergence and visceral organ cross-sensitization. Even the role of DRG in neuromodulation of nociception is a novel topic in visceral and chronic pain, we hope to convince the scientific community that the DRG is an active structure rather than passive. The future studies may reveal more neuroplastic changes at the level of first-order sensory neurons. New hypotheses will drive translational research that should improve the outcomes of clinical interventions to relive patients from suffering.

5. Discussion

Pain is a complex and personal experience. Chronic visceral pain affects mood, and social and professional life. A delicate balance between biochemical and physiological changes and cognitive approaches is the most appropriate strategy to study clinical aspects of nociception. Pain in women can originate due to inflammation of a pelvic organ (gut, uterus and bladder) that can heighten the sensitivity of noninflamed organ that are innervated by the same afferent neuronal pathway [12]. Commonly, the overlapping of pelvic pain occurs between the lower gut, uterus and urinary bladder. Common convergence of different visceral primary afferents onto one spinal secondary neuron transmitting signals to the supraspinal nuclei can either synthesize or attenuate intrinsic cellular functions via activation of $P2X_3$ receptors by ATP and TRPV1 receptors by capsaicin within the L_1-S_3 DRG neurons. Furthermore, in addition to ATP, prostaglandin E2 (PGE2) is synthesized and realized during distention and contributes to hyperalgesia. We showed that PGE2 enhanced calcium responses induced by pronociceptive molecules such as ATP and capsaicin [2]. Together, our studies opened up a new paradigm of neuroplasticity: modulation of primary sensory neurons by sex steroids that may lead to structural changes within DRG. Estrogen can gate primary afferent nociceptors to enhance or decrease nociception.

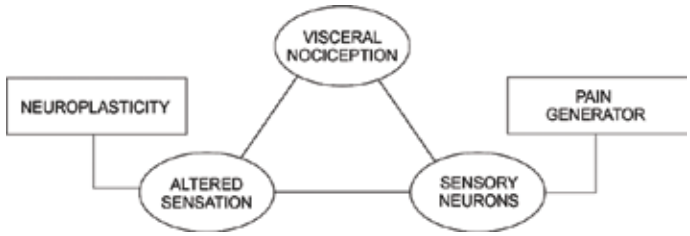

Figure 1. Conceptual model of regulatory mechanisms mediating neuroplasticity of visceral nociception at the level of primary afferent sensory neurons.

Pelvic pain is very subjective and thus difficult to standardize for any scientific modeling since its etiology affects different systems. In addition to nervous, urinary, gastrointestinal, reproductive and psychological systems are involved. Nociceptive behavior is highly complex: the affective experience leads to avoidance and often protective escape. New data will hopefully lead to the development of effective gender-specific therapies. Involvement of peripheral nervous system in mediating and/or regulating chronic visceral pain associated with nociception through structural and physiological changes at the level of DRG is confirmed. The new principle of neuroplasticity at the level of peripheral nervous system is important to understand the etiology of many chronic diseases associated with visceral pain (**Figure 1**). Peripheral sensitization at the level of primary afferent neurons (pain generator) leads to neuroplastic changes with major structural alterations. The observation that 17β-estradiol increased survival of DRG neurons [13] put this sex steroid hormone as potential neuroplastic modulator of sensory afferent neurons. Estradiol may act as transmitter molecule by changing excitability of DRG [14]. DRG neuroplasticity also contributes to hyperalgesia [15]. Noteworthy, treating pain can restore normal nervous system function. As with all new stories, the unusual concept gets most attention of medical and clinical communities. We convey the message by driving translational science into the new horizon and propose a multicomponent conceptual model of neuroplasticity associated with functional disorders.

Acknowledgements

This work is supported by National Institute of Health grant: SC1 NS 063939 (PI—Victor Chaban).

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Chapter 2

Plastic Adaptation: A Neuronal Imperative Capable of Confounding the Goals of Stem Cell Replacement Therapy for either Huntington's or Parkinson's Disease

Michael I. Sandstrom, Kevin A. Anderson, Naveen Jayaprakash, Parnit K. Bhupal and Gary L. Dunbar

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71790

Abstract

Although stem cell transplant therapy offers considerable promise for deteriorative diseases, the efficacy of its application may be mitigated by endogenous compensatory mechanisms in the host brain. Plastic compensation follows neurodegeneration, beginning at its very onset and minimizing early symptom expression. As researchers attempt to correlate symptom remission with the ability of transplanted cells to adopt specific cell phenotypes, they need to be vigilant of the possibility that competing, local compensatory effects may be altering the outcome. Clearly plastic compensatory mechanisms could confound desired transplant-derived improvements by supplanting the beneficial contributions of the transplants. As circuit-level adaptations occur, more explicit explorations of their relevance to neuronal transplantation success are needed. Conceptual models of undirected transplanted cells adopting preconceived appropriate roles require revision. The notion that newly transplanted neuronal precursors will incorporate themselves into host circuitry with mutual cooperation across both parties (i.e., transplant and host) without some symbiosis-promoting mechanism is naïve. Undirected local circuits could react to newly transplanted additions as intruders. We advocate that appropriate signaling from transplanted cells to the host environment is required to optimize the therapeutic relevance of transplantation. This review surveys critical signaling mechanisms that might promote symbiotic interdependence between the host and new transplants.

Keywords: stem cells, transplantation, Parkinson's disease, Huntington's disease, adaptive plasticity, development

1. Introduction

For several years now, efforts have been underway to examine and refine technology associated with promoting the incorporation of pluripotent stem cells from various origins following transplantation into the brains of patients suffering from various deteriorative diseases [1–3], or to test the viability of such treatments using experimental animal models [4–6]. The typical pattern of findings associated with clinical efforts is initial moderate symptom improvement, followed by either resumption of symptoms over time or highly variable therapeutic outcomes [7–9]. Common arguments raised for the mechanism underlying inconsistent effectiveness are that either the transplanted cells are not merging sufficiently with the host brain due to a timely competition-related synaptogenesis process, that transplanted cells are not surviving in the harsh environment of the host due to immune-system/inflammatory host responses, or both [8–11] (also see the extensive review by cell type [12]). While evidence for these arguments certainly exists, it remains unclear whether those arguments cover all the relevant possibilities that threaten the longevity of the transplanted stem cells' utility. One potential threat to the long-term efficacy of this treatment, or to stem cell transplant therapies in general, which is frequently overlooked, would be *plastic adaptation*. Briefly, plastic adaptation represents a multitude of cellular responses that occur with the apparent role of maintaining cellular homeostasis, yet within the nervous system also supports the maintenance of a sort of dynamic status quo in which compensatory changes adjust the actions or response capacities of local healthy neurons in support of a superseding circuit-associated need. Plastic adaptation also occurs within the physiologically healthy brain in order to adjust for novel needs, supporting changes such as long-term memory, habit formation, and other sorts of behavioral adaptation of organisms to new surroundings and demands (for examples see [13, 14]). A surge of inquiry into what are now known as epigenetic mechanisms supports the notion of a clear capacity of cells to respond to environmental stimuli by generating enduring changes in their genetic expression [15–18]. This, combined with numerous demonstrations of more transient receptor plasticity [19–22], defines neuronal cells as versatile in both short- and long-term periods in adjusting to their neurochemical and electrophysiological circumstances at their membranes and within their nuclei, respectively. Following transplantation, it is likely that plastic adaptation responses could occur in both populations of neuronal cells of concern, either the transplanted cells or the surrounding host cells that likely interact with the transplanted cells.

During nervous system development, the mechanisms that guide the distributions of cells and their connectivity offer a far more forgiving flexibility when compared to the harsher, more demanding adult environment we face when attempting to correct deterioration with transplants [23–26]. Growth distances for neurites are shorter given the smaller neuropil, and more overt chemical gradients support pathfinding [27–29]. A developmental neurogenesis surge supports self-repair in the event of cell destruction because phenotype commitment is guided by a progressive fulfillment of niches and feedback signals once niches are filled [30–34]. Differential neuronal responsibilities within developing circuits are coaxed into existence in the context of an enhanced adaptive plasticity on either side of synaptic clefts, where each contributes to phenotype adoption of the other while it is determined what they

might contribute to the developing circuit [35, 36]. As needs are met, postsynaptic neurons decrease their encouragement of subsequent equivalent connections by adapting their signaling [37–40]. How new afferents drive or control action potentials contributes significantly to circuit behavior, depending on factors as subtle as the proximity of synapses to a target neuron's trigger zone, while on the postsynaptic side the development of the trigger zone may modify when and how action potentials arise [41–46]. Incorporation into circuits relates to both identity and survival as neurons develop. As the neuronal phenotype is established, developing cells become increasingly dependent upon both afferent and efferent connections to other neurons. Neuronal fate seems to result from aspects of stimulation in the context of neurotrophic factors such as brain-derived neurotrophic factor (BDNF). Excitatory postsynaptic potentials known to increase intracellular calcium seem to participate in driving developmental determinations. This was shown by a series of experiments performed on precursor cells *in vitro* where calcium chelation blocked the establishment of neuronal phenotypes normally induced by either electrical or NMDA-glutamate stimulation in conjunction with BDNF [47–49]. Thus, precursors that receive insufficient controlling input to engage their activity likely adopt non-neuronal, glial, or support cell status, modifying or diminishing their contribution to circuits. When growing neurons establish connections to other neuronal populations, this provides them with target-derived trophic support that staves off programmed cell death that is likely to occur in its absence [50–53]. Surviving long enough to establish contributions is of course also important for transplanted populations, but evidence indicates that this is easier in the more forgiving context of development. A very useful series of explorations documenting how transplantation faces diminished success as the host ages was thoroughly documented in a mini-review by Sally Temple [54]. In addition, often the younger and less "experienced" or "committed" precursor cells are shown to more easily adapt into their transplanted roles than similar, yet older, populations [55–57]. In other contexts, such as the ability to properly generate blood cells following bone marrow transplants, younger donors seem to yield more successful results than older donors, indicating this age-dependency is not limited to neuronal populations [58, 59]. The similar goals of establishing appropriate cell populations to fill various niches following transplantation suggest if the environment were less competitive or more accommodating, and cells were guided by the more overt signals available during development, the process of incorporation would be more straightforward. In the adult brain, the mechanisms of plasticity engage to maintain the continuity of established function with mechanisms to prevent deviation from working systems; otherwise all nervous systems would constantly deteriorate into chaos. Thus, while similar concerns are present with transplantation, (i.e., coaxing the new cells to make useful and appropriate contributions to established circuitry), we cannot expect that new additions will naturally get swept into correct and working interactions the way they do during development.

To tease out the contributions of plastic adaptation to the success or longevity of stem cell transplantation therapy, it seems there is a need to expand inquiry further than whether transplanted cells develop into neurons, survive, or form mutually integrative connections with endogenous neurons. It appears equally important to determine how the cell populations influence each other and how each population adapts to this influence over time. There may be important clues

to the mysteries surrounding the impermanence of replacement therapy in how these populations adjust to the presence of the *other*. This chapter was written to consider current knowledge about plastic adaptation as it pertains to the act of incorporating transplanted neuronal cells or precursors into a damaged host brain. In addition, this review represents a general call for more direct inquiry into this subject in future efforts to explore and hone such a promising therapeutic technology. If plastic changes compromise the capacity to maintain symptom-suppressing benefits of these transplants, solutions to this will likely require more than tracking the quality and longevity of behavioral benefit or the anatomical persistence of the transplants over extended periods. Success may be enhanced by recognizing the ongoing patterns of plasticity with which transplanted neuronal cells must cooperate to earn the opportunity to contribute. Given that the age of both the cells transplanted and the host into which they have been transplanted are relevant to their incorporation and therapeutic efficacy, it appears that the capacity to adapt into the new environment depends on factors or signals from both elements that need to be understood to support moving forward intelligently with this therapeutic endeavor. The remainder of this review will address concerns regarding the host adaptive responses to the transplant as well as the transplant's adaptive response to the host that ought to be considered in this regard, focusing largely on efforts with Huntington's and Parkinson's disease.

2. Achievements of "successful" transplantations

Therapeutic support derived from neural transplantation likely necessitates circuit-level reconstruction so that certain missing neurobehavioral actions are restored. However, it is important to acknowledge that circuits can be supported by either the addition of new neuronal contributions that might restore disconnected components or by bolstering the inherent capacity of compromised circuits to adjust or compensate. The brain's inherent capacity to compensate for damage/disruption or "repair itself" is considerable and likely the reason why physical or occupational therapies support function restoration. Trophic support and other general support of persisting residual circuit components, receptor sensitivity adjustments, sprouting, and several other inherent mechanisms contribute to reparation (for an extensive review of these mechanisms see [60]). These trophic or supportive contributions can be accomplished by non-neural cells or glia that likely contribute mostly indirectly to neuronal circuit actions. In fact, Blurton-Jones and colleagues [61] demonstrated that transplant-derived BDNF was eventually responsible for supporting cognitive improvements in a rodent Alzheimer's model by promoting enhanced synaptic density in the hippocampus between preexisting neurons. Thus, it appears that either the transplanted cells become active contributors to the circuit or they support the existing circuit that itself seems to engage compensatory mechanisms supporting at least partial function. Therefore, it appears beneficial to respect that plastic adaptation persists as an ongoing process, regularly promoting positive improvements in functional circuits, and that a *successful* contribution of transplanted stem cells to existing neural circuitry necessitates a recognizable supportive contribution to this endeavor. It is our overarching concern that transplant efforts do not typically respect this context, usually holding a more direct circuit reconstruction as paramount with the presumption that the host brain will somehow also recognize our clinical perspective and modify ongoing adaptive mechanisms accordingly. When

this does not happen, we appear surprised that transplantation efforts impede healthy behavior restoration over time, or show diminished effectiveness over time—but we should not be.

While beyond the scope of this chapter, we nonetheless feel it is important to also acknowledge the prominence of neuronal circuit dependence on both use and the ongoing local actions of the immune system. It is likely that a repetitive drive on the circuit due to the person or animal engaging in systemic practice to recapture the skill they once had also supports circuit-level adaptation. This is likely why Parkinson's patients who regularly move and push themselves to actively engage compromised limbs rather than remaining sedentary reap clinical benefits from those actions [62, 63]. It is intriguing to consider these effects in the context of their ultimate therapeutic mechanisms which promote restoration or strengthening of key circuits with positive contributions to adaptation efforts, as well as the fact that once a part of a circuit, transplant-derived neurons may require practice to become proficient in their established roles. Of course, immune rejection and the broader context of inflammation also enter into this equation, given the common desire to utilize transplants in the context of deteriorative diseases or trauma-related damage. While it is arguable that convincing the immune system not to overreact has been extensively studied as a factor in this context (e.g., [64]), the inflammatory response certainly has the capacity to tailor the very adaptation mechanisms we will discuss (e.g., [65]). For extensive reviews of the reciprocal interactions between neural systems and inflammatory systems relevant to plasticity see Di Filipo and colleagues [66], or Xanthos and Sandkuhler [67].

Do the new additions engage with the existing circuits in positive adaptation-enhancing ways? Along the way it appears that there are adaptations on both sides that might enhance or diminish this relationship. If the adaptations diminish this circuit-supporting relationship, then the ability of the new transplanted cells to continue their presence and effectively support positive behavioral improvements will likely be lost and the clinical efforts of transplantation will likely be considered insufficient or transient. Alternatively, if the adaptations that occur enhance the circuit-supporting relationship while avoiding interfering with ongoing adaptive efforts, the success may extend further than the initial witnessed improvements into continuous ongoing improvements, rather than plateauing at some yet incomplete recovery. In this chapter we will divide our appreciation of transplantation-related plasticity as new cells establish roles contributing to existing yet compromised circuits first into whether the endogenous circuit adopts the newcomers as team players, and second whether the transplanted cells adopt the roles required of them to contribute to the circuit or not.

3. Adaptation of endogenous host tissue to neural transplantation

Although adult neurogenesis was overlooked in the past and neuroscientists were convinced that new neurons were not produced beyond the early stages of development, it has now been demonstrated conclusively that there are select regions in the brain that regularly accept new neurons into established circuits that are derived from precursor neuroblasts that retain mitotic capacity throughout our lives, and divide asymmetrically to produce new neurons as

daughter cells (for review see [68]). Two key areas that benefit from this neurogenesis would be the hippocampus and olfactory bulb, and the degree of this natural new neuron incorporation depends on the activity levels generated in these regions. Specific functions that rely upon neurogenesis include new learning for the hippocampus [69], and rich olfactory sensory experience for the olfactory bulb [70]. As this occurs regularly in an activity-dependent manner already, it stands to reason that neuronal precursors transplanted into these regions would be more likely to receive signals encouraging their incorporation into either the hippocampus or the olfactory bulb, and in fact, this seems to be the case [71, 72]. Yet in other regions such as the striatum (the main input structure of the basal ganglia), the capacity of endogenous neuronal progenitors to become neurons seems reduced compared to exogenous transplantations [73]. The answer to why this distinction exists has been a top priority among those of us who foresee more successful replacement therapies. While the whole picture is not available, what seems clear is that there is an interactive relationship between the endogenous host cells and transplanted cells at the center. The so-called "neurogenic" regions (hippocampus and olfactory bulb) where replacement happens regularly as part of the natural progression throughout our lives would likely not be a useful target region for clinical transplantation for any key neuronal disorders, given that their potential for reconstructive replacement remains high. Yet we might initially presume that the mechanisms encouraging incorporation, such as the guidance molecules used and trophic factors encouraging survival as connections are established, or the afferent connections grown into and onto the transplant cells as the afferent component, may follow rules similar to transplant events elsewhere.

When precision is required in the placement of axon terminals, it would seem that the parameters for what might be considered functional success would be correspondingly more restrictive or demanding. Here it is appropriate to briefly describe how establishing a wide range of general chronic dopamine can provide considerable benefit in Parkinson's disease, and the distinction between "open" diffusion-capable release mechanisms versus "closed" synaptic connections circumscribed by glial borders. Due to the chronic widespread levels of dopamine persisting in extracellular space, simple diffusion-based neurotransmitter delivery is often discussed without emphasizing the more nuanced details of precise release. To illustrate, in the case of dopamine loss in Parkinson's disease, the standard drug levodopa promotes endogenous release to higher global levels without significant dependence on direct synaptic integration of the remaining endogenous dopamine neurons, as a large majority of these are gone when this treatment is prescribed (presumably after at least 70% of the endogenous innervation deteriorates). Also, dopamine-lesioned experimental model animals have been improved by treatment with synthetic slow-release nanoparticles [74] or transplantation of genetically modified fibroblasts [75] that likely neither need, nor have the capacity to respond to, afferent control. In this context these treatments, as well as the dopamine systems considered, are seen as utilizing volume transmission or "open" synapses that tend to increase release levels over larger areas, based either on simple diffusion mechanisms or low-level chronic stimulation. By contrast, there are systems that rely on comparatively local transmission or "closed" synapses that are locally circumscribed by glial cells to certain synaptic junctions, and usually depend much more heavily on the timing of inputs for their function. Systems utilizing volume transmission would, by this definition, present an ambiguity to whether they necessitate as much acceptance into the network [76, 77]. So long as they provide the requisite compound this

contribution may suffice, at least initially, in bolstering the circuit in question, though release would need to take place in key areas to be effective. Dopaminergic inputs seem to exhibit both of these characteristics ("open" and "closed"). This concept, commonly overlooked in the clinic, will be expanded upon later.

Another aspect of transplant-related plasticity is the extent to which neurons are transplanted into a typical or atypical host environment for the neuron type that is their intended end-point for the current therapy. It is clear that during early fetal periods, useful progenitor populations can naturally undergo sufficient prior developmental modification to become predisposed to becoming a certain common neural type and can be found in regionally distinct populations in the fetus. For example, cells from the lateral ganglionic eminence show a high propensity to become GABAergic striatal neurons [78], or cells from the fetal mesencephalic region show a high propensity to become dopaminergic type neurons (e.g., [79]). Specific developmental trajectory predispositions can also be coaxed from progenitor cell populations *in vitro*, where the approximate mitogens, epigenetic cues, and morphogenic signals are maintained and progressively modified to encourage specific phenotype development trajectories [6, 80, 81]. Once neural developmental predisposition is established, it is perhaps fair to suggest that there are some host regions in which predisposed neurons would thrive as they would be placed into a "familiar" environment (i.e., a *homotypic* host region; e.g., GABAergic medium spiny predisposed neurons transplanted into the striatum) and some host regions that would *not* represent environments that might foster familiarity (i.e., an *ectopic* host region; e.g., dopaminergic destined neurons transplanted into the striatum). Precedent for this homotypic versus ectopic distinction has been set [82, 83]. Keep in mind that this distinction is made to capture the regional relations of predisposed neurons for certain locations, and functional benefit concerns are secondary.

For years, neuroscientists have been studying the anatomy of neuronal populations of various types that produce different neurochemical compositions throughout the brain and the corresponding afferent connections that grow into and drive activity in these different regions. Establishing appropriate afferent drive onto the neurons that are transplanted would be a clear sign that the circuit into which the transplanted cells need to merge has accepted them as part of the equation. Clearly then, when placed into a homotypic host region this sort of acceptance would be more likely based on the proximity of the transplanted cells to appropriate afferent input that such cells need to be driven properly by the host brain architecture. The prime example of this sort of transplant that has shown considerable acceptance into the host circuit and was extensively characterized by Klas Wictorin in 1992 is the intrastriatal transplant of striatal-predisposed precursor cells obtained from the embryonic day 14–15 fetal lateral ganglionic eminence following an excitotoxic lesion of the host striatum [84]. The extensive host innervation of this transplant along with the extensive growth and integration of the transplant with the host in the context of circuit re-establishment was dramatic, long-lasting, and seemed to contribute considerable support to the lesioned circuit as seen by neurobehavioral improvement. Wictorin indicated that the initial destructive lesion to destroy local endogenous striatal neurons is crucial for enabling the sort of host integration seen, as the absence of such a lesion (i.e., transplantation into an intact striatum) yielded far less integration [85]. It stands to reason this would occur because afferent inputs would find greater ease in filling an open void or niche so long as it maintains a general presence after

the lesion. A transplant without deteriorative or destructive loss would also be unnecessary because, as stated, the transplant is meant to restore the lost contribution. Wictorin describes a considerable ingrowth of afferent inputs from the host brain into the transplant with cortical, thalamic, nigral dopaminergic, and serotonergic inputs from the raphe that showed extensive yet differential degrees of penetration into the graft [84]. Also relevant was the point made about how regions that did not appear "striatum-like" seemed far less capable of inducing dopaminergic ingrowth and that specific transplants of cerebellar or cortical tissue into the excitotoxin-lesioned striatum of adult rats yielded no such dopaminergic innervation. Electrophysiological experiments demonstrated that these innervations of the graft were synaptically functional, supporting host-originating cortical drive [86, 87], and functional dopaminergic modification of GABA release from transplanted cells into the globus pallidus and the substantia nigra reticulata [88].

Although speculative, it is possible that even if the neurons transplanted into the striatum that became GABAergic did not grow extensively into the host tissue and participate more fully in the host basal ganglia circuitry, some circuit support might be established by enhancing only local GABA release from these neurons with limited incorporation as exclusively interneurons. It has become clear that in the context of the Huntington's disease condition, even prior to the major deterioration of striatal cells, there is considerable and abnormal spontaneous activity within the dorsal striatum [89, 90], and it appears that overactive glutamate release or diminished reuptake transport of glutamate is at least partially to blame for this [91, 92]. Under these circumstances, a considerable disruption of striatal function might arise due largely to this main input region being considerably noisier than normal (electrophysiologically speaking), and under such circumstances the proper selection of outputs would necessarily become challenged. If transplanted cells were simply driven by locally increased glutamatergic inputs or the ambient glutamate levels, after which they proceeded to feed back onto local medium spiny projection neurons in a manner that minimized this noise, some presumed information processing capacity might be restored, despite the lack of full integration.

To highlight the behavioral relevance of induced electrophysiology on transplanted cells, our laboratory initiated a project that involved preliminary transduction of transplant-destined neuronal precursor cells harvested from the subventricular zone of neonatal rats (P1 to P2) with Channelrhodopsin-2 (ChR2). This receptor construct allowed for rapid and exclusive optogenetic stimulation of these cells as they became functional neurons activated by blue light. The construct also contained a transgene with a synapsin promoter as well as code for enhanced yellow fluorescent protein (EYFP) for visualization post-euthanasia. It was our interest to explore the propensity of these transplanted cells to incorporate with the circuitry of the otherwise intact dorsal striatum in a manner that would allow a movable skull-mounted iontophoresis/single-unit electrophysiology electrode with fiber optic light incorporation to locate transplanted cells by slowly moving dorsoventrally across the striatum of a freely-moving rat and searching for units that would respond to various local stimulations. There were three stimulation types that could be generated: iontophoresis of glutamate, stimulation with 473 nm blue light, and behavioral stimulation. The advantage of this strategy was that only cells transduced with ChR2 (i.e., the cells to be transplanted) would show photosensitivity to blue light. Several interesting findings arose from this work. Previous work with a similar iontophoresis electrode (modified merely to allow the inclusion of a narrow fiber optic

cable for light stimulation in the present study) had clearly shown that rats with intact striata exhibit largely low levels of spontaneous activity [93]. Thus this technique that uses pulled 4-barrel glass electrodes (recording via 3 M NaCl, and iontophoresis of 0.25 M glutamate with a 0.25 M NaCl balance barrel and a narrow fiber-optic cable delivering blue light through the final barrel, wiring and fiber-optic cable connected to a combined swivel apparatus above the chamber) took advantage of a movable electrode holder allowing multiple exploratory passes through the dorsomedial striatum while monitoring the extracellular field for potential signs of single unit activity. Iontophoresis of glutamate was our global stimulant capable of activating any striatal neuron in proximity whether it originated endogenously or from the transplant.

We went in expecting a low yield of photosensitive units given established findings that transplanted neurons tend not to incorporate well in an intact striatum (e.g., [84]). In our experiments, each animal received approximately 40,000 neuronal stem cells as 8–10 neurospheres per animal except "controls." The distribution of behaviorally responsive units was similar to previous work with similar electrodes employed without light [93] among controls. Among our main findings was that none of the units that responded to light or light/glutamate combinations also responded to the behavior of the animal. This suggests that although glutamatergic inputs from both cortex and thalamus synapsed functionally with grafts placed into the striatum following an ibotenic acid lesion [84], we saw little evidence of the corresponding freely-moving-animal incorporation that likely would have generated behavior-induced responses in our localized and verified transplanted cells. Of the 40,000 potential contributors placed directly into the pathway of our electrodes, we recorded from approximately 20 light-sensitive cells per animal at 2 weeks and then found a far smaller number (approximately 2-3) at 4 weeks. However it was not the case that the neurons derived from transplants were unable to form connections, as on multiple occasions with the subjects tested at 2 weeks post-transplantation we witnessed responses of what we predicted were spontaneously active endogenous units that were clearly inhibited during light stimulation (see **Figure 1**). None of these light-induced inhibitions were found at 4 weeks, in part because spontaneous activity was also harder to find at this later date. However, this finding and the fact that 2–3 out of the average 20 light stimulation events yielded this sort of response at 2 weeks but not at 4 weeks also suggests the possibility of temporary local synaptic connections being formed and then lost between the periods explored. While it is possible that a certain lack of drive (evidenced by the lack of behavioral drive on light-activated units) may have contributed to their demise as well as inflammatory or immune responses, another finding from this study was particularly intriguing. During the search for EYFP-expressing units at the final stages of these experiments, rats euthanized after explorations at 4 weeks contained far higher levels of fluorescent units merging into the olfactory-destined rostral migratory stream (see **Figure 2**). Newly produced neurons from the subventricular zone (where our neural stem cells were originally harvested) initially follow the edges of the lateral ventricles and then proceed ventrally into the stream headed for the olfactory bulb [94]. Fluorescent cells were consistently found to be incorporated into this system in a more rounded and presumably migratory state that, while still potentially responsive to both light and glutamate stimulation, would not be expected to have incorporated host glutamatergic drive that has been associated with driving CAM kinase II responses and the corresponding cessation of migration and synaptic arbor development [95].

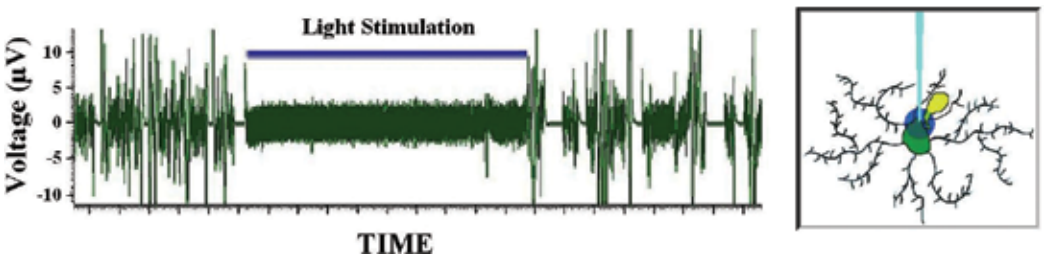

Figure 1. Spontaneous unit activity inhibited by light. Here a spontaneously active unit from within the striatum was inhibited by light. We predict that the connectivity was such that a transplanted (thus light-responsive) unit had adopted a GABAergic transmitter type and when it became activated by local light in sufficient proximity was induced to synaptically suppress the unit it had synapsed upon as depicted in the insert. *Insert Diagram*: Pulled glass electrode depicted descending from top. Darker sphere at electrode tip represents fiber-optic cable-derived blue light stimulation emanating from electrode tip. Small interneuron to electrode right depicts EYFP-expressing transplanted cell presumably sufficiently close to be excited by blue light but not to contribute to recorded *activation* response typical of "direct" stimulation. The recorded medium spiny cell, juxtaposed to the electrode tip, represents the spontaneously-active neuron providing recorded activity that was otherwise insensitive to the blue light barring GABA influence elicited from the sensitive transplanted cell connected to it.

From this study we concluded that when transplants are placed into the less-hospitable intact striatum it is possible that far fewer neurons make synapses with the local host and that when they do at earlier stages, these synaptic connections are far from permanent. It is likely that the unique proximity of the electrode, spontaneously active cell, and photosensitive cell depicted in **Figure 1**, that we hypothesize would elicit the witnessed inhibitory responses, would be serendipitous under the most opportune conditions, particularly considering the scarcity of spontaneously active striatal units within the intact brain (harder to find in general; [93]). Yet the consistency of the findings at 2 weeks and the complete lack at 4 weeks suggests that at least some transplanted units made only temporary synapses that disappeared later either due to cell death or resumption of migration. This may occur when the signals indicating the propensity for circuit interaction by the endogenous host cells are weaker, and there is subsequent reduced effort to formulate more robust and permanent interactions. Therefore, the presumption that once neurons form a synaptic relationship, the newly transplanted cells are somehow

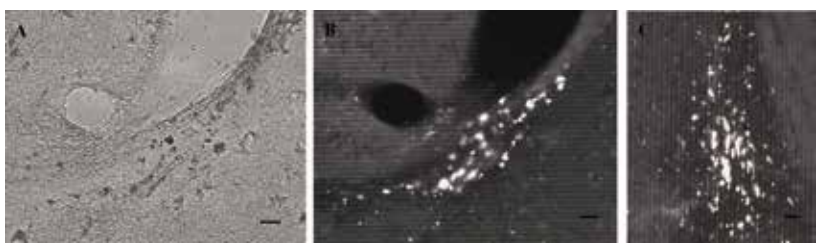

Figure 2. Fluorescent migrating cells at 4 weeks. Shown is a clear mass of cells that had migrated along the ventricles toward the bottom portion of the ventricle seen in both bright field (A) and the fluorescent images of B and C showing migrating transplant-origin cells fluorescing brightly in this location, outside of the recording area of the dorsal striatum. Line segments in each image represent 100 μm. C represents a different region of this clustering from a separate, similarly-treated animal focused at a deeper level of the rostral migratory stream. Bottom of ventricle not seen in C but is just above the upper left corner of image.

safe and likely permanently established is not supported; yet it seems to underlie the persistent sentiment that after certain periods of time, the mere existence of neuronally integrated and transplant-derived neurons in the host tissue represents a *successful* fix for the destruction or deterioration of host tissue. Such reasoning is fallacious and ignores the intricacies and constantly-shifting nature of even seemingly well-established intact neural circuitry.

Others who have transplanted into the intact striatum using lateral ganglionic eminence-derived cells witnessed a correspondingly diminished interaction with the striatal circuit. In fact, following such transplantations, Magavi and Lois [96] found a greater degree of growth into and synaptic integration with orbitofrontal cortex and the claustrum than with either striatal or nigral connections, indicating also the inability of the striatum to attract connections into the homotypic basal ganglia circuit. It is standard procedure that experiments investigating the degree of host incorporation depict dendritic arbors and other signs of likely synaptic input following transplantation, but it would be misleading to indicate that whatever snapshot taken in post-experimentation histology is a fixed and permanent condition. It would run contrary to what we know about natural endogenous synaptic plasticity to believe that any fixed depiction of synaptic status remains a permanent or "set in stone" phenomenon, as we know endogenous synapses are constantly dancing with each other, exchanging connections regularly due to competitive interactions [97]. To compete and participate in this drawn-out request for a place in the circuit, it would be important that sufficient drive is established and, after the driving elements (e.g., corticostriatal or thalamostriatal inputs) are relieved of their targets by prior lesions, there would be a likely increase in terminals seeking destinations, and this is lacking in the intact striatum. It stands to reason that neurons without such drive might continue to migrate until they can position themselves to receive it. Clearly a considerable effort is engaged by both corticostriatal and thalamostriatal afferents to synaptically integrate with striatal grafts that follow target-destructive excitotoxic lesions as well as transplanted neurons that grow far more extensive integrations into the basal ganglia circuitry [84].

As mentioned above, extensive dopaminergic ingrowth occurs from grafts of fetal progenitors into a lesioned striatum, indicating that not only does glutamatergic host innervation likely drive this population, but this population is also modulated by dopamine in a host-controlled manner. Despite this capacity, thus far, most experiments exploring the viability of transplanting cells as a treatment for Parkinson's disease have targeted cells predisposed to become dopaminergic ectopically into the striatum rather than the homotypic substantia nigra. The rationale behind striatal transplantation of these dopaminergic-destined cells rather than transplanting the cells into the substantia nigra region is largely because of the expectation that neurons transplanted into the substantia nigra region would not be able to grow axonal extensions sufficiently through the relatively inhospitable terrain of the adult brain to deliver the needed dopamine into the striatum. Also, striatal transplants would likely provide comparatively more dopamine in the target region. This concept was formulated by Anders Björklund and his collaborators [98, 99] as the idea of dopaminergic tissue transplants for Parkinson's disease was initially proposed. Previously described limitations to extensive axon growth through the adult CNS would clearly support this notion. Thus, the large majority of the experiments exploring replacement transplantations for Parkinsonian circumstances targets the dorsal striatum and would fall into the category of ectopic host destinations.

The question remains largely open how such cells, when they become dopaminergic neurons, will integrate into circuitry, given that they are typically stimulated at their cell body level by glutamatergic signals entering into the substantia nigra. In fact, the substantia nigra seems to receive active control inputs originating from the subthalamic nucleus and the somatosensory/motor cortex, both of which are touted as being capable of initiating rapid responses to salient events [100]. By comparison the cortical drive on striatal neurons tends to be highly converged on any individual medium spiny target from across relatively wide regions of the cortex, an organization that would require high collaboration between multiple regions to activate any single striatal neuron [101]. This common convergence is likely to be partially responsible for the relative silence observed within the striatum while otherwise intact animals are at quiet rest. The chances that cortical input into the striatum would even closely approximate that obtained from the cortical and subthalamic input to the substantia nigra is therefore low to begin with, let alone the serendipity that would be necessary to result in all such transplanted dopaminergic neurons stimulated by the same subset of glutamatergic afferents. This would severely challenge the precision of drive on these transplanted neurons, with terminations more haphazard across the transplanted population, resulting in a much more *constant* degree of afferent stimulation.

Explorations of the corticostriatal input into dopamine-predisposed grafts of fetal mesencephalic tissue have yielded mixed results from inputs that appear to take on the morphologic appearance of nigral cortical input (thick fibers giving off thin collaterals; [102]). Another point raised more recently by Braak and del Tredici [103] is that striatal medium spiny neurons tend to lose their spines over time during the ongoing pathology of Parkinson's disease and that dopaminergic inputs in the intact striatum of otherwise healthy subjects seem to interact in a complicated modulatory manner on spine shafts while the corticostriatal terminals engage their tips. As this arrangement is progressively lost, the ability of dopaminergic grafts to successfully interact with the main projection medium spiny efferents may also be jeopardized. If the drive on grafted dopaminergic neurons in the striatum is not well controlled, the ability to duplicate distinct periods of phasic release that mark events of behavioral significance may be missing from the grafted dopaminergic neurons.

Most synapses engage plastic mechanisms that adopt diminished responses to non-dynamic and unchanging levels of drive in a manner similar to the way sensory systems habituate to consistency. We know that rats given large unilateral 6-OHDA-induced lesions of dopaminergic input to the striatum tend to respond within days to apomorphine stimulation in a manner that depends upon postsynaptic modifications that establish "supersensitivity," and that there are modifications of dopamine receptors related to this occurring for extended periods following the lesion [104]. However, it has also been shown using equivalent lesions in mice that their rotation intensity diminishes over time when their lesioned hemisphere is continuously treated with apomorphine using an osmotic pump, suggesting that these modifications that support the supersensitivity *compensation* are reversible when sufficient dopaminergic stimulation remains persistent [105]. Processing the degree of postsynaptic responsivity to dopamine levels with a behavioral assay is common, since it is likely that adjustments in dopamine receptor sensitivity are continuously occurring in response to the degree of stimulation in a manner that stabilizes responses over time (e.g., [106]). It is interesting to note that, although very popular in the literature, recording diminishing rotation in response to transplantation has been deemed more distinctly inadequate

for processing transplants as only a very small boost in dopamine-producing capacity (such as 100–200 surviving transplanted neurons) seems sufficient to eliminate amphetamine-induced rotations by providing both chronic amphetamine-driven dopamine in general to the dorsal striatum [83]. Keeping this in mind, the ongoing adjustments in postsynaptic, and potentially presynaptic responses as well, are likely to reduce the dynamic responsiveness of the transplant-established dopamine system as seen by researchers, cautioning us about the inadequacy of drug-induced rotation in capturing underlying recovery dynamics [107].

The insufficiency of phasic release restoration also likely underlies the inability of Parkinson's patients on L-Dopa replacement therapy to rapidly adjust to ongoing motoric demands [108]. To accomplish a relative surge in dopamine release at a critical behavioral juncture such that the presence of dopamine provides sufficient ongoing support during more emergency situations, such as the need to escape from entrapment or predation, or falling into a body of water and needing to swim, phasic firing of nigrostriatal neurons occurs. The fact that gap junction connections have been found between nigral dopaminergic neurons and that electrophysiological behavior of the nigral population as a whole maintains consistency indicative of such electrotonic coupling [109, 110] suggests that wide ranging locations within the anterior striatal targets receive temporally consistent bursts as a result, in time with the events necessitating dopaminergic modulation. This phasic firing was recorded by Wolfram Schultz from dopamine neurons in the primate ventral tegmental area in his famous experiments that showed the cues responsible for generating increased drive on these mesolimbic neurons shifted from the initial pure reinforcement toward environmental cues *predictive* of the reinforcement and/or the risk associated with the reinforcement [111–113]. It is likely that what engages phasic drive among those neurons that support proper motivation in the arena of learning conditioning is driven in a manner somewhat distinct from the phasic drive on dopamine neurons that serve movement-related calculations within the dorsal part of the striatum. As we approximate human viability of transplantation, it is perhaps fair to mention that the borders of these two dopamine systems within the broader striatum of primates may not be as simple as their general projection parameters, and there is considerable overlap between the ascending dopamine systems (as described in [114]); there has nevertheless been a relatively consistent distinction made in the functional attributes of the projections. The apparent overlap may support the anecdotal events we have heard of where an immobile Parkinson's patient can initiate movement toward the exit of a building should this patient hear warnings of "*fire*" being exclaimed locally, though a stress-related release would be phasic.

Striatal cholinergic interneurons of the large aspiny variety are more likely to be tonically active for larger proportions of time than the main population of medium spiny GABAergic neurons, so their contributions to the ongoing processing within the region can be described as dynamic. These interneurons are controlled in a complicated way by dopamine, glutamate, and local GABA signals. Upon deeper scrutiny, the dopaminergic control of these large aspiny cholinergic neurons has been shown to involve differential employment of glutamate co-released from dopaminergic terminals along with dopamine between dorsal and ventral striata, rendering these regions distinct in how acetylcholine is driven [115]. The common understanding of the interaction between dopamine and acetylcholine in the striatum is that it is inverse, such that phasic bursts of dopamine lead to phasic pauses in ongoing activity among the large aspiny

striatal cholinergic neurons. The inverse responsivity to reward in the striatal systems driven by the dopaminergic input akin to what Schultz recorded from was clearly demonstrated by Morris and colleagues [116] in their work that looked at the dopamine responses simultaneously with the cholinergic responses. The complexities of that interaction and the manner in which it may in fact capture an extensive range of guidance information has been thoroughly described (e.g., [117]). Acetylcholine is unique compared with other transmitters in that the enzyme acetylcholinesterase that is abundantly expressed externally breaks the molecule down rapidly and restricts the domain of effectiveness to localized regions. This is relevant to our story because acetylcholine modulates dopamine release at the terminal level [118], adjusting the release that may otherwise be driven by afferent stimulation at the cell body level. In fact this level of local cholinergic control is likely to be driven differently by thalamostriatal versus corticostriatal origins of glutamatergic drive [119], providing those two differential systems unique access to this key transmitter input. On top of this, it has become clear that glutamatergic input also controls local dopamine terminal release in a receptor-dependent manner such that dopamine release may well be modified by both local striatal (reviewed in [120]) and distal nigral/VTA mechanisms. As described before, these inputs find their way in and around the spines expressed by the medium spiny neurons such that glutamatergic inputs to the spine tips get molded by dopaminergic, cholinergic, and GABAergic inputs engaging spine or dendrite shafts (see **Figure 3**) to maintain the proper final output signal that proceeds through the basal ganglia. To be effective, the timing of release would need to be carefully controlled as well as proximity. It is clear that these temporal dynamics contribute to the utility of each differential transmitter contribution, and to the overall effectiveness of the projecting efferents carrying signals to further destinations in the basal ganglia loops, as this aspect of basal ganglia function has been reviewed and explored extensively [121–123].

In the context of the present review, it is relevant to pause and ask ourselves if ectopic transplants of dopaminergic neurons into the striatum, during the course of or following extensive destruction due to Parkinson's disease, can approach the dynamic control present in the otherwise intact system. These transplanted dopaminergic neurons would likely be connected to rather haphazardly by corticostriatal or thalamostriatal glutamatergic, local cholinergic or GABAergic interneurons, and already lack the over-arching control of phasic release that is typically driven at the substantia nigra. The local control features described above have led investigators to suspect that dopamine release within the striatum might be *considerably independent* of nigral control [124], further supported by both anatomical evidence of striatal-derived fibers growing into grafts [125], and electrophysiological evidence showing approximately 50% of grafted mesencephalic cells being activated by frontal cortex stimulation [126]. However, none of these supporting findings suggest any clear resumption of the temporal dynamics of dopamine modulation in a manner that might be expected to fully restore behavioral versatility. Not only this, but such ectopic transplantations would likely lead to a dispersion of neuronal soma due to migration throughout the striatum that, while often seen as a positive attribute given the likely corresponding breadth of dopamine contribution, would also render the temporal release control much more regionally distinct. The previous point about gap junction connectivity between dopaminergic neurons suggests that at least initially, electrophysiological phasic firing is driven in a more unified manner that is more likely to be

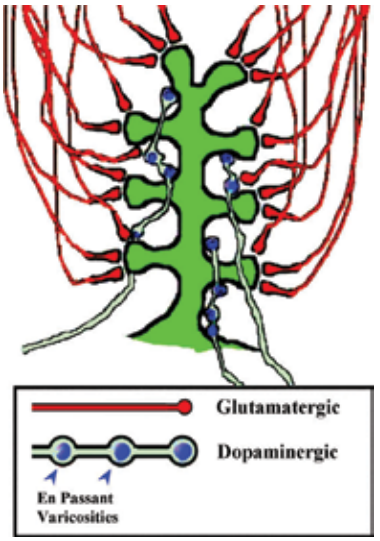

Figure 3. Simplified standard medium spiny dendrite arrangement. The prominent central aspect represents a medium spiny cell dendrite expressing standard dendritic spines. Onto this, many narrow glutamatergic inputs are depicted as synapsing onto the tips of dendritic spines, while dopaminergic input travels upward juxtaposing en-passant varicosities onto spine or dendrite shafts, modulating receptive states in a coordinated manner. Dopaminergic inputs are temporally enhanced by coincident phasic stimulation at the level of the nigra along with electrotonic coupling of nigral neurons. Proximity of cholinergic and GABAergic inputs not shown but each transmitter contribution influences the other either directly or indirectly through their converging influence on the medium spiny striatal efferent.

behavioral-event-related rather than based on local control, with local control as a secondary mechanism. As diffusion of the released dopamine occurs between such contributing neurons, this differential release control would likely establish a new consistent ambient chronic level, lacking a temporal relationship with sensorimotor events that dopamine is meant to modify, providing progressively decreasing phasic relevance to the circuit over time. At this point, if the host system has not adapted in ways that reduce dynamic sensitivities, such dopamine contributions may elicit disruptive effects, such as dyskinesias [127, 128], though other research calls this into question [80, 129]. Certainly the diminished temporal control of striatal dopamine release would be a reason for diminished support of dynamic behavioral control, and might be argued to underlie the longevity of a graft's therapeutic effectiveness.

4. Adaptation of transplanted neural cells to the endogenous host tissue

It is nearly impossible to distinguish between host-to-transplant and transplant-to-host communication because the interactions between both populations are so intimate. However, for the flow of this review, we decided to first address mechanisms of concern regarding modifications engaged by local host cells such as their growth into the transplant and efforts to exert control. Now, we turn our attention to the manner in which transplanted cells likely

recognize what they should do and how they grow into the host with an effort to exert control. As indicated previously, there are certain pro-generative regions such as the olfactory bulb and the hippocampus that are known to accommodate transplantation of new neural cells into their architecture more readily. These structures engage a system already established to continue adding new neurons into their circuits with adjustments according to demand regularly throughout adult mammalian lives, while other structures more commonly experiencing disease-driven deterioration are, unfortunately, less accommodating. Certain practical matters come into play when arranging a protocol for transplantation that limits how developmentally committed the transplant-destined population will be, deviating from the ideal circumstance of, for example, generating pure populations of dopamine and cAMP-regulated phosphoprotein (DARPP-32, marker for the classic resident medium spiny type) expressing GABAergic neurons for striatal transplant in Huntington's disease. While it is often an implicit goal to develop purified populations of only desired cell populations for transplantation, the context of "normal" developmental phenotype adoption seems to be modulated quite handily by local glial cells. If not somehow biochemically prevented from doing so, pure stem cell populations also typically develop into mixtures of neurons and glial cells in a manner approximating that expressed developmentally, with more glial cells than neurons, because the signaling molecules responsible for producing this distribution derive from the manner in which neuronal populations distinguish from epithelial populations with both distal and surface interactions [130]. By comparison, the more purified populations of specific neurons raised *in vitro* by various groups [131], would require considerable artificial manipulations compared with natural development.

As described above, previous transplant efforts have utilized populations of cells that have already developed a predisposition toward neuron types that have been responsible for some cells thriving in homotypic versus ectopic target destinations. Thus, when working *in vitro*, complete pre-differentiation and purification of specific neuronal populations requires extensive prior modification, and these efforts seem to benefit from the inclusion of developmentally-consistent proper local glial cell support [132–134]. *In vivo*, this supporting role seems largely adopted by astrocytes that enhance survival [135]. Neuronal differentiation seems to occur in conjunction with an effort to extend neurites and seek connections; a sensible concept given the previously-described importance of being connected to, and making connections in, the developing organism. After culture, the cells in question need to be lifted from their culture conditions and placed into a delivery mechanism (typically a syringe), for transplantation. It has been pointed out that primary neuron cultures might be particularly sensitive to the trypsin dissociation step and suggestions that more gentle procedures, such as papain, for dissociation of these cells have been made in recent scientific communications [136, 137], along with the notion that extensive floating cultures also face challenges. Therefore, researchers frequently opt for transplantation prior to full neurite extension and interconnectedness, even prior to full neuronal commitment, with the presumption that remaining neuronal commitment will occur *in situ*. Transplants often occur with suspensions of dissociated individual cells or of neurospheres (for review of neural sphere transplant contributions see [138]). If final neuronal commitment occurs after transplantation then, to some extent, this process will be guided by environmental cues within the host tissue.

Clearly, to adopt a specific neuronal fate with exclusive neurotransmitter-releasing properties, there are specific genes that must be expressed, as each phenotype would require either exclusive gene expression to produce or post-translationally select specific neuropeptides for release, or to process precursors enzymatically to generate classic neurotransmitters. These genes and immediate products have generated a new phenomenology that captures the immunohistochemical characterization of cell types in modern histology. It has, however, become abundantly clear that gene expression responsible for this is controlled by complicated internal processes that, despite early presumptions to the contrary, are not permanent or irreversible. The more prominent of these involve histone manipulations such as acetylation or methylation of DNA. This leads to the DNA source of specific genes becoming buried within inaccessible Gordian-like knots, which may be unwound to allow for DNA transcription under certain contexts. Current technology maintains the clear capacity to take committed cells and reverse this genetic commitment to generate what is now called "induced pluripotency," essentially restoring a precursor status to cells, even after they have become a more standard somatic type. In order for this induced pluripotency to be remotely possible, as well as subsequent guided induction of specific types of neurons from such cells, some genetic propensities must remain, even in cells that have outwardly adopted noticeably distinct fates. Early explorations of neuronal phenotype commitment involved explorations of the bizarre and seemingly extreme tendency of sympathetic neurons that ended up targeting the sweat glands to *convert* from a noradrenergic to a cholinergic phenotype [139]. Considerable controversy surrounded the search for the target-derived factor that was responsible for inducing this switch, which was clearly necessary given the cholinergic receptor population of the gland, and research centered on a cytokine family molecule [140]. The capacity of neurons to switch transmitter expression or take on more complicated forms of expression during development is broader than this [141], but these examples clearly demonstrate that the mechanisms underlying neuronal phenotype determination remain versatile and responsive to external signals, even after neuronal differentiation. This suggests that precursor cells would harbor an even more versatile capacity to properly respond to host signals and, as such, merge into the circuit in a regionally attentive manner.

The process of becoming a neuron is sensitive to the degree of electrical stimulation in that the corresponding calcium increases tend to facilitate neuronal differentiation [47]. In the case of the earlier stage fetal development following more complex anterior-posterior differentiation of the neural tube and the establishment of prosencephalon, mesencephalon, and rhombencephalon, more distinct neuronal populations begin to emerge due to specific morphogen combinations and temporal sequences of exposure [142, 143], with certain regions producing environmental signals conducive to specific neuronal subtypes. As glutamatergic neurons distinguish from GABAergic neurons within regionally distinct sub-areas of the subventricular source of new neurons, basic helix-loop-helix transcription factors are induced to kick in, such as neurogenins, leading to a glutamatergic fate, and Mash-1 along with the "distal less" homeodomain genes presumably induced into expression by positional signals Dlx1 and Dlx2 that seem to promote a GABAergic fate, with the degree of initial neurogenin or Mash-1 expression a seemingly deciding factor [144]. When cells that are transplanted into the early developing nervous system are evaluated for the expression of regionally specific markers, such as

these transcription factors, they seem to express themselves as if they pay little heed to their ectopic location. For example, one experiment demonstrated that only about 6% of the transplanted cells that took up residence in the striatum expressed Dlx immunoreactivity, indicating a GABAergic trajectory, while 37% of the transplanted cells residing in the tectum, a region typically devoid of this marker, expressed this marker [145]. Such data suggest that there may be a longer-term set of guidance steps that feed into promoting the regionally specific neuronal phenotypes that include an earlier need for cell juxtaposition interactions that generate homeodomain predilections prior to the final departure of neurons from the cell cycle. The circumstances seem somewhat different in vitro with populations of neural stem cells, because when growth factors are carefully removed from such populations within N2B27 media (conducive of neuronal differentiation), the large majority of neurons (over 80%) produced adopt the GABAergic phenotype [146]. Another informative embryonic-to-embryonic transplant study that broadens the developmental factors associated with phenotype decisions was performed by Magrassi and colleagues [147]. They found that when ganglionic eminence-derived neurons clustered together as aggregates, they supported each other in maintaining their GABAergic phenotype fate while, by contrast, neurons that migrate into ectopic locations as individuals may adopt alternative fates guided by local signals. The capacity of cells that are predestined to adopt alternative fates based on responding to positional signals has been demonstrated in that they are able to adopt cortical-like morphologies when they migrate into the cortex, presumably as individual neurons [148]. In fact, at this early point in transplantation evaluation, outcome assessments based largely on the morphology of neurons indicated that transplantation into any region seemed to be guided by local cue phenotype induction toward locally appropriate fates (e.g., [149]). These days such assessments are largely considered insufficient, and a more marker-specific immunocytological phenotype determination is encouraged. When these are evaluated with the subventricular-zone-derived adult neural stem cells and their common migration trajectory into the olfactory bulb, it has been suggested that differential phenotypes or phenotype-restrictions might begin to be established quite early, prior to migration to the destination, given the diversity of expressions despite common local cues within the bulb [150]. However, a more recent hypothesis-driven review compiled by Sequerra and colleagues [151] suggests that the capacity for true phenotype guidance from local cues can be quite extensive, such that environmental circumstances can differentiate between glutamatergic and GABAergic phenotypes and manipulations of morphogen expressions, such as sonic hedgehog, by blocking it in ventral locations or ectopically expressing it in dorsal locations can "dorsalize" neuronal phenotypes in ventral sectors or "ventralize" them in dorsal sectors respectively. Transplantation of small numbers of embryonic stem cells into various regions and subsequent specific tracking of resulting neurons indicates that within the intact mouse brain there is a regionally distinct capacity to promote the incorporation of new neurons that is largely progressively lost with age, but when neurons merge into the circuit during more accommodating developmental periods, they typically adopt regionally appropriate functional contributions.

What about the projection potential of transplanted neurons as they attempt to integrate with the host? As described before, Wictorin and his collaborators [84] explored the placement of presumed striatal-predestined rat embryonic ganglionic eminence-derived grafts into the striatum of adult rats following excitotoxic lesions in this same-target location. They witnessed

significant growth into the basal ganglia circuitry with the majority of the graft adopting a GABAergic phenotype and projecting *myelinated* axon growth into the host globus pallidus, with only a few projections showing retrograde transport indicating they reached the substantia nigra reticulata. Interestingly in his review, Wictorin [84] also mentions control studies performed in which cerebellar precursor tissue was transplanted into the striatum instead of the ganglionic eminence-derived cells, and that this ectopic transplant resulted in considerably diminished outgrowth and diminished integration with either glutamatergic or dopaminergic host-derived afferent ingrowth. The migration of neurons was even affected when transplants were placed into the developing neonatal striatum in a restricted manner when cerebellar precursor tissue was used instead of striatal precursor tissue, indicating the relevant guidance cues are established early [152]. As might be imagined, hindbrain (rhombencephalon) precursor tissue, transplanted into the adult cerebellum after excitotoxic lesions in that region, adopts several local phenotypes and seems to grow extensively into this region, recapitulating that circuit to an arguably regionally specific, yet similar, degree [153]. This indicates that homotypic versus ectopic concerns are more universal and relevant to multiple regions. Interestingly, human-derived precursor cells transplanted into the rat brain have also been described as growing more extensively into the rat host than do either rat- or mouse-derived precursor cells, though they seemed also to be sensitive to being placed within a homotypic domain (striatal into striatum) versus an ectopic domain (cerebellar into striatum), once again expressing significantly reduced growth into the latter host location [154]. It is intriguing to speculate about how human-derived neural precursors attain a more prominent and extensive host integration into rat host tissue when the signals presumably inspiring growth are likely distinct, though it has been speculated that human cells harbor a propensity to grow for greater distances before target-derived signals are expected while exhibiting a relative insensitivity to growth-inhibiting signals that are produced by the host. The bottom line message of this section is that cell populations seem to acquire, and become limited by, their: (1) neuronal status where they depart from the mitosis cycle, (2) neurotransmitter phenotype that limits their range of influence, and (3) regional predilection that bolsters their contribution to the circuit when they recognize "home" and diminishes contributions from cells delivered elsewhere. This regional predilection has been described above for striatal, or ganglionic eminence-derived neurons, transplanted into the striatum. Apparently, it is also relevant to dopaminergic neuron transplants of fetal ventral mesencephalon, which typically includes both nigral (A9) and ventral tegmental (A10) "type" neurons and for which the ability to successfully re-innervate the striatum is far superior among the nigral type, both anatomically and in terms of behavioral support [155–157]. It seems clear that there are niche components integrated into neuronal phenotypes that extend beyond merely the transmitter they express.

So what does this say about the ectopic dopaminergic cell transplantation into the striatum and the idea that transplant contributions will be more successful if placed within their target region? These efforts do require some background explanation. Parkinson's disease has been understood as mainly a loss of forebrain or more specifically striatal dopamine for most of its history. Although the specific temporal and spatial actions of striatal and greater basal ganglia neurons have been better understood for quite some time, there has been a corresponding lack of attention to the temporal dynamics of the dopamine provisions to that system in the clinical world, presumably because the tools available seem to work without a need for such a

concern. The virtue of the most common pharmaceutical treatment, Levodopa, seems to derive largely from ensuring a more consistent dopamine presence. Dopamine agonists, also used as a pharmacological treatment, likely linger outside any strict temporal parameters in that they are likely removed only by diffusion. Animal models benefit from rudimentary delivery mechanisms that also largely appear to maintain dopamine presence with little to no dynamic shifting according to "need," as might be expected of the phasic attributes of an intact dopamine system. Clearly the previously-mentioned movement tests that reveal the insufficient temporal precision of behavioral control with classic treatment has heightened awareness of the concern [108]. Nevertheless it is readily apparent that dopamine cell transplantation for the Parkinson's patient remains largely conceptualized as a more sophisticated delivery system for dopamine that may become increasingly necessary as the ongoing deterioration of dopaminergic neurons diminishes the patient's capacity to convert Levodopa into dopamine. The enzyme aromatic L-amino acid decarboxylase is necessary to complete this conversion step and experimental animal tests indicate that the effects of Levodopa both depend on this action and that serotonergic neurons within the brain, which also harbor this enzyme, may be capable of supporting continued benefit from the drug [158]. Dopamine neuron transplantation into the striatum likely provides improved benefit beyond this serotonergic neuron involvement in that transplanted cells would be capable of growing into greater proximity, and that their terminals would maintain an improved reuptake transport control of the corresponding dopamine released that serotonergic neurons would lack. Nonetheless, for the reasons mentioned in the previous section, the absence of controlled dynamic modification would develop into a problem over time as the greater circuit compensates, and should be attended to as clinical strategies are formulated.

Presumably behavioral support would be improved if derived from dopamine release from more fully reconstructed dopaminergic projections from a grafted nigra into the striatum as there would be improved potential for dynamic temporal control by more "appropriate" afferents. Despite the fact that most reviews of transplants for PD mention this point (e.g., [155]), to our current knowledge, despite several apparent successes in establishing nigra-to-striatum re-innervation from nigral dopaminergic grafts [159–165], there have been no systemic assessments of the afferent control of these grafts established by the host. Clearly the main interest at present with such a grafting strategy is to ensure the dopaminergic reconstruction extends across the inhospitable terrain of the adult brain from the nigra to the striatum. Likely due to the expression of considerable disruptive signals within the adult CNS and the need for more continual support during the growth process, initial efforts to coax this reconstruction from homotypic nigral-placed grafts were unsuccessful in breaching the divide, though even these relatively nigral-restricted grafts did provide some behavioral support [166], likely due to the importance of dendritic dopamine release within the nigra [167]. In fact, the neuronal populations upon which the dendritic dopamine release likely plays its role are GABAergic neurons in the reticulata, and efforts to transplant GABA-producing neurons into this region have also demonstrated some behavioral benefit, presumably by somehow expanding the repertoire of this basal ganglia output region [83, 168]. Such an effect speaks volumes, questioning the precision of the disinhibitory feedback loop formed by striatal efferents to the nigra reticulata and

on to the ventrolateral and ventromedial thalamus, understood to form the basis of motor program selection provided by the basal ganglia [169]. These GABAergic transplants into the nigra also may contribute benefit by increasing the local suppression of noise as described previously for the seemingly noisy striatum. As efforts expanded, it became clear that considerable neurotrophic support was necessary and this was either provided by "bridge" tissue grafts that might be likely to release such compounds, such as Schwann cell type cells, or the local cells were induced to release these compounds by viral transgenic expression (e.g., [82, 160, 170]). This strategy renders the CNS territory through which the new dopaminergic fibers must grow more hospitable, presumably also providing retrograde support signals, inspiring continued growth, and staving off the previously-described cell death that results from the lack of connectedness during this growth journey.

It is notable that while many of these strategies were being tested, a well-recognized age dependency was revealed in that even significant dopamine-depleting lesions performed on young and neonatal animals yielded only mild or dramatically diminished behavioral deficits [171, 172]. At the same time, these animals, when grown to adults, still depended upon dopamine for their locomotor behavior, albeit in an altered way [173], and produced sufficient but diminished levels of striatal dopamine to accomplish this [174]. Perhaps the enhanced plasticity supporting this maintenance of dopamine-dependent behavioral control was derived from the natural expression of neurotrophic factors that maintain a higher presence during early postnatal periods of development [175]. A general protection of dopaminergic neurons has been shown to derive from glial-derived neurotrophic factor (GDNF) in particular [176]. In fact, it has been determined that developing nigral dopaminergic neurons depends considerably on GDNF for their survival and maintenance by the establishment of a conditional GDNF knock-out mouse that exhibits clear dopaminergic disruption-related hypokinesia and diminished tyrosine hydroxylase among dopaminergic neurons once GDNF production is blocked during adulthood [177]. The age-dependency factor, regarding the dopamine system, has also been demonstrated in the ability to incorporate dopaminergic transplants. Efforts to unilaterally transplant dopaminergic fetal grafts into the substantia nigra on postnatal days 3, 10, and 20 into rats that had received bilateral 6-OHDA lesions on postnatal day 1 resulted in the intriguing finding that transplants given on postnatal days 3 and 10 showed evidence of nigrostriatal regrowth or fuller incorporation into that circuit, while those receiving transplants on postnatal day 20 did not [178]. It seems GDNF and BDNF may cooperate, to some extent, in supporting dopaminergic cells, as BDNF has also been used successfully to promote a sparse re-innervation of the striatum from a nigral-targeted graft [179]. The neurotrophic factors that seem to play supportive roles expand considerably when observed in the light of what supports the original production of the medial forebrain bundle during development [180].

Coaxing the growth-trajectory environment to also express adhesion molecules that new growth cones might grow along has also been considered (e.g., [170]). The sorts of glial cells or other tissue, which are often added to the equation of a "bridge," are generally not those known to be disruptive to axon growth such as astrocytes and oligodendrocytes. In fact when the medial forebrain bundle pathway is observed for regrowth following axotomy, sprouting of new axons is considerably enhanced by removing glial cells from the growth path by use of

a glial toxin [181]. Among the putative interfering variables to this sort of existing cell regrowth are heparan sulfate proteoglycans, chondroitin sulfate proteoglycans, and keratan sulfate proteoglycans that are derived from activated astrocytes that surround lesions [182]. Developing dopaminergic neurons of the substantia nigra must sprout axons that grow anteriorly for substantial lengths to reach their target termination zones. Also, anatomists have recognized a substantial formation of synapses *en passant* among these and other monoaminergic neuron types, suggesting multiple way stations occur within target structures prior to establishing classic terminal boutons, each subject to various degrees of local control [183]. Their extensive growth trajectory requires growth-promoting and cell-death-diminishing signal molecules during axon extension, particularly when transplants are placed during adulthood when the road is longer. Thus, regrowth from such posterior-ventral origins likely depend on the presence of cellular *guideposts* along the way that might break up the full growth required of the nigrostriatal tract into growth stints that are supported by retrograde feedback signals, as well as the removal of potentially interfering substances derived from activated glia. The involvement of glia in the diminished propensity to grow extensive connections from posterior regions may also depend on the manner in which the original lesion is created. It may be that the neurotoxins used in animal models to induce dopamine-depleting lesions (e.g., 6-OHDA, MPTP) exacerbate glia, resulting in more activation of astrocytes and thereby interfering with regrowth (see [184, 185]). However in most idiopathic cases of Parkinson's disease, there is a distinct lack of reactive astrocytes during the course of deterioration or afterward [186, 187], indicating that the contributions of chondroitin sulfate and other growth-interfering responses might be lower in this condition, despite a clear insufficiency of dopaminergic regrowth. Nevertheless, the indication that reactive astrocytes may linger for up to 90 days following 6-OHDA administration [185] is intriguing when the rat 6-OHDA treated model system is considered because usually transplantation is performed prior to that time in those animal models.

Homotypic transplant placement may also be promoted in the context of dopaminergic cells, given that their qualities may be guided more substantially by local cues, as well as gaining from local afferent control. During development, the local ventral midbrain environment seems to contribute considerable epigenetic guidance to newly generated neurons in the form of morphogens. One of these morphogens that has been classically associated with ventral development beginning at the neural tube stage is sonic hedgehog (for review see [188]). The two prominent locally secreted factors that drive dopaminergic phenotype development are fibroblast growth factor 8 (FGF-8) and sonic hedgehog [175], leading to internal genetic expression of Nurr1 and Ptx3 transcription factors that further establish phenotype delineation. This is likely why those two secreted factors are used in protocols that guide the development of dopaminergic phenotypes from more pluripotent precursors *in vitro* (e.g., [189, 190]). When ventral mesencephalic-derived embryonic stem cells are left to develop freely in culture, many of them develop as dopaminergic, but there is also a mixture of phenotypes that might be expected from the ventral midbrain or hindbrain such as serotonergic and GABAergic neurons. Efforts to improve the yield of dopaminergic phenotypes have produced multiple proposed protocols involving different steps that replicate different aspects of developmental phenotype adoption. For example, one of these uses the Wnt signaling to influence developing neurons at the location of the developing nigra. Wnt signaling seems to be established to differential degrees in the developing nervous system, in large part by

cell to cell contact information and signal gradients that get established during the course of progressive commitment in gene expression. Specifically, inducing the transcription factor known as Wnt5a via transfection, following sonic hedgehog and FGF-8 exposure, seems to generate greater dopaminergic phenotype yields than sonic hedgehog and FGF-8 alone [191]. In addition, bone marrow derived stem cells seem to require a neuronal-enhancing, region-specific environment characterized by low oxygen, retinoic acid, and continuous neurotrophin-3 stimulation, as these in combination with the aforementioned sonic hedgehog and FGF-8 stimulation seem to enhance dopaminergic phenotype expression further [192]. All this indicates that there are specific local environments that would induce phenotype commitment based on regionally-specific combinations of factors that are provided in the appropriate sequence during development, and these remain in a sort of residual form still capable of supporting, albeit at a more limited degree of commitment, in the adult structure. The capacity of dopaminergic neurons grafted into the nigra to acquire afferent control remains understudied, but this capacity would likely be higher than that of ectopic transplants into the striatum. If gap junction connections could also be established with the local endogenous dopamine neurons of the nigra, this could enhance temporal pattern production substantially. Of course, if there are ongoing deterioration-inducing challenges among the Parkinsonian endogenous dopamine neurons this could induce the closure of gap junction connections, due to sensed pH or calcium changes, as a protective response [193]. However, given the circumscribed positioning of the dopaminergic neurons within the nigra following the transplant, it would seem a far more straightforward incorporation process regarding afferent stimulation in general than what would otherwise be required within the striatum.

Multiple placement transplants have been performed using animal models that have shown more substantial support for behavior. Experiments performed by Mukhida and colleagues showed considerable improvement in behavioral control with dopaminergic-destined fetal ventral mesencephalic transplants into the striatum, substantia nigra, and subthalamic nucleus that seemed to improve behavioral recovery better than the typical single transplant alone [194]. Clearly there may be a benefit to such extended transplantation but there are two major issues drawing the practicality of such strategies into question. First, transplantation of cells into one area in human patients is already a significant procedure, fraught with considerable risk and expense. The idea of multiple sites of transplantation would need to be justified by not only significant movement restoration but also in long-term viability beyond the 5-week, post-transplantation assessments commonly used. Second, given the concerns raised in this review, each ectopic transplant performed is likely to both provide some distortion in the temporal dynamics of delivery and also would perhaps block the more successful growth and penetration of the homotypic aspect. How well would new nigrostriatal terminals grow into the striatum if there are already local striatal dopaminergic terminals competing for CNS real estate in the same region? Given the clinical limitations and the likely extended growth time that would be required for nigrostriatal restoration, it may be prudent to consider formulating temporary neurons that could be progressively eliminated as fibers reach the striatum that could maintain a "substitute" dopamine presence. The concern with dual transplants (both in the nigra and the striatum) is that striatal transplants would likely diminish the growth or synaptogenesis drive among incoming nigrostriatal growth cones in a manner similar to what seems to occur among striatal neurons transplanted into the intact

striatum (establishing limited interactions with the host as a result). At this point, developing transgenic transplantable cells with pharmacologically inducible properties may be able to accomplish this temporary substitute goal. Initial inefficient support might be maintained during the growth process and this might be progressively and selectively removed as dopaminergic growth from homotypic regions reaches the area.

5. Concluding remarks

Plastic adaptation was described above as representing a multitude of cellular responses that occur with the apparent role of maintaining cellular homeostasis, yet within the nervous system also support the maintenance of a sort of dynamic status quo in which compensatory changes adjust the actions or response capacities of local healthy neurons in support of a superseding circuit-associated need. We understand that various CNS circuits establish the capacity to process a wide range of information with various degrees of versatility that presumably evolved to provide stability in some areas of common reliance and flexibility in areas where learning functions occur regularly and synaptic adjustments are correspondingly at higher demand. Neurons appear to undergo adaptations as they attempt to enter a circuit, and the environmental guidance for the control contributed by new additions extends to various degrees backward into the history of the newly added cells in question as it signals what it can provide and encourages host connections while it negotiates for acceptance into the host circuit and the privilege of contributing. As neurons do this during development, their relative pluripotency diminishes toward the eventual niche they enter into and it is highly likely that new neuronal contributions transplanted into these circumstances go through similar steps as they adapt to the roles they play. The long-term viability of additions requires that a utility anticipated by the circuit is fulfilled or the host circuit may adapt the addition out of relevance like an efficient social system isolates and eventually eliminates an influence perceived as disruptive. As an example, a long-term neurotransmitter lingering without dynamic change could come from leaky or malfunctioning neurons, so it would benefit a circuit to recognize this and diminish postsynaptic responses until the signal once again exceeds noise. Synaptic negotiation during development of the mammalian neuromuscular system, which has been more accessible and easier to manipulate with experiments, shows a series of back and forth messages that eventually culminate in the muscle fiber accepting one motoneuron terminal and rejecting other applications for the job (see [195] for detailed discussion of this process). It is likely that whether neurons incorporate into CNS circuits depends upon their capacity to apply themselves and on whether the corresponding job has already been taken, as indicated by the diminished success of transplants into adult intact CNS structures achieving synaptic incorporation. While it is possible for neuronal precursors to be conditioned in a manner that promotes certain wanted phenotypes, the ability to properly incorporate into a workable circuit is challenged when they are placed into an ectopic environment as described above. To draw an analogy to human socialization, it's as if the cells in question either have, or are given, an agenda that may or may not merge with the agenda of the local host circuit. The mechanisms in place that promote apoptosis, in this context, are a useful and positive contribution to the overall circuit despite the fact that the death of cells seems unfortunate. Neurons in various deteriorative diseases adopt abnormal activities. In fact, the whole basis

of deep brain stimulation, as derived from earlier therapies for Parkinson's disease, was to render excessive and aberrant activity quiescent (see [196, 197]). It is important that our clinical efforts consider the adaptive nature of the host tissue, into which we desire our transplants to be incorporated as this strategy will meet with greater long-term success and fewer potentially disruptive side-effects that generate additional, unwanted measures into the equation if these concerns are not accounted for from the outset.

Acknowledgements

Support for this work was provided by the Central Michigan Neuroscience Program, the Central Michigan University Office of Research and Sponsored Programs, the John G. Kulhavi Professorship, and the Field Neurosciences Institute.

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Chapter 3

Neuroplasticity in Young Age: Computer-Based Early Neurodevelopment Classifier

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.70894

Abstract

Neurodevelopmental syndromes, a continuously growing issue, are impairments in the growth and development of the brain and CNS which are pronounced in a variety of emotional, cognitive, motor and social skills. Early assessment and detection of typical, clinically correlated early signs of developmental abnormalities is crucial for early and effective intervention, supporting initiation of early treatment and minimizing neurological and functional deficits. Successful early interventions would then direct to early time windows of higher neural plasticity. Various syndromes are reflected in early vocal and motor characteristics, making them suitable indicators of an infant's neural development. Performance of the computerized classifiers we developed shows approximately 90% accuracy on a database of diagnosed babies. The results demonstrate the potential of vocal and motor analysis for computer-assisted early detection of neurodevelopmental insults.

Keywords: brain development, neuroplasticity, early neurodevelopment classifier, brain injury, computer-assisted diagnosis, tracking algorithm, ferns algorithm, early motor & vocal expression, Kinect, premature babies

1. Introduction

1.1. Brain development: Cortical Subplate

Neurodevelopmental syndromes, a continuously growing issue, are impairments in the growth and development of the brain and CNS which are pronounced in a variety of emotional, cognitive, motor and social skills.

Fetuses at 33–41 weeks' gestational age recognize properties of their mothers' voice and their native language [1, 2] suggesting that neural networks connecting circuits which process

afferent sensory information (such as voice and pain), basic efferent vocal expressions, and high brain function (attention, memory, learning) are already being formed [3–5].

In early fetal life a transient structure develops in the subcortical future white matter, situated between the intermediate zone and the developing cortical plate: the Cortical Subplate [6]. The Subplate is thickest at around 29 weeks' postmenstrual age (PMA), and is absorbed gradually until around 4–6 months' post-term, with relocation of fiber terminals into the cortex, in the order of "first to come last to go" [7–9]. Most of its afferent and efferent connections run through the (future) periventricular white matter. The size and duration of the Subplate visibility increases along mammalian evolution and reaches the peak in human fetuses, concomitantly with an increase of cortical fiber complexity. Hence it is considered a recent phylogenetic structure that expanded to enable the increasing complexity of cortical circuitry [10].

The Cortical Subplate serves as a relay station for the growing neural protrusions of the developing cortical neurons and as an integrating element that synchronizes neural network activity by distributing signals effectively in the developing cortical plate [11, 12]. As such, Subplate neurons are essential in creating the accurate wiring and functionality of the cerebral cortex: Subplate neurons create preliminary transient synapses between Thalamic axons (carrying mainly sensory inputs) and their targets in the forming cortical layer number 4 [13].

Subplate neurons, with their multiple excitatory, inhibitory and neuromodulator synaptic connections, are key elements to affect cortical development and maturation [14–16]. In the time window of their existence, the Subplate circuits are sensitive to hypoxic injury [17], which may lead to long lasting impact on brain development and functional deficits: sensory, cognitive, social, motor. The time window of Subplate circuits' high activity is also the time window when young infants born premature make their first coping out of utero.

Premature birth rate is around 10% of all deliveries in average; it is a continuously growing phenomenon (WHO, 2012) and is the second common cause of death among infants worldwide (after Pneumonia). Recent medical advances enable increasingly more premature infants to survive, however, many of them are in high risk for brain injury [18–21] and neurodevelopmental impairments [22, 23]. For example, babies may develop Periventricular Leukomalacia, which may cause severe, long term damage to brain tissue [24–27]. Early assessment and detection of developmental impairments is crucial for early and effective intervention, as early identification will support initiation of early treatment and may minimize neurological and functional deficits. The babies participating in this study project were born premature and participated in this research during Cortical Subplate activity time window.

1.2. Vocal expression

A preliminary model of neural mechanism of baby vocalization was called "the brainstem model", based on reports about vocalization of anencephalic human infants whose cerebral hemispheres were vastly non-functional [28]. Further studies with baby primates and mammal pups showed elevated activation in additional brain areas during early vocalization, including the Peri-Aquaductal Grey, cerebellar Vermis, Thalamus, anterior Cortical Cingulate Gyrus, Amygdala and the neural pathways connecting the components of Limbic system [29–31].

Early studies of infant cry were conducted with babies considered at risk for Developmental impairments, and babies with identified disorders [32–34]. More recent studies have described acoustic features (Formant, Pitch, etc.) of infant cry in babies at risk for poor developmental outcome due to perinatal risks or medical complications, such as hyperbilirubinemia, prenatal substance exposure, lead exposure, or evidence of brain damage [35–38]. Thus, baby's vocal characteristics are regarded as an indication of neurobehavioral insult.

Neonatal vagal tone is an indicator for balanced, ripe function of the autonomic nervous system [39] that has shown to predict the infant's neurobehavioral and cognitive development and social-emotional adaptation across infancy and up to 6 years of age [40, 41]. Neonatal vagal tone was also found to predict the degree of mother-infant synchrony at 3 months [42]. Moreover, vagal tone is expressed in the baby's vocalization and in infant oral neuromotor competence; the production of vocalization or cry is the result of air being forced through the vocal tract, over the larynx and through the vibration of the vocal folds. Pitch frequency, the base repetition rate of a sound waveform, is dictated by vocal cord vibration, vagal tone and autonomic regulation. Formants (the resonant frequencies of upper airways) reflect breath, utterance and soft palate synchronization. Indeed, vocal features may be indicators of an infant's brain development.

Here we present a new approach to early developmental diagnosis of young infants, comprised of computerized vocal analysis and a database of double blind diagnosed premature born babies. The clinical diagnosis of the babies which comprise the database consists of Spontaneous movements and oral neuromotor competence.

1.3. Motor expression: spontaneous movements and oral neuromotor competence

Markers of neural development can be observed and quantified in the newborn and young infant using the quality of spontaneous general movements (employing the "General Movements assessment tool", GM) [43, 44], and oral neuromotor competence (employing the "Neonatal Oral-Motor Assessment Scale", NOMAS) [45].

The assessment of the quality of spontaneous general movements is a sensitive, age specific neurodevelopmental tool, which in particular teaches about the integrity of complex supra spinal neural circuits. Spontaneous General Movements are first identified early in fetal development, produced by deep brain circuits and before afferent stimuli exist [46]. From about 34 weeks' postmenstrual age, three consecutive age specific characteristic forms of Spontaneous General Movements appear, reflecting maturation and reorganization of subcortical and cortical brain circuits [44, 47]. Spontaneous General Movements are composite movement patterns comprising head, trunk, arms and legs, with variable movements and trajectories, and they disappear when goal directed movements appear at the age of 3–4 months' post-term [48]. Significant relationships between abnormal general movements at 1 and 3 months and cerebral white matter abnormalities on MRI in preterm infants were demonstrated [49], supporting the concept that abnormal spontaneous general movements reflect developmental damage to cerebral white matter and sub cortical plate neural circuits [50]. Additionally, assessment of spontaneous general movements has predictive power for developmental

outcome at school age for major disorders (cerebral palsy), and minor developmental disorders [51–53].

Moreover, neural development, as well as later developmental outcome, is reflected by the newborn's feeding patterns, as these require synchronization of neural circuits that master integrated sucking-swallowing-breathing ability and normal vagal tone [45, 54–56]. Sucking reflex develops at 16 weeks in utero, coordination of suck/swallow appears at 32–34 gestational weeks, and coordination of suck/swallow/breath appears at 37 gestational weeks or later [57–59]. Suck/swallow/breath coordination and rhythmicity control requires involvement of many brain circuits, including afferent and efferent fibers of cranial nerves (IV, V, VII, IX, X, XII), brain stem Lower Medulla nuclei (Ambiguus, Solitarius, Hypoglossus) participating in Bulbar circuits, sensory supra-bulbar fibers and motor cortical circuits [60]. Problematic feeding patterns occur frequently in babies born premature, in neonatal encephalopathy, chronic lung disease, after intra-uterine drug exposure, abnormal somatosensory balance, structural abnormalities and pain. The NOMAS scale is used for assessing either breast feeding or bottle feeding (exhausted human milk or formula) behaviors [45, 56, 61].

NOMAS and GM performance reflect integrity of the neural circuitries of the Cortical Subplate and together they may give complementary information for better prediction of developmental outcome [64, 65]. Hence both tools were employed here to clinically evaluate infants' neural development and create the database for the computerized motor and vocal algorithms.

1.4. Rational

As described above, early detection of brain insults enables early beginning of intervention, which may minimize neurological and functional deficits. However clinical experts seldom are available in remote or poor populations. Previous works have shown that motion and vocalization can be used as a diagnosis tool for health and development, using various methods to develop an automated system for characterizing pathologies [64–67]. However, these methods require complex classifiers and long training time. Therefore, in this work, we introduce accessible, simple and cost-effective assisting tools for a convenient early diagnosis of infants, requiring only recorded samples of infant vocalization and motor performance.

2. Computer-assisted early developmental assessment

2.1. Babies' reference developmental status

Tracking of infants' neurodevelopment was conducted after parents signed informed consent. Participating babies were recorded using Kinect for windows of Microsoft (formerly PrimeSence). Double blind neuromotor diagnosis was conducted using GM (General Movements) and NOMAS (Neural Oral-Motor Assessment) tools. The tools were shown to be highly correlated [62, 63] (**Figure 1**).

NOMAS	GM4 (Normal Optimal)	GM3 (Normal Suboptimal)	GM2 (Mildly Abnormal)	GM1 (Definitely Abnormal)	Sum
Normal	1 (3%)	5 (12%)			6 (15%)
Disorganized		12 (29%)	19 (46%)		31 (75%)
Dysfunctional				4 (10%)	4 (10%)
Total	1 (3%)	17 (41%)	19 (46%)	4 (10%)	41 (100%)

Figure 1. Correlation between babies' spontaneous general movements and feeding behavior.

GM diagnosis referred mainly to Complexity, Variability and Fluency of babies' motor performance, and was classified as normal (2 grades: optimal, sub-optimal) or abnormal (2 grades: mildly, definitely).

NOMAS diagnosis referred mainly to functionality and synchronization of suck, swallow and breathing, and defined as normal, disorganized, or dysfunctional.

2.2. Computerized analysis of babies' motor expression

Young infants with brain injury have typical neuromotor performance, expressed in synchronized movements, relative dominance of upper or lower limbs, asymmetry, absent or abnormal fidgety general movements, and more. These higher order motor features have been shown to have clinical correlations.

2.2.1. Tracking algorithm: following a 'Cloud of points'

Existing skeletal joint tracking tools [68–70] were originally developed for the gaming console and humans bigger than one meter, and are not suitable for tracking of babies' joints. In addition, the background surface on which the baby is lying is too close to the baby to allow the algorithm to perform stably, the morphology of the baby consists of round shape silhouette contours rather than sharp angular joints; and the delicate, high/low tone spontaneous movements performed by babies inherently made joint tracking unstable and inefficient. In order to solve these issues, a new tracking method was implemented, consisting first on volumes and then computed baby skeleton. First, estimation of the baby's movement was based on the normalized volume occupied between the segmented body and the background, using the depth stream.

$$V_i(t) = \frac{v_i(t)}{\min v_i(t)_{t=1,\ \ldots,\ N}} \tag{1}$$

where $v_i(t)$ is the overall volume occupied by the baby in video i and time frame t.

Background of the baby was erased (using body segmentation), spatial alignment and temporal synchronization of sensor beams (RGB camera and depth sensor) were performed. Due to filming angles, a depth-rectification was also implemented.

Tracking algorithm followed changes in babies' volume occupied between the segmented baby's body and background, using shape descriptors known as Hu central moments [71] and the depth stream.

2.2.2. Identification and selection of motor features

Feature extraction is conducted according to high order movement parameters:

- Complexity and variation are calculated as spatial and temporal motor variability.
- Fluency is calculated as one over the jerkiness (third time derivative) of the limbs' trajectories.
- Predictability is calculated according to predictive information which is the mutual information between the position at time t and position at time $t + 1$. In other words, the more predictable the trajectory, the higher the predictive information.

Several statistics were computed for the normalized babies' volume: variance, jerkiness (third derivative), predictability of normalized volume variation (1st derivative of volume) using Hurst exponent [72] to distinguish system's randomness:

1. The variance

$$var(V_i) = \frac{1}{N-1}\sum_{t=1}^{N}\left(V_i(t) - \mu(V_i)\right) \tag{2}$$

where $\mu(V_i)$ is the average of the normalized volume over time. The variance of the normalized volume is negatively correlated to the developmental diagnosis.

2. Average absolute jerkiness of the normalized volume

$$jerk(V_i) = \frac{1}{N-3}\sum_{t=1}^{N-3}\left|\frac{\partial^3 V_i(t)}{\partial t^3}\right| \tag{3}$$

the jerkiness is also negatively correlated to the developmental diagnosis.

3. Wavelet coefficients obtained with the continuous wavelet transform (CWT) of the signal. Hence, given a signal $x(t)$ and a basic wavelet $\psi(t)$ for a shifting parameter b and a scaling parameter a:

$$X_w(a,b) = \frac{1}{|a|^{1/2}}\int_{-\infty}^{\infty} x(t)\overline{\psi}\left(\frac{t-b}{a}\right)dt \tag{4}$$

As we were interested in the high frequency content of the signal, so a symlet wavelet with a center frequency of 0.66 Hz was chosen and averaged the coefficient obtained with scales $[9.0, 9.1, \ldots, 10]$ over the entire time series.

4. Predictability of the normalized volume variation (i.e. first derivative of the volume). The Hurst exponent H is used to determine the predictability of a time series and to distinguish random from non-random systems [74]. It is defined as:

$$\mathbb{E}\left[\frac{R(n)}{S(n)}\right] = Cn^H \text{ as } n \to \infty \tag{5}$$

where:

- $R(n) = \max(x_1, \ldots, x_n) - \min(x_1, \ldots, x_n)$ is the range of the first n values,
- $S(n)$ is the standard deviation of the first n values,
- $\mathbb{E}$ is the expected value
- n is the number of points in the time series
- C is a constant

the Hurst exponent was positively correlated with the developmental diagnosis.

2.2.3. Developmental classification according to motor features

A machine learning algorithm was applied to analyze the clinically correlated features, according to samples from train series (clinically diagnosed by a specialist).

A linear estimator was trained using step-wise linear discriminant analysis (SWLDA) to estimate the developmental diagnosis from input features, using the following computed features for each video: variance of the normalized volume, average CWT coefficient of the normalized volume and Hurst exponent of the normalized volume variations (adding the average absolute jerkiness of the normalized volume degraded the performance). So for each video i, we have a vector of features:

$$X_i = [var(V_i), c(V_i), H(V_i)] \tag{6}$$

A linear decoder was trained the following way: for each combination of 12 videos, 9 videos were included in training set and 3 videos in testing set, which leaded to 220 training runs with each different combination of training samples. This means that each video was tested 55 times using different training datasets. The following model was fitted on each run using step-wise linear discriminant analysis (SWLDA):

$$\hat{d}_i = X_i w + b \tag{7}$$

where $\hat{d}_i$ is the estimated developmental diagnosis, w are the weights of the predictive model and b the bias term of the prediction.

In order to evaluate the performance of diagnosis classification, Baby videos were divided according to clinical assessment as normal neurodevelopment (grades 3, 4) and abnormal neurodevelopment (grades 1, 2). Prediction of the test videos for each run was plotted against the developmental diagnosis. In order to evaluate the performance of computer classifier, threshold was applied to the estimated diagnosis.

We define: true positive (TP): correct diagnosis of non-healthy baby; true negative (TN): correct diagnosis of healthy baby; false positive (FP): false alarm (type I error); false negative (FN): missed non-healthy baby (type II error).

We then have three different performance assessment:

- Accuracy: $\frac{TP}{TP+FN}$
- Sensitivity: $\frac{TN}{TN+FP}$
- Specificity: $\frac{TP+TN}{TP+FP+FN+TN}$

The classification performances for different threshold values are showed in **Figure 2**. The classification performance for threshold value of 2.3 is 87% with sensitivity of 73% and specificity of 98%. This value is close enough to the clinical division between normal and abnormal neurodevelopment. Motor system architecture up to this stage is shown in **Figure 3**.

2.2.4. Tracking algorithm—from a 'Cloud of points' back to joints in a skeleton

Our aim is to develop specific fine-tuned tools for specific syndromes. Tracking a 'cloud of points' does not allow direct translation and fine tuning of clinical criteria related to specific body parts into mathematical phrases. Hence the first stage giving a result "normal" or "abnormal" neurodevelopment is important but not final.

The Kinect sensor is using a predefined skeleton model to return the joints' positions based on the depth data. There is no option to inject new rules and adjust the predefined joint distances which are not suitable for infants' dimensions, as there is no access to all the internal functions that process and output the human skeleton. Hence we would be unable to use the inherent skeleton as a reference for how the babies' skeleton is reconstructed. Instead, we use the Random Ferns [73–75], and Random Forests [76] algorithms.

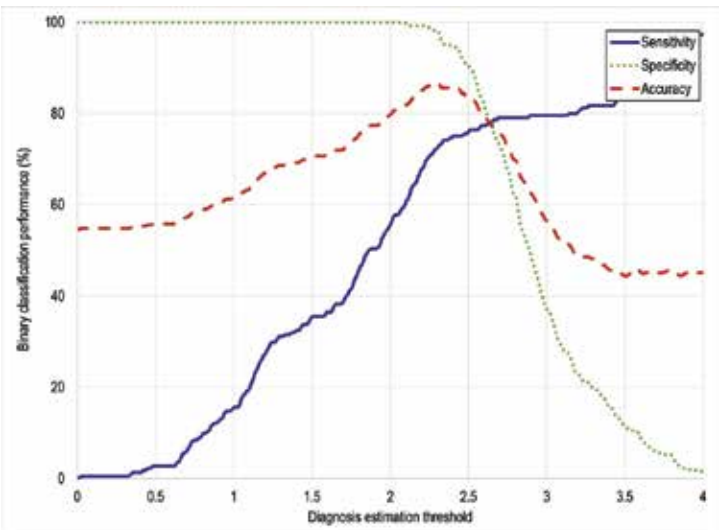

Figure 2. Binary classification performance against threshold value.

http://dx.doi.org/10.5772/intechopen.70894

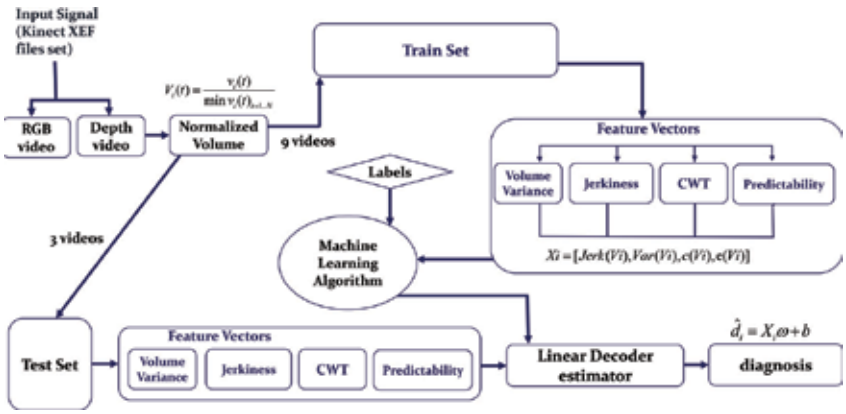

Figure 3. Motion system architecture.

Random Decision Forests combine binary depth comparison features to assign a body part label for each input depth pixel; based on the detected body parts, the joint positions are expected. However, the training of the forests is computationally very intense and not efficient for a large amount of data. In order to simplify the training procedure and to flexibly adapt the system to different application requirements we use Random Ferns which are an efficient and robust alternative to random forests in order to find the 3D positions of body joints in single depth images.

We use Random Ferns for melding binary depth comparison features and create a pixel wise body part classifier. Based on the body parts, we estimate the positions of the body joints. Then each pixel of the body is allocated to a body part class and joints' positions are deduced by calculating the mean of the cluster of each body part. A large amount of labeled data for training was created, using an open source three dimensional model of a baby's body [77]. Open source software [78] and CMU motion capture dataset [79] were used for animating the model in various postures. Depth images were then created from the model and body part label was allocated to each pixel. The virtual camera viewpoint for generation of our depth images is frontal views of the virtual body, corresponding to the babies in our real data. The babies' reconstructed skeletons are then classified using machine learning algorithm with the existing and additional high motor features which directly reflect early signs of specific brain injuries.

2.3. Computerized analysis of babies' vocal expression.

2.3.1. Identification and selection of vocal features

For processing of vocal signal and extraction of distinguishing acoustic features that are correlated with brain development, baby recordings were divided to frames of 15 ms and segments of 0.3 s with 0.15 s overlap. This way each segment contains 20 frames and enables good detection of time varying phenomena with minimal loss [80].

The most prominent vocal features extracted from frames were:

Pitch frequency—the frequency of vocal cord periodic vibrations when the baby sound is produced. A typical Pitch range in babies is 200–450 Hz, hence frames of 15 mill sec would contain 3 pitch periods, the minimum needed for reliable pitch detection [81–83].

Formant—the dominant resonant frequencies of the air flowing through the oral and nose cavities during baby's vocal expression. Typical frequencies of healthy baby formants are 1100 Hz (F1), 3300 Hz (F2), 3500 Hz (F3) [37]. The first three formants were extracted.

Spectral centroid—gives an estimate of the spectral content of the voice frame, with typical value of 1000 Hz for healthy babies, was calculated for each frame using FFT.

Dominant frequencies—first second and third quarterly frequencies of each frame are the frequencies above which 25%, 50%, 70% of the vocal energy resides.

Mel-Frequency Cepstrum coefficients—were extracted in order to better estimate the spectral envelope of the vocal signal, using a logarithmic scale for the frequency axis (in order to imitate human pitch perception) [84].

Linear Predictive Coding coefficients—represent the spectral envelope of the signal using a linear predictive model. LPC coefficients are mostly used for speech compression and encoding; and are plausible also for infant vocal analysis. We extracted the first three LPC coefficients from each frame.

The most prominent vocal features extracted from segments are changes in Pitch contour that were shown to correlate with developmental disorders in infants [85].

Glide—defined as a steep rise or fall of Pitch contour of at least 600 Hz in 0.1 s.

Vibrato—defined as rapid falling and rising of Pitch contour. Vibrato was detected from subsegment groups containing runs of more than 2 positive/negative pitch differences larger than 3 Hz. These were summed and normalized to define Vibrato intensity [80].

Modes—describe a continuous temporal state in vocal segment, in which the pitch contour is either in a certain range or cannot be clearly detected (as in aperiodic signal). Two modes were extracted—phonation (pitch is up to 750 Hz), and hyper phonation (pitch is above 1000 Hz [85].

Melodies (falling, rising, flat)—describe the general trend of pitch contour, as rising, falling or flat. Vocal expression containing a majority of one melody may reflect a developmental impairment [86–89]. Cry melody was calculated using derivatives of the pitch contour, when a positive value reflects a rising trend in pitch contour and a negative value reflects a falling trend, both of over 50 Hz. When pitch contour derivative was smaller than 50 Hz melody was defined as flat.

According to the RELIEFE iterative feature selection algorithm [90], the most prominent features for correct categorization of training samples in the developmental classification, are short time energy, the third formant, the vibrato feature and the melodies (falling, rising, flat).

2.3.2. Developmental classification according to vocal features

After babies' reference developmental status ("data base") had been quantitatively categorized as "normal" (44%) or "impaired" (56%) (**Figure 1**), a machine learning approach was employed with the babies' vocal recordings. Computerized vocal classification was based on computer analysis of the vocal features extracted from babies' vocal expressions, against the clinical diagnosis of the premature infants (data base). vocal system architecture is shown in **Figure 4**.

http://dx.doi.org/10.5772/intechopen.70894

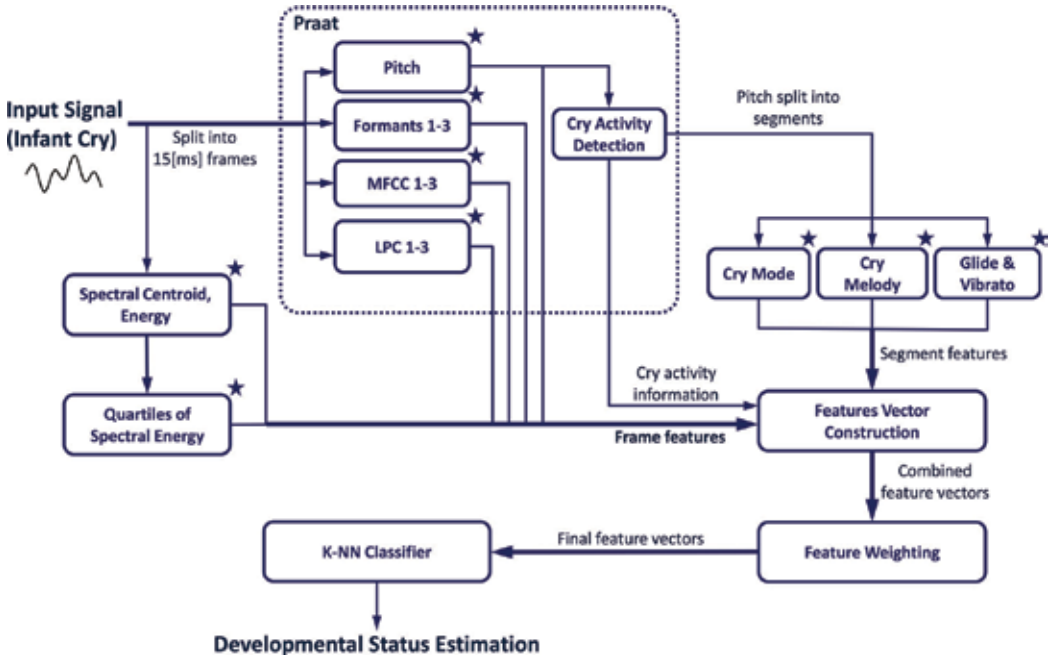

Figure 4. Vocal system architecture (star labeled boxes define feature extraction block).

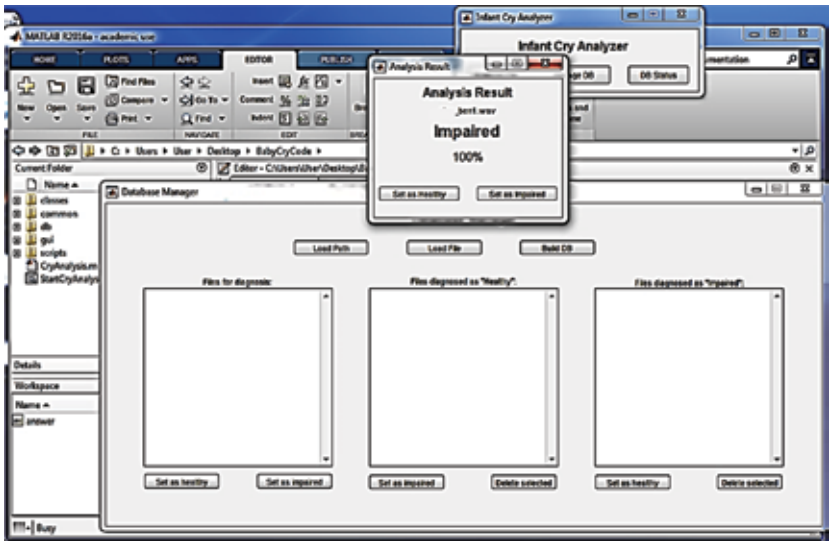

Figure 5. Infant Cry Analyzer algorithm in work.

For the machine learning process, a k-NN algorithm was employed. In the training phase each frame was represented as a 22 dimensional feature vector, according to the ten vocal features described above. In the training set vocal signals were divided into disjoint sets so that each piece of vocal recording would appear only in one set. System performance was evaluated

using 5-fold balanced cross validation. The system classified correctly 89% of the babies. The percentage of babies' vocal signals falsely classified as "impaired" while diagnosed "healthy" was approximately 9% (type 1 error, false positive, alpha) and the percentage of babies' vocal signals falsely classified as "healthy" while diagnosed "impaired" was 2% (type 2 error, false negative, beta). Infant Cry Analyzer algorithm in work is shown in **Figure 5**.

3. Conclusions

The results verify the correlation between early motor & vocal features and infant neurodevelopment. Performance of classifiers show approximately 90% accuracy on a database of diagnosed babies. The results demonstrate the potential of neuromotor and vocal computerized analysis system as an assisting tool in early detection of developmental insults. We currently characterize and group specific features which are early signs of specific brain injuries. Ultimately this system can be widely used in remote clinics, leading to earlier diagnosis of developmental insults and early intervention.

Acknowledgements

We wish to thank: the parents of the babies for their trust and cooperation. Chava Kasher (Sheba MC), Dr. Amir Kushnir (Poriah MC), Moran Moskovich (Ben-Gurion university), Dr. Guillaume Sikard, Amit Oren, Avi Matzliach, Rami Cohen (the Technion), Dr. Oren Forkosh, Dr. Goren Gordon (Weizmann Institute). Marjory M. Palmer (NOMAS International), for their contributions. The National Institute for Psychobiology in Israel, for their generous support.

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Chapter 4

Brain Reorganization in Late Adulthood: Rapid Left-to-Right Switch of Handedness Through Memory-Drawing Training

Lora T. Likova

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76317

Abstract

The neural correlates of hand preference are still debatable, and the very few studies on the mechanisms of enforced change of handedness from left to right are all restricted to early childhood. We were able to address the question of retraining handedness in late adulthood for the first time, well outside the accepted critical period for brain plasticity, through a unique training utilizing the complex motor task of blind memory-guided drawing, in a totally blind, congenitally left-handed man. Ten hours of this Cognitive-Kinesthetic Drawing Training, which the author initially developed to drive neuroplasticity in blindness rehabilitation, was sufficient to generate a profound switch in the cortical lateralization of motor control. This study provides new insights into the neuroplasticity of motor control architecture. The results are of high relevance to the long-standing debate about the sources of hemispheric asymmetry. The unprecedented effect on handedness of the rapid Cognitive-Kinesthetic Drawing Training implies a powerful potential of this training for further rehabilitation domains, such as the rehabilitation of stroke or trauma affecting hand control.

Keywords: neuroplasticity, drawing, training, learning, memory, neurorehabilitation, lateralization, left-handed, handedness, Cognitive-Kinesthetic training

1. Introduction

The neural correlates of hand preference are still debatable, and the very few studies on the mechanisms of enforced change of handedness are all restricted to handedness switching from left to right in early childhood [1]. The question of *retraining* handedness in *late adulthood,*

well outside the accepted critical period for brain plasticity, has not been previously studied. The author was able to address this question for the first time by driving neuroplasticity through a unique training on the complex motor task of blind memory-guided drawing, in a congenitally left-handed man who had become totally blind 10 years before. The unprecedented effect on handedness of the rapid Cognitive-Kinesthetic Drawing Training—which the author initially developed for blindness rehabilitation [2–7], implies a powerful potential of this training for further rehabilitation domains, such as the rehabilitation of stroke or trauma affecting hand control.

Left-handers are often excluded from neuroscience study cohorts in order to focus on a more uniform population. However, left-handed individuals represent a substantial portion of the human population, and therefore, it is important to account for this aspect of neural coding in order to better understand brain functioning [8]. Most studies have found that, in both right- and left-handers, movements of the preferred hand activate mainly the contralateral hemisphere [9–18], whereas movements of the non-preferred hand tend to result in a more balanced pattern of activation in the two hemispheres, indicating greater involvement of ipsilateral cortex [1, 12]. For example, it has been found that right-handers had greater activation in the left premotor area for either hand [13], indicating a general dominance of the left hemisphere in motor function, whereas left-handers showed a symmetrical of activation in the premotor cortex contralateral to the moving hand (either left or right). A parallel pattern of such a contralaterality for either hand in the right-handed, but not in the left-handed, was found in another brain region—the sensorimotor cortex [19]. It should be noted, however, that there are still many discrepancies in the literature, which are often attributed to differences in experimental design, including the type of motor task.

Does forceful switching from left-to-right handedness in adulthood change the patterns of cortication activation in left-handers or not? How much of the observed inter-hemispheric patterns are entirely genetically predetermined or can be affected by experience, such as training? There are only a few studies addressing these questions, with divergent results (e.g., [1, 16, 20–24]).

2. The Cognitive-Kinesthetic Drawing Training

The Cognitive-Kinesthetic Drawing Training is a noninvasive approach to blindness rehabilitation that the author has developed based on a novel conceptual paradigm [3–6]. It utilizes a special protocol of *memory-guided drawing*. My previous studies show that this training affects a widely distributed brain network, including both lower-level regions, such as the primary visual cortex (even in the blind), and higher level regions as the hippocampus or a swath of temporal cortex regions [6]. It also enhances top-down connectivity from the hippocampus and other memory-related regions such as the perirhinal cortex [25–26] to early visual areas.

The results from my previous study [6] also revealed the remarkable learning dynamics of functional reorganization in the hippocampal complex and the temporal-lobe object processing hierarchy over a two-month-long consolidation period. In particular, the hippocampal pattern

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of profound *learning-based transformations* was strongly reflected in the primary visual cortex (V1), with the memory retrieval function showing massive growth as a result of the Cognitive-Kinesthetic memory training and consolidation, while the initially strong hippocampal response during tactile exploration and encoding became almost nonexistent. Furthermore, the inferior temporal cortex manifested a striking *alternating patch structure* [6] (**Figure 1**, bottom panel) reminiscent of the face and object patches reported along the temporal lobe [27]. However, in my study, the differentiation was a function of the *temporal evolution of learning* changes, that is, it was reflecting the effect of training *over time (*instead being a function of face/object category). This cascade of alternating discrete regions also underwent a radical *sequence of transformations* as a

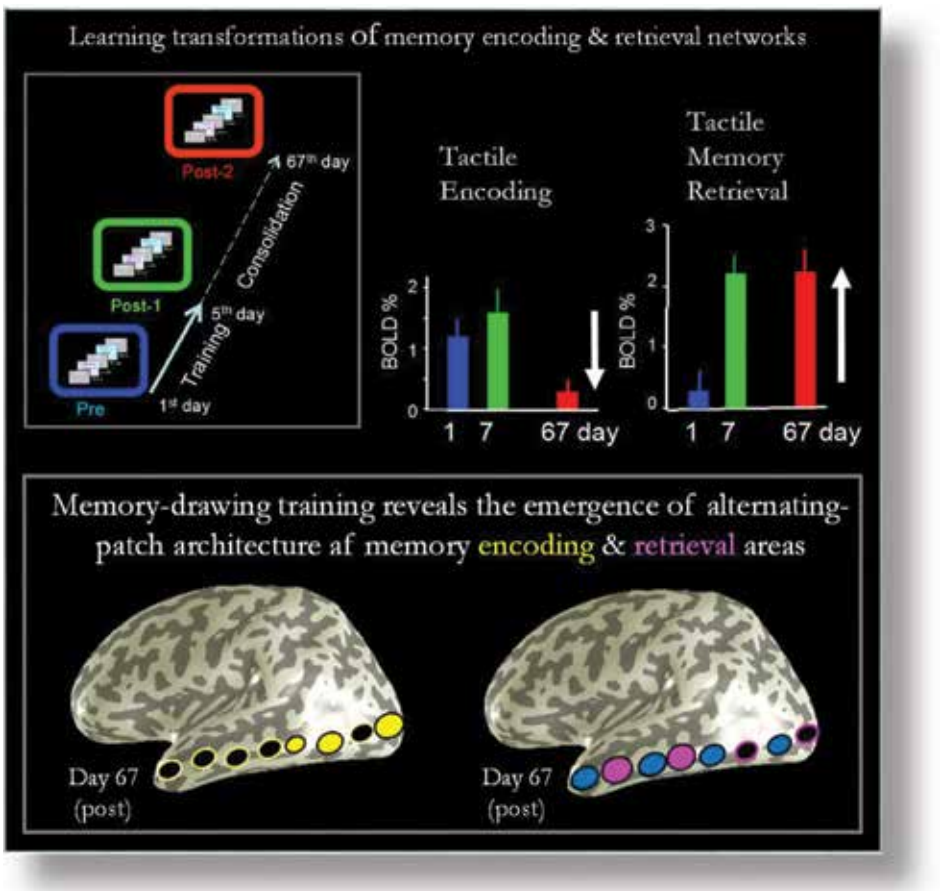

Figure 1. Learning evolution along IT driven by the Cognitive-Kinesthetic memory-drawing training. **Upper panel:** Left: Experimental design, including 3 fMRI assessments: i) a pre-training (*pre/blue*), ii) an immediate after training (*post1/green*), and iii) two months after consolidation period with no training (*post2/red*) assessments. Right: Example of the dramatic reorganization of BOLD responses in an IT region for the *tactile encoding*, and the *tactile memory retrieval tasks* from Day1 (pre-training), to day 7 (immediately post-training) to day 67 (2 months after training). **Bottom panel:** Alternating-patch structure of dissociated, largely non-overlapping encoding and retrieval regions after consolidation (day 67; after training). Left: Memory-encoding task. Yellow—areas still active after consolidation; black—areas not activated anymore. Right: Memory-retrieval task: Purple—areas that become active; blue—suppressed areas; black—areas not involved anymore (after Likova [6]).

function of the *stage of learning*, toward a *complete functional specialization* in terms of either *encoding* or *retrieval* after consolidation. Several distinct patterns of this learning evolution *within* each of the patches (see, e.g., in **Figure 1**, top panel) implied a complex reorganization of the object processing sub-networks throughout both the *training* and the following *consolidation* period.

3. Generalization of drawing-learning effects

While there have been many cross-sectional comparisons of blind and sighted capabilities, the only research focused on *interventions* to enhance basic spatial-cognition abilities in people with blindness has been that based on my Cognitive-Kinesthetic drawing training. This intervention has been shown to improve *spatial memory* and *memory-guided spatiomotor coordination* to a dramatic extent. Although it is typically assumed that drawing is dependent on vision, previous work indicates that individuals with congenital blindness are able to learn to draw over some unspecified time period that often may take years [28–30]. My studies have shown, however, that everyone—blind, sighted, or visually impaired—can learn this skill in only a few hours through an appropriate training, such as the Cognitive-Kinesthetic methodology [3–4, 31–33].

I have further hypothesized that the improvements from the Cognitive-Kinesthetic training would *transfer*, or—*generalize,* to a wide range of *untrained* basic spatial-cognition abilities that extend well beyond the drawing task *per se* [6]. "Basic" abilities were conceptualized as those that are foundational to other tasks, such as the ability to perceive, and remember object features, textures, spatial configurations, and patterns, together with abilities for spatial analysis and new concept learning. My rationale for this *transfer of learning*, or *Generalization of Learning,* hypothesis derived from the fact that the act of drawing complex images from memory "orchestrates" multiple spatial-cognition abilities [2–3, 31–33]. A recent study confirmed my Generalization of Learning Hypothesis [34] by showing significant improvements in a large standardized battery of untrained cognitive tests [35–36] for the blind and low vision following the 10 hours of Cognitive-Kinesthetic training in a cohort of congenitally blind and severe low-vision participants.

4. Switching of handedness as a form of learning effect?

In the earlier cited studies, the Cognitive-Kinesthetic Drawing Training was designed and applied as a noninvasive intervention for a rapid enhancement of *spatial memory*, *spatial cognition* in general, and precise *memory-guided motor control* in both the blind and the sighted. The memory drawing protocol in the form developed for this training, fully engages the whole *perception-cognition-action loop* [3–4], which was a key element of my *conceptual framework* underlying the training. (Note here the expansion of the traditional "perception-action" loop to include the central component of "cognition" (**Figure 2**), as I believe it is critical to its generalization to the gamut of spatial-cognition abilities.)

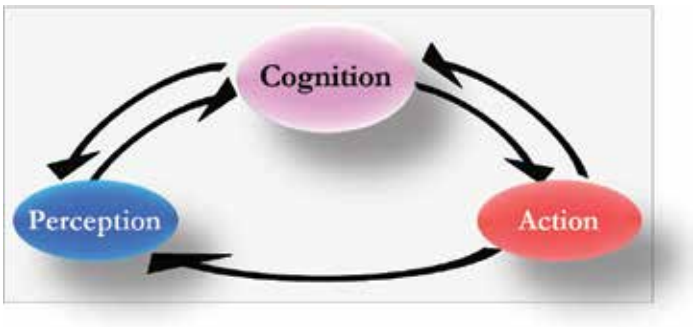

Figure 2. Perception-Cognition-Action Loop. Note the inclusion of the *cognition* module as a central mediator into the traditional perception-action loop [4].

In this chapter, we ask whether the learning effect of this training could extend to the ***action*** component of the processing *loop* involved. Specifically, can it drive reorganization of motor-architecture for *switching handedness*?

5. Methods and procedures

5.1. Participant and the Cognitive-Kinesthetic Training

The participant was a 57-year-old male who had full vision until age of 47, when his vision began declining in one eye and then the other, and he was diagnosed with Leber's hereditary optic neuropathy. Within a year, he was blind, seeing only some light in the far periphery. He had been left-handed since birth. The participant gave informed consent for the experimental protocol, which was approved by the Smith-Kettlewell Institutional Review Board as in full conformance with the Declaration of Helsinki.

After only a total of 10 hours of the Cognitive-Kinesthetic Drawing Training (2 hours/day for 5 days [3]), this left-handed blind participant learned to develop detailed and robust *memory representations* of *haptically* explored (with the ***preferred/left*** hand) raised-line depictions of complex images, such as human faces and objects, in order to draw them with his ***non-preferred/right*** hand. Thus, in order to generate the structured motor output of the drawing, he had to learn how to use these enhanced haptic memory representations to *replace* his *lost "eye-hand coordination"* by a *"memory-hand coordination"* mechanism now that he was blind.

In the process, this blind participant learned to *draw freely* with his *non-preferred/right* hand, guided *solely* by the haptic memory acquired with the other hand. This man had never been able to draw well even with his preferred/left hand while still sighted, so he and his family were greatly surprised by this successful outcome.

> *I never could draw very well … That's why it's very interesting to me that I would've been the person that did not have drawing skills before, and to be able to do something like this now .., wow, it is exciting - you have thought me drawing better than I could when I could see … and - to do this with my right hand …!.*

In an additional session, he subsequently practiced drawing the *already* memorized images with his preferred/left hand.

5.2. Experimental design

A key component of the study was measuring whole-brain functional MRI (fMRI) activation before and after applying the Cognitive-Kinesthetic Drawing Training, allowing us to determine the neuroplastic changes in a *causal* framework (**Figure 3**).

As in previous studies with the Likova Cognitive-Kinesthetic training method, fMRI was run *before* and *after* the training for a battery of *raised-line* models of faces and objects as the drawing targets in a three-task block fMRI design [3–4]. The three tasks were as follows: *Haptic Exploration* (*HE*) involving perceptual exploration and encoding in memory of the raised-line model to be drawn; *Memory Draw* (*MD*)—the task to draw this model freehand, guided solely by the encoded haptic memory; *Scribble* (*S*) was a negative memory-control and motor-control task for the hand movements alone. Each task duration was 20 s, with a 20-s baseline condition (*Rest*, *R*) intervening between tasks. Importantly, as opposed to the usual null periods, the participant not only rested motionless but was also instructed and practiced to clear any memory or imagery from awareness ("*blank-mind*"). The start of each task or rest interval was prompted by an auditory cue. The whole task sequence with interleaved rest intervals (*R, HE, R, MD, R, S, R*) was repeated 12 times in each 1.5-hour fMRI session using a new face or object image for each repeat. The *HE* task was always performed with the preferred/left hand. The *MD* and *S* tasks were performed with the non-preferred/right, and additionally, with the preferred/left hand in separate scans.

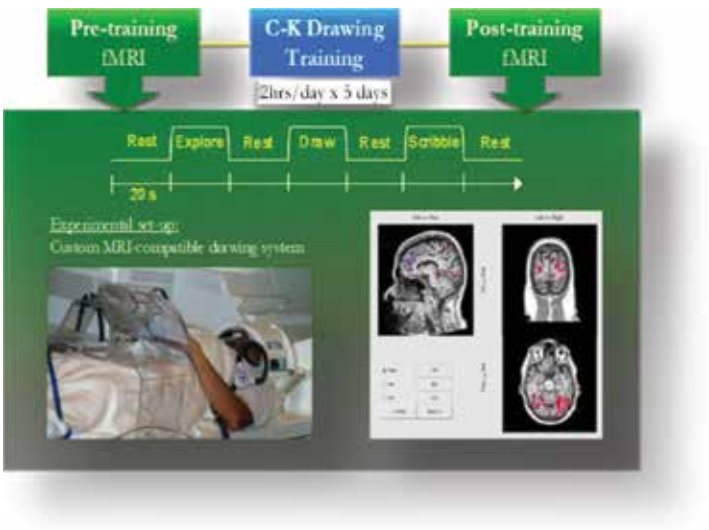

Figure 3. Experimental design. The rapid Cognitive-Kinesthetic drawing training (2 hours/day × 5 days) was preceded and followed by fMRI scans. In the scanner, three tasks were performed in a block paradigm: *Haptic Exploration, Memory Draw*, and *Scribble*. Each task and interleaved rest period were 20 s in duration. An innovative MRI-compatible lectern (lower left) provided for tactile stimulus presentation and both nonvisual and visual drawing. Functional brain activation is color coded in red (lower right).

5.3. Innovative experimental platform

The overall experimental platform integrated a number of innovations, such as, a *multisensory magnetic resonance (MR)-Compatible Tablet system*, and a novel type of parametric brain mapping—*Categorical-Change Maps* [37] that we developed especially for the purpose of assessing *brain plasticity changes* as a result of a causal intervention, and the *Cognitive-Kinesthetic Training*.

The custom MR-Compatible Tablet system (**Figure 3**) allows for participant-controlled tactile-stimulus presentation for haptic exploration and drawing in the scanner. This system consists of a plexiglass lectern extending across the participant's lap, topped with a dual-slot height-adjustable surface [3]. In the left slot was the raised-line drawing stimulus to be haptically explored during the *HE* task, and in the right slot was an MR-compatible electronic drawing tablet (EMS Medical Systems, Bologna, Italy) to be used during the *MD* and *S* tasks. Between scans, the participant was instructed how to remove the topmost raised-line drawing stimulus (which was just explored and drawn) from the left slot and place it by their side, exposing the next stimulus in the prescribed sequence. Participants used a plastic stylus to draw and scribble, with the movement of the stylus across the drawing tablet being recorded and presented in real time to the experimenters on a display in the control room. Auditory cues were presented through MR-compatible headphones (Resonance Technologies, Salem, MA). Our custom MR-compatible tablet system allowed participants to draw comfortably on the plastic lectern across their torso/lap without moving their head. Additionally, during scanning, the participant's head was stabilized in the head coil with firm but comfortable padding around all sides to minimize movement.

5.4. Brain imaging data acquisition and preprocessing

Functional MRI data were collected on a Siemens Trio 3 T magnet equipped with a 12-channel head coil (Siemens Healthcare, Erlangen, Germany). BOLD responses were obtained using an echo-planar (EPI) acquisition (TR = 2 s, TE = 28 ms, flip angle = 80°, voxel size = 3.0 × 3.0 × 3.5 mm) consisting of 35 axial slices extending across the whole brain. Preprocessing was conducted using FSL (FMRIB Analysis Group, Oxford, UK) and included slice-time correction and two-phase motion correction, consisting of both within-scan and between-scan six-parameter rigid-body corrections. To facilitate segmentation and registration, a whole-brain high-resolution T1-weighted anatomical scan was also obtained for each participant (voxel size = 0.8 × 0.8 × 0.8 mm). White matter segmentation in this T1 scan was conducted using FreeSurfer (Martinos Center for Biomedical Imaging, Massachusetts General Hospital) and gray matter was generated with the mrGray function in the mrVISTA software package (Stanford Vision and Imaging Science and Technology, Palo Alto, USA). The Stanford package mrVISTA allows us to estimate the neural activation amplitudes for each task within respective regions of interest (ROIs) using a standard general linear model (GLM) procedure for each task regressor applied to the average signal across all voxels within each ROI.

5.5. Categorical-Change parametric mapping: a novel concept and methodology for the assessment of brain plasticity changes

In studies on brain plasticity, it is critical to be able to fully assess functional brain *changes* due to either an intervention, a natural development, or other causes, such as loss of vision.

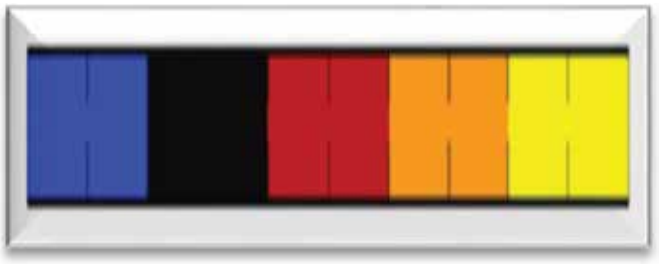

Figure 4. Color coding for Categorical-Change mapping in the case of *positive baseline* activation. *Orange*: No significant change; *Red*: Reduced but still positive activation; *Yellow*: Increased positive activation; *Black*: Lost activation; *Blue*: BOLD signal inverted from positive into negative.

We have conceptualized a system of ***brain-change categories*** and developed a novel type of voxel-wise parametric mapping that can provide the needed *multifaceted assessment of neuroplasticity* [37], and thus, bridge a major gap in this field. This is based on (1) assessing the activation (in each voxel of the brain) during an initial state (e.g., *before* training; *baseline*) and (2) the change in activation (e.g., *after* training) ***relative*** to that baseline.

In the current study, we employed a subset of the categorical-change mapping to visualize **at once** *all five possible categories* of post-training change (or lack thereof) of any ***positive baseline*** activation prior to the state change or intervention.

The color coding for novel type of maps is shown in **Figure 4**. If an activated region did not undergo any significant change relative to the initial state, it is visualized in orange; if its positive activation was increased—in yellow; if it was reduced but still positive—in red; if the activation was lost—in black; while if the sign of the BOLD signal was inverted from positive into negative reflecting a changed in the nature of processing, it is shown in blue.

Note that we have developed the categorical-change mapping to assess the ***full spectrum of possible changes***, relative to a given pre-intervention state. In other words, this mapping tool can also be applied to brain regions that in the ***baseline*** state have ***negative*** BOLD signal, or have ***no activation*** at all. These two options are beyond the scope of the present analysis, however.

6. Results

6.1. Drawing qualities

A total of only 10 hours of the Cognitive-Kinesthetic Drawing, spread out over 5 days, led to dramatic motor control changes in this *congenitally left-handed* blind participant, who was able for the first time to obtain a highly precise control of his *non-preferred right* hand.

The scope and quality of this unexpected new ability of the *non-preferred* hand is illustrated in **Figure 5**. The *central panel* shows a comparison of his pre-training versus post-training drawing

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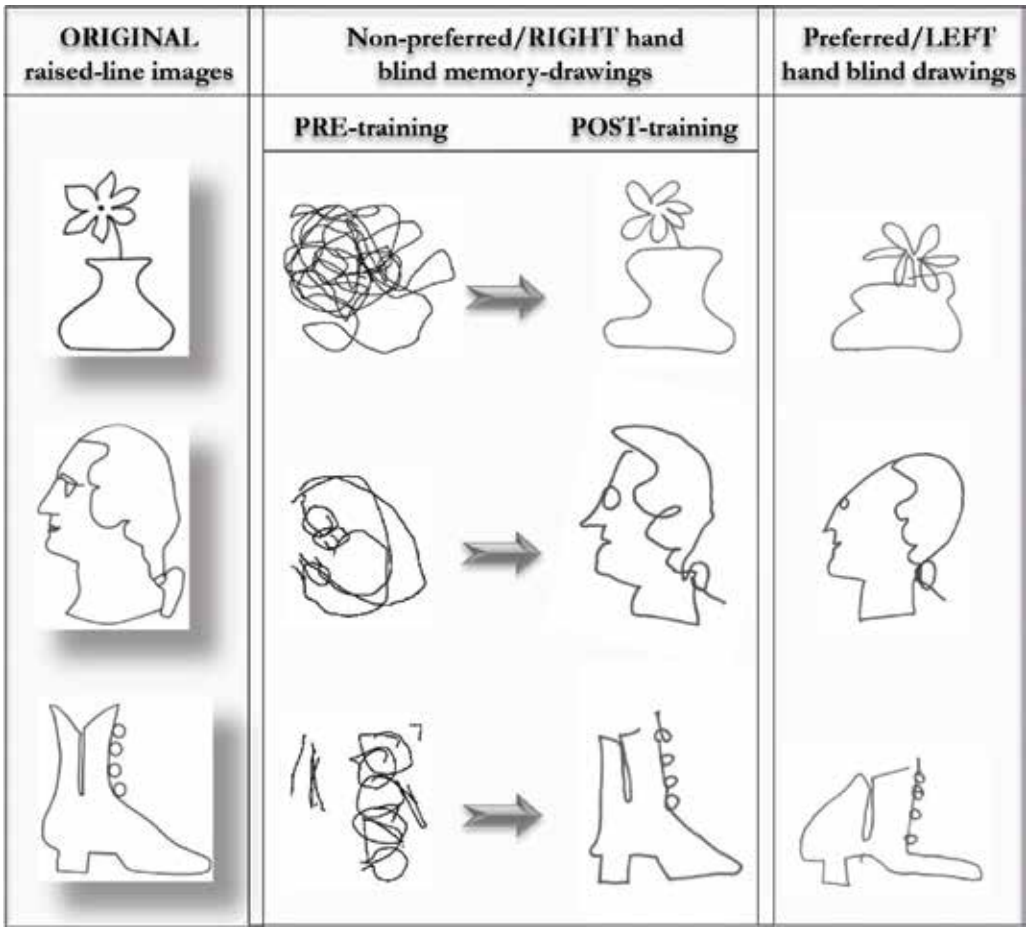

Figure 5. Examples of drawings made by the left-handed blind participant, who underwent the Cognitive-Kinesthetic drawing training. **Left panel:** Raised-line originals used in the haptic exploration and memorization phase. The exploration was always done with the preferred/left hand. **Middle panel:** Drawing from memory with the ***non-preferred/right*** hand, showing the dramatic improvement *from pre-training to post-training*, despite the fact that this hand has never been used before for drawing, writing, or any other habitual motor activity. **Right panel:** Drawings with the ***preferred/left*** hand. These drawings are guided by the same memory that was guiding the other hand; the memory per se, however, was based always on the haptic exploration of the originals with the preferred/left hand. Note that the two hands seem to express two different personalities.

done with the right (non-preferred) hand. Note, these are performed entirely *non-visually*, guided solely by the *memory from the haptic* exploration of the raised-line originals shown in the *left panel*. Note that the exploration was always done with the *preferred/left* hand.

After completing the training of the non-preferred/right hand, the participant had a session of practicing drawing with his all-life preferred (but untrained on the blind memory drawing) left hand. He was then asked to use the untrained left hand to draw the same images guided by the *already* acquired memory (**Figure 3**, *right panel*). Because the left hand had been the dominant one for almost six decades, and moreover, as the haptic exploration and memorization phase

(*HE*) was always done with this hand, the expectation would be for these to provide definitive advantages for *left*-handed drawing.

Conversely, in the main study (drawing with the non-preferred/right hand), the fact that the image information was gathered through exploration with the opposite (left) hand, sets the expectation that the *right*-hand drawing would be at a disadvantage. However, this seems to be the case only *before* the training. Note the rapidly achieved dramatic improvement ***from pre-training to post-training*** for the right hand (**Figure 3**, *middle panel*) despite this disadvantage, and despite the fact that his right hand had never been used before for drawing, writing, or any other habitual motor activity. It is thus surprising, that the *post-training* reproductions with the right hand resembled the originals better than those done with the whole-life-preferred left hand (**Figure 5**, *right panel*). Note again that both phases of the process—*haptic memory encoding* and *retrieval for memory drawing*—were done without the involvement of any vision in this blind participant.

Although the drawing quality and similarity are evident to the human eye, we further assessed the drawing quality by *bi-dimensional regression analysis* [38]. First, for each original image, landmarks were set at unique points that could be easily identified by the naked eye in the original figures and the resulting drawings. Second, bi-dimensional analysis was run for the correspondence between landmarks on the original images and those available on their reproduction by drawing. The specific measure for analyzing the quality of drawings was the fit of an affine bi-dimensional regression (expressed as Fisher-Z values of the respective Rs). The number of landmarks depended on the complexity for each template image.

The bi-dimensional regression scores indicated an improvement averaging about a factor of six *from pre- to post-training* accuracy with the trained hand. Consistent with the perceptual evaluation done earlier, the *post-training* bi-dimensional regression values were significantly higher overall for the non-preferred (but Cognitive-Kinesthetically trained) right hand versus the preferred but untrained left hand, even though the left hand was the one used in acquiring the spatial memory that guided each of the hands along the drawing trajectories.

Interestingly, although the line stability, and image completeness produced by the preferred/left hand were very good, the accuracy of reproduction with this preferred but untrained hand was lower by about a factor of two relative to the strong improvement with the training of the non-preferred/right hand. What was even more surprising was that, stylistically, it could be said that the two hands seemed to express two different personalities.

6.2. Brain plasticity driven by the Cognitive-Kinesthetic Drawing Training

6.2.1. Baseline A: the activation in the brain network engaged by the left hand in memory drawing as baseline

The fMRI recordings run *before* and *after* the training provided a measure of the neuroplastic functional changes underlying the behavioral improvements. To assess not simply *what* has been changed, but ***how*** was it changed and what specific *categories of change* had occurred in the cortex, we used our novel approach of Categorical-Change parametric brain mapping described earlier.

Using the categorical-change parametric mapping, **Figure 6** shows the types of changes that happened in the cortical network activated during memory drawing with the preferred/left

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hand (*baseline*), when the non-preferred/right hand instead performed the same memory drawing task either *before* training (*top panel*) or *after* training (*bottom panel*).

6.2.1.1. Pre-training (top panel)

The architecture of the *baseline network* (used as a mask) indicates that the movements of the *preferred/left* hand activated predominantly its *contralateral*/right hemisphere, as expected.

As also expected, the categorical-change mapping shows that *before* training, the drawing movements of the *non-preferred/right* hand resulted in a more ***balanced inter-hemispheric*** pattern of activation, indicating preservation of the greater involvement of the ipsilateral (right) hemisphere, consistent with previous studies on switching handedness (see Introduction). This result demonstrates that in its attempt to perform such a complex and precision-demanding motor task *before* training, the non-preferred/right hand continued to depend on the functional architecture of the preferred/left hand. Third, the figure shows that all motor, premotor, and sensorimotor regions in *both* hemispheres that were engaged by the preferred left hand were engaged to an even higher degree by the non-preferred hand.

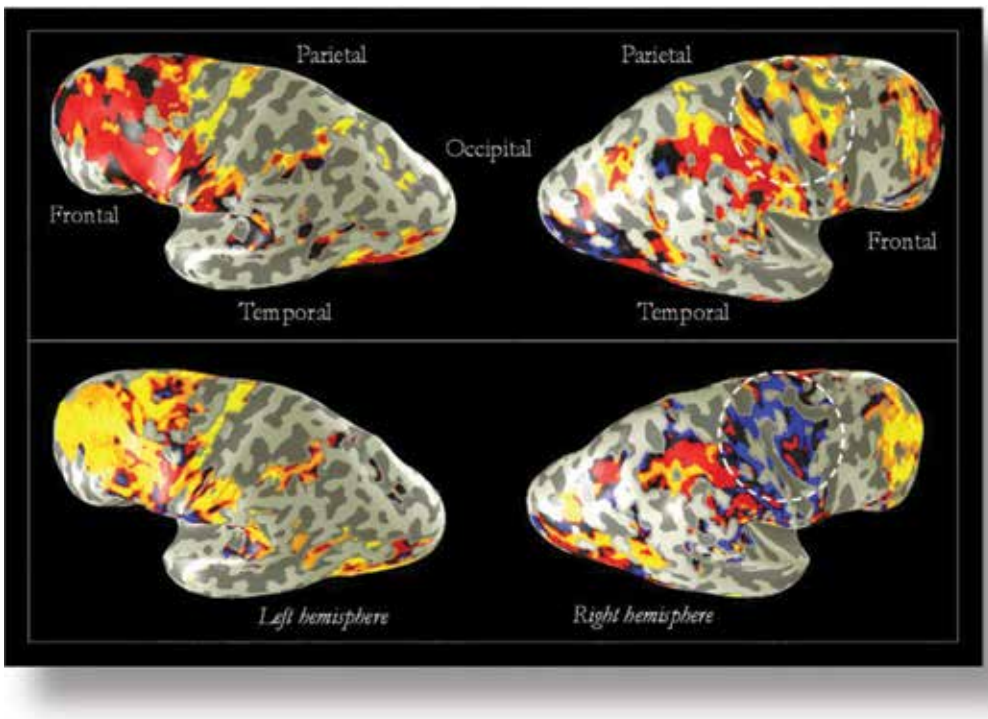

Figure 6. Categorical changes in cortical activation relative to that for memory-guided drawing with the untrained left hand *(baseline)*. The *positive* activation in the brain network engaged by memory drawing with the *left hand* was used as the ***baseline*** for the comparisons in both panels. The differences from that *baseline* for the non-preferred/right-hand activation *before training* are shown in the top panel; *after training*, they are shown in the bottom panel. The voxel-wise changes are presented on inflated views of the lateral surfaces of the left and right hemispheres. Color coding as in **Figure 4**: *Orange*—No change relative to baseline; *Yellow*—Increased positive signal; *Red*—Decreased positive signal; *Black*—Reduction to no significant signal; *Blue*—Negative signal.

6.2.1.2. Post-training (bottom panel)

Remarkably, after the Cognitive-Kinesthetic training, we observed a dramatic reorganization of motor architecture of the *non-preferred/right* hand toward a strongly expressed *contralateral* (left hemisphere) dominance. This previously unobserved reorganization is also clearly confirmed by the categorical-change map analysis in Section 6.2.2. below, where the *pre-training right-hand* network was used as baseline.

6.2.2. Baseline B: the activation in the brain network engaged by the non-preferred/right in memory drawing before training as a baseline

In this section, the network activated by the non-preffered/right hand during MD was used as the basline in the analysis. Consistent with findings from Section 6.2.1. above (see **Figure 6**), the categorical-maps shown in **Figure 7** confirm both the *bilateral* pattern of (positive) activation of the *non-preferred* hand *before* training (used as the baseline mask) and the ***transformation*** of this *bilaterality* into a *strong contralaterality* as a result of training. Another striking finding from the categorical comparison in **Figure 7** was the *massive suppression* (blue) of the BOLD responses in the motor and premotor cortex of the *ipsilateral*/right hemisphere. Furthermore, contrary to what may be expected, this happened in conjunction not with an increase but with an almost *unchanged (orange) or even reduced (red)* activation in these motor control regions of the *contralateral*/left hemisphere relative to pre-training. In other words, the increased contralaterality (left hemisphere greater than right) was *not* caused by increased contralateral/left activation but by an *ipsilateral suppression*, in spite of the fact that this right hemisphere has been the dominant one for the *entire life* of this participant.

It is noteworthy that drawing, particularly if it is solely guided by memory as in the Cognitive-Kinesthetic training applied here, is a highly complex task *orchestrating* a wide

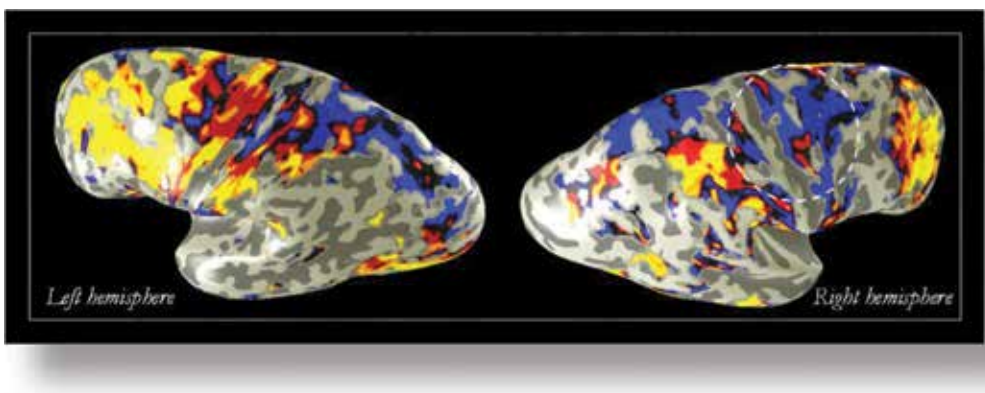

Figure 7. Rapid switch of handedness. To establish the categories of ***training-induced*** changes in the cortical network controlling the ***non-preferred/right*** hand during *MemoryDraw*, our categorical-change mapping was used with the *pre-training* activation pattern of the ***right*** hand as a ***baseline*** in a comparison with its activation ***after*** the right hand underwent the 10 hours of ***training***. The ***training-induced*** categorical changes in the ***functional architecture of the non-preferred right*** are shown on the lateral surfaces of the left and right hemisphere. Color coding as in **Figure 4**: *Orange* — No change relative to baseline; *Yellow* — Increased positive signal; *Red* — Decreased positive signal; *Black* — Reduction to no significant signal; *Blue* — Negative signal

range of perceptual, cognitive, and precise motor functions, thus engaging widely *distributed networks* throughout the brain; their detailed analysis, however, is beyond the scope of this chapter.

6.3. Patterns of hemispheric asymmetry

To quantitatively assess and compare the hemispheric patterns of activation across conditions, we applied the approach used in [1] of comparing the number of voxels, or—volume, activated in each condition. We, however, significantly expanded this approach by taking both positive and negative voxel activations and considering them separately. The voxel numbers were calculated for the conjunction of the motor, premotor, supplementary motor, and somatosensory cortices. The respective FreeSurfer ROIs were used to define the respective cortical regions for quantitative analysis.

Figure 8 shows that both the preferred/left hand (*left panel*) and the non-preferred/right hand pre-training (*middle panel*) conformed to pre-existing models: (1) the activation for the left hand was *predominantly contralateral* (right > left; see *left panel*), whereas (2) a more *balanced, bilateral* pattern of activation was observed for the drawing movements of the *right hand (middle panel)*, indicating a greater ipsilateral involvement.

The *right panel* of **Figure 8**, on the other hand, reveals a radical reorganization in the motor control architecture of the right (non-preferred*)* hand as a result of the Cognitive-Kinesthetic drawing training. The bilateral pattern of (strongly positive) activation *before* training (*middle panel*) rapidly changed into a strongly contralateral (left hemispheric) pattern *after* training (*right panel*).

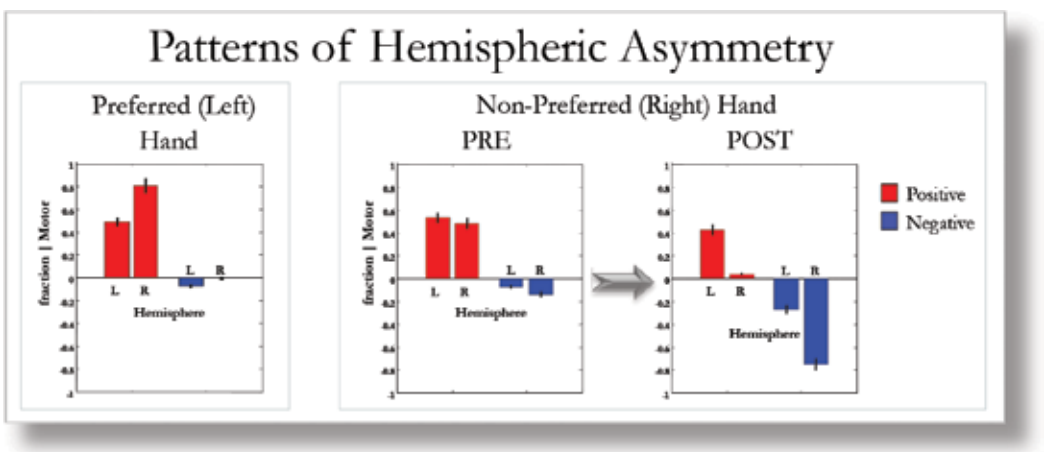

Figure 8. Cognitive-Kinesthetic training effects on the pattern of inter-hemispheric interactions. Three distinct patterns of inter-hemispheric interaction were observed. The distributions of positive (*red*) and negative (*blue*) voxels per hemisphere (*L, left; R, right*) in the conjunction of the motor, premotor, supramotor, and somatosensory cortices during memory-guided drawing is shown for the preferred/left hand (*left panel*), the non-preferred/right hand *pre*-training (*middle panel*), and the non-preferred/right hand *post*-training (*right panel*).

7. General discussion

It is generally accepted that, in both *left- and right-handed* subjects, the preferred hand is controlled mainly by the hemisphere *contralateral* to that hand, whereas the *non-preferred* hand is controlled by *both hemispheres*. In relation to *left-to-right switches* in handedness, the switched individuals have been found to share features of both left-handers and right-handers regarding their motor control architectures. ([1], see Introduction).

Before training, the results for both the *preferred*/left and the *non-preferred*/right hand conformed to these preexisting models: the *preferred*/left hand produced predominantly *contralateral* activation, whereas the *non-preferred*/right hand produced a more balanced *bilateral* activation, indicating control by both hemispheres.

After training, however, the bilateral pattern expected in switchers was not observed any more. Instead, the non-preferred/right hand underwent a strong training-based reorganization of its motor control architecture, so as its bilateral activation pattern radically changed post-training into a contralateral one. Remarkably, this *contralaterality* (left hemisphere > right hemisphere) was caused not by increased contralateral (left) activation but by a massive suppression in the ipsilateral (right) hemisphere; it is particularly surprising that this happened despite the fact that the right hemisphere has been the dominant one since birth.

These findings show for the first time that the dominance of the preferred hemisphere can be rapidly overturned, and that this can happen even in late adulthood after decades of established dominance. Note that, until now, despite long-standing efforts across many disciplines to achieve a fully-fledged hand switching in left-handers, the best that has been achieved has been to engage the contralateral left hemisphere without being able to overturn the ipsilateral right hemisphere control [1, 12]. The fact that the Cognitive-Kinesthetic Drawing Training was able to transform the bilateral into a definitive contralateral pattern, and to do so effectively and efficiently, implies a serious deficiency in the current knowledge on motor control plasticity, and the need for enhanced investigation into this process. Moreover, the power of this memory-driven motor training to rapidly drive motor-control plasticity, in addition to the previously shown effects on memory and spatial cognition, for example [2–5, 39–41], suggests strong involvement of cognitive mechanisms in this process, as codified earlier by the introduction of the "perception-cognition-action loop" concept.

The resulting neural reorganization in this congenitally left-handed individual was correlated with similarly remarkable enhancement in the memory-drawing performance of the non-preferred hand, which post-training resembled the original much better than pre-training, and moreover, significantly better than the experienced preferred hand. This was particularly unexpected because the left hand had several additional advantages. First, the *haptic exploration* of the originals was *always* done with the *left* hand, thus providing a direct perception and encoding of this hand's movements along the lines of that image. In contrast, the non-preferred right hand *never* received any direct encoding of the trajectory but for planning and execution of the drawing trajectory it had to use the memory image developed through the other hand. Second, the nature of the left-hand exploration phase represents a strong form of *dual* memory

encoding for that hand. There was no difficulty or delay, however, to successfully use thus acquired memory for guiding drawing with the right hand. It should be noted that, in the drawing phase, the hand under training receives multifaceted, Cognitive-Kinesthetic feedback, which affects the initial haptic memory, corrects and sharpens it, thus adding another layer of enhancement and embodiment to the overall encoding.

An important practical implication of these findings is that the effects of the Cognitive-Kinesthetic Training can generalize over the full "perception-cognition-action loop" involved throughout the process, which suggests its usefulness not only in the domains of spatial cognition and memory rehabilitation but also in motor control rehabilitation as well.

8. Conclusions

This study is the first to show results that contradict the models of the nondominant hand always being controlled by both hemispheres, as had been previously thought. It is particularly remarkable that this brief memory-guided drawing training was able to switch lifelong handedness, overturning almost six decades of dominance of the right hemisphere by inducing profound suppression in the previously dominant hemisphere. In terms of handedness research as a whole, the study suggests a critical role for functional mechanisms, such as inter-hemispheric competition, as opposed to an inherent structural predetermination in hand dominance. The results are consequently of high relevance to the long-standing debate about the sources of hemispheric asymmetry. The unprecedented effect on handedness of the rapid Cognitive-Kinesthetic Drawing Training also implies the powerful potential of this training for further rehabilitation domains, such as the rehabilitation of stroke or trauma affecting hand control.

Acknowledgements

This research was supported by the National Eye Institute at the National Institute of Health (RO1EY024056) and National Science Foundation (NSF/SL-CN: 1640914) awarded to Lora Likova. The author would like to thank Christopher W. Tyler for helpful discussions on the manuscript, Kristyo Mineff for assisting with the drawing training, Spero Nicholas for helping with the functional Magnetic Resonance Imaging (fMRI) data collection, and preprocessing.

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Chapter 5

Music and Brain Plasticity: How Sounds Trigger Neurogenerative Adaptations

Mark Reybrouck, Peter Vuust and Elvira Brattico

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.74318

Abstract

This contribution describes how music can trigger plastic changes in the brain. We elaborate on the concept of neuroplasticity by focussing on three major topics: the ontogenetic scale of musical development, the phenomenon of neuroplasticity as the outcome of interactions with the sounds and a short survey of clinical and therapeutic applications. First, a distinction is made between two scales of description: the larger evolutionary scale (phylogeny) and the scale of individual development (ontogeny). In this sense, listeners are not constrained by a static dispositional machinery, but they can be considered as dynamical systems that are able to adapt themselves in answer to the solicitations of a challenging environment. Second, the neuroplastic changes are considered both from a structural and functional level of adaptation, with a special focus on the recent findings from network science. The neural activity of the medial regions of the brain seems to become more synchronised when listening to music as compared to rest, and these changes become permanent in individuals such as musicians with year-long musical practice. As such, the question is raised as to the clinical and therapeutic applications of music as a trigger for enhancing the functionality of the brain, both in normal and impaired people.

Keywords: music, neuroplasticity, ontogenetic development, adaptation, connectivity, neurorehabilitation

1. Introduction

Going to concerts, listening to music in your living room, singing together or even playing an instrument is part of most people's everyday life. Recent research indicates that apart from just changing the current mood this may have long-lasting influences on the brain. This contribution, therefore, describes how music shapes the brain as the outcome of

interactions with the sounds. These interactions can be multifarious, as in the case of performing, listening or mentally imaging music, but they all show complex and widespread activity in many areas of the brain. This activity, moreover, is related to training, previous exposure, personal preference, emotional involvement and many other modulating factors related to the cultural background and biological repertoire of each individual [1–7]. Musical training, moreover, is related to structural changes within auditory and motor areas of the brain and reinforces functional coupling of these regions during musical tasks as evidenced by many neuroimaging studies [8–10]. These changes have been observed also in white-matter tracts, such as the corpus callosum, the corticospinal tract and the arcuate fasciculus [11–13]. Studies (particularly those with a longitudinal design) showing the causal relation between the brain changes and the duration of musical training have convinced some researchers to consider musical training as a model for investigating practice-related brain plasticity in humans [14].

Music is a powerful stimulator of the brain. Acoustically, it consists of time-varying sound events that are characterised by a large number of features—more than hundred features can be computationally extracted that are tracked by several regions of the brain [15]. Many low-level features, such as timbre and pitch, are partly processed in Heschl's gyrus and the right anterior part of the superior temporal gyrus, in which the primary and non-primary auditory cortices are located [16, 17]. Besides auditory cortices, also motor regions, such as the supplementary motor area and the cerebellum, are involved during musical activities, including both playing and listening. Due to audio-motor coupling that is necessary for playing an instrument, listening is influenced by the motor demands intrinsic to musical practice, even to the extent that this would become manifest also in the brain responses to music listening alone [18, 19]. Moreover, practising and performing music is a complex, multimodal behaviour that requires extensive motor and cognitive abilities. It relies on immediate and accurate associations between motor sequences and auditory events leading to multimodal predictions [10, 20, 21], which engage broad networks of the brain [16, 22, 23]. Music training has thus been associated with changes in the brain, and some of these changes have been causally linked to the duration of the training, which makes the musician's brain a most interesting model for the study of neuroplasticity [9, 24]. This holds in particular for performing musicians, who provide a unique pool of subjects for investigating both the features of the expert brain and, when considering the length of the training, also the neural correlates of skill acquisition. Musicians' training and practice require the simultaneous integration of multimodal sensory and motor information in sensory and cognitive domains, combining skills in auditory perception, kinaesthetic control, visual perception and pattern recognition [25, 26]. In addition, musicians have the ability to memorise long and complex bimanual finger sequences and to translate musical symbols into motor sequences (see **Figure 1**). Some musicians are even able to perceive and identify tones in the absence of a reference tone, a rare ability termed absolute pitch [27, 28].

The brain changes that musical training entails are numerous and well-documented [2, 3, 5, 9, 24, 26–34]: they involve brain regions important for auditory processing, coordination of fast movements and cognitive control, as well as sensory-to-motor coupling mechanisms (see [35],

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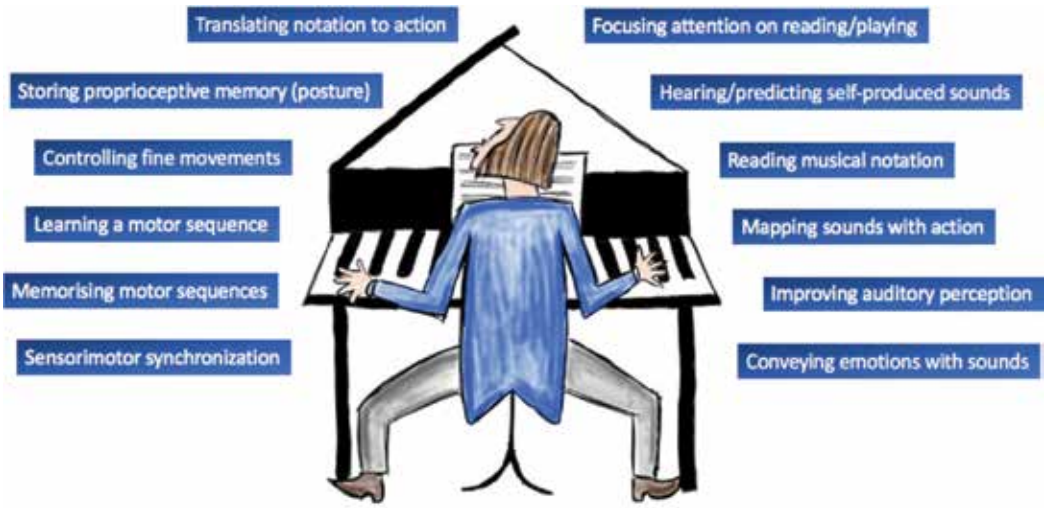

Figure 1. Illustration of several perceptual, motor, interoceptive and emotional skills that are acquired during musical training.

for an overview). While some of these changes might be what characterise individuals that decide to undertake a musical profession, and hence might exist at birth, others could be a direct result of training, as suggested by the significant relations between years of training and brain measures (e.g., [36]), as well as by longitudinal designs recording brain responses before and after music training (e.g., [29, 37]).

Here, we propose that the differences observed between the brains of musicians and non-musicians can be attributed to neuroplastic adaptations responding to the challenging demands of musical practice. Alternative explanations are also possible, such as that the differences exist even before training in those individuals that choose music as their profession, but accumulating evidence points at a causal relation between music training and brain changes. The behavioural correlates of these differences are multiple and can be seen especially in childhood (e.g., [38]). Besides, it has been shown that music may be beneficial in relation to a number of symptoms in several kinds of impairment, such as epilepsy, Alzheimer's disease, Parkinson's disease and senile dementia (see [39] for an overview). Hence, it is possible to conceive of dealing with music in educational, clinical and therapeutic terms.

In this contribution, we first expose the concept of adaptation, both from the phylogenetic and ontogenetic points of view. We then narrow down this concept by putting forward the hypothesis of music-induced neuroplasticity, with a first distinction between macrostructural and microstructural adaptations. Thereafter, we consider the reorganisation of the brain as the outcome of learning and skill acquisition, both at a structural and functional level of description with a major focus on the adult musician or listener as a model for the interaction between ontogeny and phylogeny. This latter, further, is considered from the point of view of network science with a major focus on the role of resting-state networks. Clinical and therapeutic applications, finally, are envisioned also.

2. Phylogenetic and ontogenetic claims: the role of adaptation

Brain plasticity is an adaptation to the environment with an evolutionary advantage. It allows an organism to be changed in order to survive in its environment by providing better tools for coping with the world [40]. This biological concept of adaptation can be approached from two different scales of description: the larger evolutionary scale of the human as a species (phylogeny) and the more limited scale of the human from newborn to old age (ontogeny). This phylogenetic/ontogenetic distinction is related to the "nature/nurture" and "culture/biology" dichotomy, which refers to the neurobiological claims of wired-in circuitry for perceptual information pickup as against the learned mechanisms for information processing and sense-making and immersion in a culture [41, 42].

These approaches may seem to be diverging at first glance, but they are complementary to some extent. This holds, in particular, for the here-hypothesised music-induced plasticity, which espouses a biocultural view that aims at a balance between genetic or biological constraints and historical/cultural contingencies. This places all human beings on equal ground (unity) by stating that diversity in culture is only an epiphenomenon of an underlying biological disposition that is shared by people all over the world [43]. The assumed unity is attributed to the neural constraints that underlie musical processing in general, but these constraints should not be considered as a static dispositional machinery. The picture that emerges from recent research is arguing, on the contrary, for a definition of the neural machinery as a dynamic system that is able to adapt in answer to the solicitations of a challenging environment [6]. The neurobiological approach to music, therefore, deals not only with the nature and evolution of the innate and wired-in neural mechanisms that are the hallmark of the hominid phylogenetic evolution but also with the ontogenetic development of these mechanisms [43]. As such, it makes sense to conflate neurobiological and developmental claims by taking the concept of adaptation as a working hypothesis.

The relation between adaptation and development, however, is asymmetrical in the sense that it is possible to conceive of development without adaptation, but no adaptation is conceivable without development. This development, further, can be natural, when it is the outcome of maturation, but it is possible also to intervene in its trajectory by combining development and learning. This is the case when an organism faces continued and long-term exposure to challenging environments, which triggers plastic changes in the structure and the functions of the brain. This brings us to the concept of brain plasticity, which refers to the fact that neuronal circuits are tuned in close interaction with the environment. It was introduced by William James, who defined plasticity as "the possession of a structure weak enough to yield to an influence, but strong enough not to yield all at once" [44] (p. 106). The idea was further developed by Ramón y Cajal, who claimed that to fully understand the phenomenon it is necessary to admit the formation of new pathways in the brain through ramification and progressive growth of the dendritic arborisation and the nervous terminals in addition to the reinforcement of pre-established organic pathways. The same idea was elaborated further by Donald Hebb, who proposed that neuronal cortical connections are strengthened and remodelled by experience. There is, however, another aspect of plasticity that goes beyond the level of

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synapses and that incorporates the level of cortical representation areas or cortical maps, which can be modified by sensory input and training [45]. It is suggested, in this regard, that additional neurons are recruited when they are needed and that rapid and transient alterations of cortical representations can be seen during learning tasks. Such short-term modulations are important in the acquisition of new skills, but they can lead also to structural changes in the intra-cortical and sub-cortical network once the skill has been established.

3. Neuroplasticity and music: macrostructural and microstructural adaptations

The evolutionary claims of adaptation—both at the phylogenetic and ontogenetic level—have received empirical evidence from neuroimaging and morphometric studies. In order to elucidate its underlying mechanisms, there is currently a whole body of research related to the psychobiological approach to the study of action, cognition and perception. A major claim in this research field is that the nervous system provides the immediate, necessary and sufficient mechanisms that underlie all mental processes, and that mental processes are reducible to the function, arrangement and interaction of neurons as the constituent building blocks of the nervous system [46]. This is the axiom of psychobiological equivalence, which claims an equivalence of maintained information from the neural to the psychological state [47]. The related research revolves around three major themes: (i) the localisation of functions in the brain, (ii) the representation or coding and (iii) the dynamic change or learning [46]. The first investigates which brain structures are responsive for particular processes. The second investigates how neural networks represent, encode or instantiate cognitive processes, both at macrostructural and microstructural level of description (see [48]). The third, finally, investigates how our brain adapts to experience and learning, what changes occur in its neural networks and how these changes correspond to externally observed behaviour.

The third theme—dynamic change or learning—concerns the neural correlates of skill acquisition and has been studied mainly at the level of perceptual processing and motor output. Yet, there is also the whole domain of creativity [49], musical aesthetics [6, 50–53] and human interaction [54, 55], which have been poorly investigated in relation to long-term music training. However, the topic is exemplary of a paradigm shift in current neuromusicological research, with a transition from a static conception of brain modules to a conception of reorganisational plasticity of the developing and adult brain [6]. Plasticity, in fact, is a fundamental organisational feature of the human brain, which can be modified throughout the life span in response to changes in environmental stimulation. This has been observed not only during a critical period in the developing brain but also even throughout the whole life span.

Skill learning, such as learning to play a musical instrument, can thus be used for the study of neuroplasticity. It typically starts early in life, while the brain is most sensitive to plastic changes, and continues often throughout life. It involves multiple sensory modalities and motor planning, preparation and execution systems [27, 56]. The role of environmental enrichment—being defined as a combination of complex inanimate and social stimulation [57]—on

the other hand, should be stressed also as an emerging area of research. Music, in this view, can be considered as "sounding environment" [42], which is likely to drive brain plasticity. Even in the foetal phase of development, sounds can trigger ways of implicit learning [58]. Neonates and infants also learn to talk and sing quasi-effortless as the result of mere exposure, thus demonstrating implicit learning and developmental plasticity, which is even cross-modal to a large extent, in the sense that loss of one sensory modality may lead to neural organisation of the remaining modalities [2].

Neuroplasticity is also related to the field of sensory-motor learning, with a major role attributed to the challenges of a rich environment. It favours multiple interactions with the world—both at the sensory and at the motor level—stressing the interdependency of an organism and its environment through which it "enriches its repertory of genetic adaptations with acquired dispositions that are immediately at hand and mobilizable when confronted with a situation that can be foreseen or recognized as a familiar one" [59] (p. 925).

Musical training, accordingly, may be related to sensory and motor changes in the human brain of professional musicians. As a rule, music training involves years of sensory-motor training, often beginning in early childhood, with the aim to develop an expertise in a chosen instrument or mastery over the own voice, together with an improvement of the ability to attend to the fine-grained acoustics of musical sounds, including pitch, timing and timbre [3]. The brains of musicians might adapt to the demands of their instrumental practice at two levels: the gross anatomical differences between professional musicians and amateurs or laymen, and the subtle functional differences after enhanced musical practice and/or experience, which have to be sought in ever finer modifications of synaptic strength in distributed cortical networks. As such, it is possible to distinguish between macrostructural and microstructural adaptations. The macrostructural differences related to volume, morphology, density and connectivity of brain structures are measured with magnetic resonance imaging (MRI), whereas the microstructural differences in the functional activity of brain regions are measured with functional magnetic resonance imaging (fMRI), positron emission tomography (PET) and neurophysiology (electroencephalography, EEG and magnetoencephalography [MEG]) (see [5] for an overview). It is further hypothesised that functional reorganisation may cause structural adaptation. For instance, bimanual instrument training, such as for the piano, may cause an increase in cortical functionality for symmetric areas involved in motor, auditory and visuospatial processing, as well as in the white matter tracts of the corpus callosum [20] as compared with less bimanual training (such that for the violin) and especially as opposed to laypersons.

At the macrostructural level, studies show differences in the size of the primary motor cortex size, the cerebellum, the planum temporale, the corpus callosum, Heschl's gyrus and the arcuate fasciculus, all of which seem to correlate with the ability of musicians to identify and process acoustic variations [60]. The microstructural adaptations, on the other hand, happen at the level of individual neurons and synapses, with the aim to change the efficacy of neural connectivity. In general, the brain shows adaptation to extraordinary challenges by giving birth to new neurons (neurogenesis) and glial cells and by the formation and remodelling of new connections by the outgrowth of dendrites, axonal sprouting and increasing or strengthening of synaptic connections [61] (see **Box 1** for an overview). Such adaptations have

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been studied in the context of deafferentation studies—in the case of brain lesion—and in the case of motor skill learning [62]. In the former, some cortical remodelling has been found, including microstructural changes, such as the strengthening of existing synapses, the formation of new synapses (synaptogenesis), axonal sprouting and dendrite growth. In the latter, similar changes have been found, including an increased number of synapses per neuron and changes in the number of microglia and capillaries [63], which all lead to volumetric changes that are detectable also at the macrostructural level [27, 64].

The bulk of studies on neuroplasticity has been performed in the context of within-modality plasticity, particularly in the domain of sensory and motor modalities. They aim at demonstrating the adaptive capabilities of the human brain to shape the processing of sensory stimuli or to perform motor acts after repeated sensory exposure or action [9]. With regard to the sensory modality, animal studies have shown that environmental change critically affects brain development. Experience-driven neural activity, in fact, regulates the refinement of the neural circuitry by influencing various neural processes, such as synapse formation, pruning and synaptic plasticity (see **Box 1**) with modifications in synaptic connectivity as a result [65]. This enhanced connectivity, further, acts as a basis for learning and memory through alterations at the level of neural circuits [66], such as strengthening or weakening of the synaptic links or altering their number, changes in the number or properties of postsynaptic receptors in transmitter release and the formation of new synapses. The result is an increase in synaptic strength, which may be persistent and facilitate learning and memory so that experience-dependent plasticity could involve selective changes in pre-existing brain circuits [65].

As to the enhanced auditory skills, it has been argued that they may prime the brain for the processing of musical sounds and that these skills may percolate to other domains, such as speech, emotion and auditory processing in general [67, 68]. This has been observed already in the early stages of the auditory pathway, which are located mainly in the brainstem. Musicians have enhanced temporal and frequency coding in the auditory brainstem with

Myelinisation: the acquisition, development or formation of a myelin sheath around a nerve fibre. This fatty coating serves as insulation of individual fibres to enhance specificity of connections and increases markedly the quick and accurate transmission of electrical current from one nerve cell to another.

Pruning: a process that helps sculpt the adult brain and by which neurons and synaptic connections that are no longer used or useful are eliminated in order to increase the efficiency of neuronal transmissions.

Sprouting: a process by which a neuron generates additional branches or outgrowths to establish new links between existing neurons, as seen frequently in the case of growth of axons or dendrites from a damaged or intact neuron that projects to an area that is denervated.

Synaptic plasticity: strengthening or weakening of the synaptic links either by modulating the strength of synapses or by altering their numbers.

Synaptic efficacy: changes in the number or properties of postsynaptic receptors in transmitter release and the formation of new synapses (synaptogenesis).

Adult neurogenesis: birth of neurons from neural stem cells in the adult brain. In humans, adult neurogenesis has been shown to occur only in the hippocampus (particularly in the sub-granular zone of the dentate gyrus) and in the striatum. It differs from developmental neurogenesis.

Box 1. Overview of some basic mechanisms for refinement of the neural circuitry

earlier (as early as 10 ms after acoustic onset) and larger responses than non-musicians to both speech and music stimuli. This has been shown for the onset response and the frequency-following response (FFR), i.e., a neuronal ensemble response that phase-locks to the incoming stimulus and that underlies perception of pitch as it relates to the sustained portion of a periodic sound with less or more stable frequencies [68, 69].

The role of auditory brainstem processing of behaviourally relevant sounds such as speech and music is important here. It can be measured by using the onset response and the FFR to see how the brainstem represents pitch, timing and timbre [68]. It has been shown that both temporal and spectral characteristics of sounds are preserved in this subcortical response (see [70] for an overview), reflecting the physical properties of sound with an unrivalled fidelity. As a rule, it occurs automatically at pre-attentive levels of auditory processing but is shaped by both long-term and short-term experience [71–73]. Subcortical function, moreover, is neither passive nor hardwired but interacts dynamically with higher-level cognitive processes refining the transcription of sounds into neural code. Hence, the responses do not originate merely in the brainstem but receive feedback from top-down cortical influences even at the earliest stages of auditory processing [3] via corticofugal feedback pathways [74, 75]. As such, it can be demonstrated that musical practice changes the early sensory encoding of auditory stimuli [68] relying on a top-down feedback system—consisting of efferent effects on cochlear biomechanics—that is continuously and automatically engaged to extract and represent regularities in the auditory system [3]. Musical training is thus not limited to the modification of cortical organisation but the modifications extend to subcortical sensory structures and generalise to early processing of speech and sounds in general.

Moreover, early auditory evoked responses and particularly the negative–positive complex (N19-P30) in the auditory evoked potential [76] localised in the primary auditory cortex (the anteromedial portion of Heschl's gyrus) have been found to be larger in musicians compared to amateurs and non-musicians. Moreover, it has been found that the generating neural tissue, namely the grey matter volume of the primary auditory cortex, was broader in volume for professional musicians [77] as compared to laypersons. It thus seems that music can trigger both macrostructural and microstructural or functional changes, not as separate and distinct levels of adaptations, but as phenomena that are dynamically and tightly interconnected.

4. Music facilitates neural connectivity

Music can trigger plastic changes in the brain, as evidenced by the rich history of structural and functional neuroimaging studies of the past decades. Recent advances in functional neuroimaging have furthermore provided new tools for measuring the functional interactions and communication between distinct regions in the brain and for examining their functional connectivity [78]. In an attempt to study the brain as a complex network of functionally and structurally interconnected regions, a fuller understanding of its organisation and function is proposed by relying on the contributions of network science [79], which investigates complex systems in terms of their elements and the relationships and interactions between these elements.

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Functional connectivity can be defined as the temporal dependence of neuronal activity patterns of anatomically separated and removed regions in the brain, reflecting the level of communication between them [80]. It makes it possible to examine the brain as an integrative network of functionally interacting regions and to gain new insights into large-scale neuronal communication in the human brain. Such whole-brain connectivity patterns can be studied by measuring the synchronisation of spontaneous fMRI or MEEG time-series reflecting neural activity of anatomically separated brain regions, which are recorded during rest. These resting–state networks are believed to reflect the functional communication between brain regions [78, 81] and suggest an ongoing information processing and functional connectivity between them even at rest, which is related to neuronal firing. The pattern of correlations between distinct brain areas, moreover, points at the existence of organisational networks in the brain [81], which seems to be analogous to the networks that are engaged during the performance of sensory-motor and cognitive tasks, and which are dependent upon the brain's anatomical connectivity [10]. Such spontaneous neuronal interaction has been first investigated in motor cortices but were later extended to other cortical systems, such as the visual and auditory networks, the default mode network (DMN) and attention and memory related regions. It has been suggested that at least 10–12 resting-state networks (RSNs) can be detected in the cerebral cortex in resting state, which implicates that they represent some intrinsic form of brain connectivity with temporal correlations between spatially discrete regions [82].

DMN has been related to specific brain functions, such as self-referential thoughts, emotional perspectives and levels of self-awareness. DMN is believed to be a neural circuit that constantly monitors the sensory environment and displays high activity during lack of focused attention on external events [83]. It seems to function as a toggle switch between outwardly focused mind states and the internal or subjective sense of self [84] and can be used to explore the functional connections of the complex integrative network of functionally linked brain regions, which continuously share information with each other. As such, there are interconnected resting-state neuronal communities or functional brain networks with functional communication between them. Being organised according to an efficient topology, they combine efficient local information processing with efficient global information integration with the most pronounced functional connections found between those regions that share common functions.

Overall, resting-state fMRI oscillations reflect ongoing functional communication between distinct brain regions [78], which makes them indicative of the level of cognitive functioning in general. There seems to be, in fact, a link between an efficient organisation of the brain network and intellectual performance—this is the neural efficiency hypothesis—so that functional connectivity patterns may be used as a powerful predictor for cognitive performance [85]. This resting-state connectivity, further, is not to be considered as an established and fixed property, but as a state that can be modulated by recent experiences and learning episodes, both within and between the networks they recruit. Such modulation points in the direction of a learning consolidation function of resting-state brain activity, as evidenced by the findings that high learners manifest stronger pre-task resting-state functional connectivity between the involved regions than low learners [10]. It thus seems that, even in the absence of

external stimuli or demands, the brain is constantly sharing information. It thus consolidates recent learning and maintains the association of activity of brain areas that are likely to be used together in future [86].

Initial research suggests that musical training might enhance this pattern of increased resting-state connectivity by triggering heightened connections at a functional level between those brain regions that are structurally and functionally altered as the result of training. This is manifested even during a task-free condition, pointing to the "silent" imprint of musical training on the human brain [35]. Research on the differences between musicians and non-musicians in their functional connectivity during rest, however, is still in its infancy [10, 82]. By selecting predefined seed regions for computing connectivity analysis, increased connectivity between contralateral homologue regions has been found in musicians between prefrontal, temporal, inferior-parietal and premotor areas [35]. It is to be questioned, however, whether the study of predefined regions or seed regions does not neglect residual whole-brain dynamics. However, for the seed regions for which plastic changes in musicians have been found already—as evidenced by increased grey matter volume—connectivity analyses have revealed brain areas whose resting-state time series activity was more closely synchronised with one of them. Four networks were found to supply integrative interpretations for the cognitive functions during musical practice: (i) autobiographical memory-related regions belonging to the default mode network, recruited by the encoding, storage and recall of melodies with an emotional and biographical quality; (ii) areas that belong to the salience network with access to semantic memory that is related to the storage of music in terms of verbal labels and auditory structure; (iii) regions that are implied in language processing and the resting-state auditory network and (iv) structures that belong to the executive control network, and which could subserve the motor modulation required for an emotionally expressive interpretation of music. The question whether this practice-related plasticity is triggered by local grey matter volume, however, is not yet satisfactorily resolved, in the sense that other variables may be implicated in the expertise-related resting-state functional reorganisation of musician's plastic brain [10].

5. Clinical and therapeutic applications

To stretch further our hypothesis about music-induced neuroplastic adaptation, music, as a cognitive-demanding activity stimulating neuroplasticity, may be able to slow down, arrest or even reverse the detrimental effects of ageing on learning and memory capacity of the elderly [33]. Recent studies have provided evidence that music-induced plasticity may help also to overcome neurological impairments, such as neurodevelopmental disorders and acquired brain injuries [56]. For instance, attentive music listening recruits multiple forms of working memory, attention, semantic processing, target detection and motor function, relying mainly on bilateral brain areas—superior temporal gyrus, intraparietal sulcus, precentral sulcus, inferior sulcus and gyrus, and frontal operculum—which all serve general functions rather than music-specific cortical regions [87, 88]. Complex musical tasks, moreover, engage the co-activation of many processes involving widely distributed and partly interchangeable substrates of the brain [89]. This may explain, to some extent, the sparing of some musical functions in cases of progressive

http://dx.doi.org/10.5772/intechopen.74318

destruction of some areas in degenerative diseases of the brain. This has been shown most typically in the case of Alzheimer's disease (AD), which is characterised by a general and progressive decline in cognitive function, with the first symptom as an impaired episodic memory. Music, in this case, has been reported as one of the domains in which general skill and memory are preserved in spite of otherwise severe impairment [90]. This preserved musical processing, moreover, is not limited to procedural memory but often includes also stories of music, which can be used as an effective mnemonic device [91].

Hence, music may shape the development of normal and healthy human beings over the lifespan, but its potential as a non-pharmacological interventional aid for caregivers to help the cognitive and emotional capacity of patients with neurological and psychiatric brain disorders is receiving growing interest [15]. The use of resting-state fMRI techniques, e.g., with a main focus on the default mode network, seems to be well-suited to examine possible functional disconnectivity effects in disorders such as Alzheimer's disease, depression, dementia and schizophrenia. Also, other neurogenerative diseases like multiple sclerosis and amyotrophic lateral sclerosis seem to show changed connectivity in the default network as well as in other resting-state networks [78]. This may suggest that neurodegenerative diseases would attack interconnected cortical networks rather than single regions in the brain [92] and can thus be targets of a music intervention aimed at stabilising abnormal patterns of functional connectivity between compromised brain areas.

Music has been used already as a treatment for some psychiatric and neurological pathologies, such as schizophrenic disorders, Alzheimer's disease, Parkinson's disease, cerebral ischemia, pain, autism, anxiety and depression [15]. Music, furthermore, has been reported to improve also the well-being and cognitive functions in healthy adults, such as autobiographical memory, semantic memory, language ability and cognitive functions, and to alleviate neuropsychiatric symptoms, such as agitation, apathy, depression and anxiety (see [39] for an overview). Effects of music on AD are exemplary of the mechanisms that might mediate the impact of music on human well-being. Latent benefits of musical mnemonics as an aid to standard mnemonic methods, which may seem to be insufficient for AD patients, have been reported (for a review, see [15]). The mechanisms behind these memory-enhancing effects, however, are still not fully understood, but there is strong evidence for a benefit of music as a mnemonic device in a variety of clinical settings [91]. A possible explanation is that the areas of the brain associated with music cognition are preferentially spared in the case of AD. It has been suggested that procedural memory and priming effects for musical stimuli remain intact, whereas short-term and long-term episodic memory for melodic excerpts is impaired [93].

This dissociation between memory and general performance in AD patients holds in particular for listening to their favourite songs, which seems to recruit previously encoded memories. These memories seem to support and sustain brain introspection via connectivity within the default mode network and also to effectively reprocess autobiographic and episodic memories [84]. An additional explanation for this dissociation is that in patients with general cortical and hippocampal atrophy, which impairs standard episodic learning, musically-associated stimuli allow for a more diversified encoding. Music processing, in that case, encompasses a neural network that is recruiting from multiple areas of the brain, including

cortical as well as subcortical areas. Musical stimuli and stimuli accompanied by music seem to create a more robust association at the stage of encoding and support a more composite encoding and retrieval process by inducing oscillatory synchrony in those neural networks that are associated with learning and memory [91, 94].

6. Conclusion and perspectives

Neuroplasticity is now an established topic in music and brain studies. Revolving around the concept of adaptation, it has been found that the brain is able to adapt its structure and function to cope with the solicitations of a challenging environment. This concept can be studied in the context of music performance studies and long-term and continued musical practice. It has been shown that some short-term plastic changes can even occur in the case of merely listening to music—without actually performing—(e.g., [95]) and in the short-time perspective of both listening and performing (e.g., [96]). Attentive listening to music in a real-time situation, in fact, is very demanding: it recruits multiple forms of memory, attention, semantic processing, target detection and motor function [18, 97]. As such, we propose here that music represents a sort of enriched environment that invites the brain to raise its general level of conscious functioning.

Traditional research on musical listening and training, however, has focussed mainly on structural changes, both at the level of macro- and microstructural adaptations. This has been well-documented with morphometric studies, which aimed at showing volumetric changes of target areas in the brain as the outcome of intensive musical practice. Recent contributions, however, have shown that the brain can be studied also from the viewpoint of network science. The brain, in this view, is not to be considered as an aggregate of isolated regions, but as a dynamic system that is characterised by multiple functional interactions and communication between distinct regions of the brain. Whole-brain connectivity patterns can be studied by measuring the co-activation of separate regions. Much is to be expected from the study of resting-state networks with a special focus on the default mode network. These networks seem to be indicative of the level of cognitive functioning in general and are subject to the possibility of modulation by experience and learning, both in the developing and in the mature brain. We propose that music has the potential to alter the organisation of these brain networks and enhance the connectivity of the brain, both in normal people and in those with an impaired brain.

A major emerging topic, therefore, is the tension between neurogenerative and neurodegenerative forces with the critical question as to the possible role of music as an intervening force to develop, maintain or even restore the connectivity in brain tissue. The idea that age-related cognitive decline may be slowed, arrested or even reversed through appropriately designed training or activities, such as musical practice, is supported already by some research. Moreover, the finding that the adult brain can undergo continual modifications highlights the potential of music intervention for inducing the plastic changes that can ultimately attenuate the impairments due to brain injury. Much more research, however, is still needed towards an integration of findings from neuroscience, education, music therapy and development.

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Chapter 6

Brain Dynamics and Plastic Deformation of Self Circuitries in the Dementia Patient

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71054

Abstract

Despite improved medical care that has resulted in greatly extended life expectancies, significant increases in numbers of individuals suffering age related cognitive defects is expected, making the improved understanding of normal and pathological aging an important priority. Current studies indicating that brain activity requires a dynamical architecture to preserve functional order in the face of persistent and extraneous activity suggests that cognitive impairments are likely to be closely linked to dysfunctional dynamical activity of brain systems. Cognitive impairments such as those introduced by Alzheimer's dementia (AD), that affect fundamental operational constructs like the self, are thus likely to implicate global dynamics that oversee whole brain operation. This paper explores plastic events associated with dynamical elements used in the normal construction of the self percept and the etiology of their deconstruction in the course of AD. It is proposed that the evolution of the disease involves the increasing impairment of a global dynamical operation that is normally engaged in forming a stable and coherent self image needed to flexibly engage task related, motor plans and effectors.

Keywords: brain dynamics, Alzheimer's dementia, dynamic neural fields, attractors, self circuits, oscillations

1. Introduction: the dynamical self and Alzheimer's dementia

United Nations' estimates project that by 2050 20% of the world's population will be nearly 60 or more years of age, with considerably higher percentages in developed nations [1]. Despite improved medical care resulting in greatly extended life expectancies, significant increases in numbers of those suffering age related cognitive defects, including various dementias, is expected. This reality makes the improved understanding of normal and pathological aging an important priority. Among the most promising avenues to such

understanding today are studies related to brain dynamical systems and globally operant phenomena, including self awareness and consciousness. Significantly, and unlike the activity of peripheral sensory and motoric neural processes, brain activity is ongoing and not intermittent, dynamically sustaining a wide variety of metastable and plastic states, including such perceptual phenomena as the self that are progressively impaired in dementias. Changes in brain dynamics, therefore, are clearly evoked during the evolution of these pathologies.

Although promising, brain dynamical studies have yet to find a role in studies of self representation and its loss with pathological progression. This is especially pertinent since there is the increasing realization that self circuitries underlie global phenomena that are key to individual performance. Accordingly, this chapter will consider three themes that are intended to illustrate how the loss of self representation is related to plastic changes in brain system dynamics. The first theme will build upon the traditional linkage that has been forged between brain activity and its modulation by neuroplastic events underlying its physiological expression, Donald Hebb's oft-quoted rule, what fires together wires together [2]. Expanding on this foundation it will explore how connectivity changes modulate the stability of energetically favored resonances, for example, attractors, that are naturally exploited as operational motifs of larger scale networks [3] and how these are used to build a three-dimensional self image. The second will evidence how the regulation of motif stability assists the generation of self representations, particularly during development as sensorial, somatotopic input progressively shapes the brain, creating the self percept for mature interactivity with the world. The third will contextualize pathological distortions as loss of function etiologies that can be modeled by a change of system dynamics, evidenced in the loss of function of global dynamical systems underlying construction of the self image.

The outcome of this exploration will be the creation of a portrait of brain dynamics that both reciprocally and iteratively relates brain dynamics to the evolution of self representation in dementias. It will assist in clarifying the relationships between neuroplastic mechanisms of connectivity, their modulation of brain dynamical elements that create spatial representations of the embodied self, and distortions in regulating this representation as dementias increasingly impact global operation.

2. The self and its method of representation

Conceptions of the self adopt understandings that may be colloquial or intuited, philosophical or definitional, subjective or objective, functionalist or ontological, self contained or overlapping [4]. Cumulatively, these varied understandings provide a sense of what is generally meant by the self, yet the distributed nature of the definitions that contribute to its understanding mean that the conceptions that are intended to define the self can only approximately service the notion. Nevertheless, the self is a real construct, universally appealed to by professional and public alike. John Locke provided a definition more than 300 years ago that is still widely adhered to, and often regarded as definitional for personal identity

> *'the awareness of being the same person in different places and different times'.*

This self percept is usually taken in its subjective sense, yet the elements that contribute to the percept are objective features closely related to the neural architecture that underlies its subjective expression.

The present chapter will adopt a perspective of the self that is dynamical and interactivist, that is, an operational perspective of how the individual autonomously engages the exterior world [5, 6]. It will consider the self's role in initiating and structuring the actions that the individual carries out as it negociates a variable environment, that is, its interactive dimension, that will be termed here the performative self. There are several reasons for adopting this conception, that, while not covering the rich depth of understanding that is associated with the concept of the self [4, 6], are yet likely to be both valid and useful in the context of its contribution to understanding the etiology and evolution of dementias. First, it offers a tractable construct that possesses conceptual clarity vis-à-vis its behavioral and neural expression. The self is here understood to be that individual representation that acts to secure the vital dimensions needed for viability. Second, the neural and biological foundation of this conception of the self is increasingly well understood and likely to shed light on events that are abnormal, such as cognitive diseases like Alzheimer's dementia (AD). Current evidence, for example, indicates that the self is associated with the whole of the individual, that is, it is a totality whose origin and association is linked to the entire corpus, and not restricted to an isolated domain of representation lying within the brain [7], one that is the product of the progressive integration of regionally distributed, somatotopic afferent input. Significantly, this perceptual image appears to be invested with protagonist features and to assist in the construction of an intentional image used for counterfactual affordances [8]; in other words, this 3-D body image of the self enables the individual to 'situate and to conceive of himself' in different goal-directed planning trajectories.

There is good evidence to suggest, moreover, that the body image is the neural product of dynamical systems mechanisms that serve as its operational basis. Among other findings, for example, limb positions for navigation are topologically mapped [9] as are the external trajectories navigated, where they are linked to environmental cues later recalled to guide movement in association with exploration. The neurophysiological basis of such memories appears to involve dynamical systems processes that encapsulate the memories in energetically stable activity zones, such as Hopfield attractors [10]. Taken together, the performative self appears to be a construct that represents the whole individual bodily, engages dynamical neural processes, and is needed for unitary, goal-directed activity.

This performative model thus affords a physically defined and physiologically relevant neural terrain encompassing global brain and body operation that can be explored in terms of an ongoing etiological progression. In the next sections, I will consider underlying mechanisms of normal plastic changes involving dynamical system operation related to the self and then subsequently consider how these are likely to be affected by the progression of the disease.

2.1. Neuroplastic mechanisms in dynamical systems representations

The operational perspective of the self adopted here is situated by the need to sustain a continuous stream of neural activity. Constituted as a goal directed and autonomous entity

the individual must possess a neural architecture whose operation, necessarily, remains persistently active in order to enable self directed action and maintain homeostatic autonomy. This requirement for independent and autonomous existence has clear implications for the form adopted by the neural architecture both anatomically and physiologically, a form that needs to be robustly maintained in the face of variable input activity while yet enabling behaviorally relevant responses.

Studies of brain operation are beginning to reveal how these requirements are met. Physically, they begin with the anatomical structure of the nervous system that in the brain exhibits an exceptionally high degree of recurrency. For example, nearly 95% of all brain neurons receive some level of feedback via nerve collaterals [11]. This organization creates neural topologies in which information flow cycles through preferred, low resistance pathways creating zones of stabilized activity, that is, basins of attraction to which the flow converges. What is significant about this observation is that it reveals that the brain's configuration is necessarily oriented to creating a dynamical setting where activity is used to create a functional order that resists the ravages of entropic deconstruction and is operationally reliable, that is, it can be described by mathematically ordered relations.

Constitutively, stability is the critical feature of dynamical system operation [12]. Stabilized activity is needed due to the susceptibility of information flow to external, that is, sensorial and internal, perturbing influences that are an ongoing and intrinsic aspect of brain operation. Such perturbing influences can only be circumvented by the creation of stabilized activity zones that foreclose interference to extraneous information flow that can and usually does originate in such sources. Because organisms are embodied their sensory, motor, and cognitive operations are continuously engaged in monitoring, interpreting, and responding to varied informational sources.

Stability is conferred in multiple ways that in addition to recurrency of information flow include appropriately adjusted connectivity strengths, leakage currents, glial barriers, and the like. The cumulative effect is the creation of energetically stabilized zones within an overall energy landscape that entrains and structures information flow, that have been termed attractors because of the convergence of flow to particular paths lying, appropriately, within basins of attraction. Thus, its characteristic feature is a zonal resistance to change that acts as a buffer to spurious sources of variable neural activity.

Dynamical system operation is characterized, additionally, not only by robustness and stability, but also by flexibility and the capacity for directed control. Thus, three features are incorporated into brain operation in the context of and need for accommodating ongoing brain activity that is required for sustaining interactive autonomy. Because brain activity determines motoric output energetic levels of information flow within stable zones must also remain sufficiently close to zonal instabilities in order to permit its exit from particular attractors and subsequent entry into alternative ones. This capacity for egress constitutes a principal means for achieving variable motor responsivity, a functional feature that also means that the particular form of behavioral output selected is directly related to the set of activity zones chosen. Physically, instabilities are forces of convergence that are relatively weaker in particular regions of the attractor. It becomes possible at such locations, accordingly, to induce transitions out of the stabilized zone, which

therefore constitute bifurcations leading to new energy profiles [13]. Such transitions are characteristically abrupt, for example, like those observed in the hippocampus [14], and so represent a wholly new and nonlinear trajectory to a different energy locus. This sort of dynamical patterning suggests that functionally significant, motor output is characterized by its dependence on the assembly of discrete energy sets, rather than the deconstruction and reconstruction of parts of the dynamical elements themselves.

It is in the context of such selection that the role of the self is pertinent. A capacity for flexible responsivity means that there must also exists a reproducible and reliable manner of selecting particular sets of stabilized zones in order to yield a certain type or class of motor response. This capacity, further, must operate within the context of the demands of the whole individual, that is, it must benefit the individual as a whole, hence, the requirement for a performative self, that is, a representation that can guide goal-directed actions. Increasingly, neuroscientific evidence is revealing that this dynamic construct is systemically ordered, that is, it is globally distributed and operative [15], though more focally concentrated in regions, such as the Default Mode Network (DMN) [16], a feature that may be analogized by the DNA of eukaryotic cells. This global system, moreover, very likely operates by engaging select dynamical elements, which appears to be the basis of motor responsivity [17].

Importantly, since the neural substrate for the self is itself subject to the same operational constraint for persistent activity its operational configuration must also employ dynamical system operation. Overall, a critical operational dimension of the self that flows from this structure is that dynamical activity is constructed by a layered ordering of properties that while fundamentally dependent on underlying structural elements are nevertheless operationally independent from them. For example, while the brain uses spiking mechanisms to create information flow, that is, mechanisms described by Hodgkin Huxley action potential formulations, as do peripheral nerves, these constitute lower level operative features, whereas higher level dynamical systems like attractors and their assemblies constitute, in reality, the fundamental functional elements through which the brain conducts its operations [18].

2.2. Organizational mechanisms of the dynamical self percept

The use of dynamical stability to negociate biologically relevant outcomes, via the percept of the performative self, thus introduces the question of how the percept is structured and persists over time while yet remaining sensitive to ongoing input that may either facilitate its maintenance or induce an evolution in core features. A portion of the answer to this question can be gleaned from the distinctions between mechanisms used to create information flow, like the spiking mechanisms just mentioned, and those that employ these mechanisms to create distinctively new functional elements, like attractors. This is to say that the dynamical construction of this percept is a consequence of the nervous system's ability to construct a layered and dynamical order, with the progressive appearance of functionally significant new features at progressively higher levels. Thus, a fuller answer to this question is situated by the multiple layering of dynamical elements, and so relates to the question of the plasticity of the changes that transpire at various levels. Existing evidence indicates that these changes

are likely to be multiple and to include specifically effects on connectivity modulation [11], dynamical motifs [19], higher order clustering [20], and/or symbolical representations [21].

Modulation of connectivity that transpires in recurrent networks constitutes a ground level for plastic changes that affect dynamical systems like attractors. Connectivity changes can influence signal transmission by controlling overall resistance to transmission among multiple synaptic contacts, for example. Underlying mechanisms mediating such changes via Hebbian or other processes, that is, synaptic activity dependent strengthening [2], can include molecular effects involving protein kinase modulation related to synaptic vesicle release, enhanced pre- or post-synaptic membrane conductances, increased synaptic size, and the like which modify information flow [22]. Significantly, connectivity weight changes can undergo dynamical variation with learning, habituation, or development, that is, weight changes that are mathematically described differentially by rates of change that assume different and variable time scales and which thus modulate the temporal evolution of higher order properties. For example, the time evolution of activation in a linear feed forward network described by $\Delta a_i = \lambda(net_i - a_j)$, where $net_i = \Sigma_i w_s a_s$ and w_{is} is the weight of a connection from neuron i to neuron s, creates a cascading effect of system dynamics over time [3]. Controlling connectivity changes over time, in fact, is a basic mechanism for introducing experiential effects into dynamical systems.

Such effects on higher properties are significant, moreover, for also revealing the existence of dependencies of dynamical processes on underlying network mechanics that sustain them, but which, nonetheless remain operationally and functionally independent of the connectivity arrangements themselves. This is seen, for example, in the Usher-McClelland model [3] for classification responses that are constructed from a single set of hidden neuron units receiving equal input with feedback excitation only to the units themselves while inhibitory to all other units. The significant feature of this relatively simple connectivity model is that the output of the network asymptotically approaches a steady state level as input levels to the hidden layer continue to rise over time. That is, while the output of this network depends on the existence of incoming spiking activity, it is only slightly and indirectly related to the magnitude of the activity entering the network at the onset and becomes altogether independent of its magnitude over time. Importantly, connectivity patterns are widely variable and of great complexity, not only between species, but in the structure of the human brain [23]. Thus they offer an extraordinary repertoire for introducing dynamical variation. Changes in ground state connectivity can lead, for example, to changes in the performance of given dynamical elements, or lead to the formation of new dynamical elements altogether.

The layering of properties, seen in the transition from the level of spiking activity to that of dynamical elements like attractors, is manifest at yet higher levels where dynamical units may combine to yield qualitatively and quantitatively differing sets of such elements. Shoner shows, for example, how an attractor and a divergent element like a repellor can combine to yield an entirely new attractor state [12]. Like the contributing elements, the assembled combinations are themselves robust to interference, as, for example, in the case of heteroclinic channels that are constructed from a series of attractors joined in sequence that effect unique trajectories through the dynamical state space [24]. Such combinations are potentially

used for phoneme construction, a behavioral output that can be accommodated by effecting sequences within sequences through the creation of supraordinate heteroclinic activity at higher levels, that is, a hierarchically layered operational order that is functionally pertinent.

The significance of this layering, further, goes beyond the generation of robust patterns to their manner of combination and how their assembly is regulated since the former dictates what cognitive or motor effector is actuated and the latter how this actuation is subject to control. Because dynamical systems are persistent structures, due to their basins of attraction, the individual energetic profiles they assume cannot be randomly disassembled and then recombined but instead must be joined together in combinations of units which retain their individual profiles, that is, operative motifs, within the larger assembly. What this implies is that there are preferred ways in which these motifs can be joined to achieve functionally significant groupings. Evidence for functionally significant examples of this higher order association has been demonstrated in neural networks that control mouth motion 'chewing' in the roundworm *Caenorhabditis elegans*. Network clustering analyses show that the behavioral networks used for chewing are organized operationally into higher order clustering patterns that are made from combinations of lower level network motifs [20] which appear to be coordinated by primitive synchronizing mechanisms [25].

Such combinatorial products also enable the possibility for multiscaling, that is, combinatorial groupings that repeat at higher levels, and by so doing avail the possibility for accessing an extended operational repetoire of the sort suitable to symbolic representations. Cooperative behaviors like those of clustered networks can thus be exploited to greatly extend the range of representations that can be discriminated. Building on the work of Rodriguez [26], for example, that showed that simple recurrent networks could generalize from syntactical constructs with relatively few combinations of dynamical elements—in most cases an attractor and repellor sufficed—Tabor proposed that dynamical system elements could mediate between connectionist network structures and symbolical representations [21] by evolving combinatorial possibilities that could be scaled upwards by repeating lower level organizational combinations. Such scaling, significantly, means that these possibilities mathematically adopt fractal type organized patterns, which is to say that the self organized processes also found in physical structures like snowflakes, or protein molecules, retain their functional significance at increasingly hierarchical scales.

2.3. Constructing the self percept from dynamical elements

In keeping with much evidence the body is intrinsically involved together with the brain in establishing the performative self [7]. The layering of dynamical elements described above, accordingly, is framed by the dynamic between the two that involves the reciprocal and mutually dependent flow of information effected between brain regions such as the hippocampus and the distributed sensory and motor neural network that lies beyond the brain. This relationship is necessarily constrained by the dynamical nature of brain activity, which is continuous, and the need for stability, which remains the cornerstone of dynamical operation.

While it is clear that stability is needed for basic dynamical elements like attractors, it is not immediately clear why this should be required for global brain operation such as

the self whose physiological basis seems to be underpinned by constraints beyond that of the buffering of spurious noise signals. Yet existing evidence indicates that stability and coherence are fundamental at this level as well. Hence, built into the dynamical layering is a predisposition to create coherent structures that can carry out the grander tasks of the neural architecture. Interestingly, this evidence has come from studies that explore the lack of certain cognitive capacities rather than their presence, such as the demonstration of perseveration in infants [27]. In the case of infant perseveration, that is, infant behavior when presented with an 'A not B' task, cognitive processing of the body's disposition in space is needed to relate the individual to the events of the world. This is to say that the whole body is needed to create a stable and coherent image that can be interactively and cognitively positioned in motor planning to create affordances enabling motor planning. Indeed, identity and self awareness are both linked to this embodied dimension [6, 7, 28]. For example, intentional acts in developing infants to either stand upright or to crawl, effect a differential brain mapping depending on the performative act intended, such as the acquisition of bimanual skills with the correspondent coupling of sensori-motor areas [28]. This observation reveals that task performance is related to the linkage made between the goal and the individual for whom the goal is intended, which is to say that intentional performance requires a stable representation of the individual that can be cognitively or physically mobilized as a whole.

Corbetta's [28] experiment is revealing in siting this representation to the performative dimension; that is, the representation of the self is set within the context of motor plans and is effected by sensorial, largely somatotopic, input. There appears, therefore, to be a constitutive requirement for a holistic and bodily contextualized, self representation for performance that can be employed in a wide variety of interactive and dynamic circumstances. This implies that mapping between levels must cohere, and can only do so to the extent that the dynamical elements themselves cohere and cooperatively engage to yield its global outcome. Due to the wide variety of interactive circumstances it also means that it must relate to the dynamical elements involved in effecting various acts at multiple levels that can then be reorganized according to the exigencies of interactive demands. Finally, it invokes the need for regulatory processes that are evoked by the self to accommodate the variability encountered in motor planning, that is, the regulation of the disassembly/reassembly processes that are mobilized to accommodate such possibilities.

The coherent self image is the basis on which the twin needs for stability and flexibility are satisfied and is used to dynamically frame individual motor acts in relation to the global self representation. In this way they can be understood as belonging to the self. It is in this context that Smith's [27] sensori-motor object can be understood, and also the way in which the infant perseveration studies are interpreted. In other words, the performative self arises from the need to frame the individual holistically for its interactive role, a framework that arises from somatotopic afferent input of the body schema and constituted by the three-dimensional body image [7].

Significantly, the body position undergoes its own temporal evolution that then becomes critical to the successful negociation of motor plans. This situates the juxtaposition of the whole against the elements of the body in a continually mobile frame where successive movements are always referenced to an immediately preceding spatiotemporal location, that is, at each dynamic moment there is a contrast between the spatiotemporal configuration of that which

just was and the correspondingly new position of the here and now [27]. Thus, the sensorium is always placed in the role of needing to monitor the whole body, and so also of continually contextualizing the motor planning that may transpire. Decisional acts, additionally, that are associated with motor planning, must also participate in structuring motor plans in order to generate goal-directed activity.

This is also to imply that the sense of self in a minimal sense is a *persistent* process that provides the stable ground for decisional performance. That is, we consciously select among a range of potential acts suited to achieving a particular interactive goal [8] that must be understood to be the consequence of self made motions, in order to distinguish them from a sea of surrounding and non-cohering imagery. Here the infant perseveration results are again useful for understanding the need for a coherent image that can be directed to perform various motions. Consistent with this, Frith first proposed that the sense of agency was related to a comparison mediated correspondence between self made motions and selected motor plans [29], a genre of predictive processing models [6], thereby introducing the motor planning stage as the needed dimension for generating the sense of agency. In his Comparator Model a motor command termed corollary discharge is sent to the sensory cortex in advance of the execution of motor movement so that the sensorial observation of the expected movements can enable the distinction between movements of the individual and those of the surround. However, for the motor movements to be situated as an operation that is part of a whole means that the sensorial imagery of the body must also identify the motions as flexible ones against the stability and coherency of the self image. This makes it likely, and as the perseveration experiments seem to show, that there must be a sustained awareness of the self, with its corresponding need for persistent sensory input, from which such actions begin, depart, and end. Together, the performative self, motor planning, and decisional acts appear to share intimate relations that are mediated by the dynamical processes of the neural architecture, as Varela, Thompson, and Rosch earlier intuited [30].

2.4. Self mechanisms that effect operational order

How these dimensions overlap is thus crucial to a biological understanding of how the self percept is mediated performatively over time. Evidence indicating how these events occur implicates the sensorial control of dynamical elements, which are selectively appropriated for motor planning. Matching of sensorial input with special classes of attractors termed dynamic neural fields (DNFs) appears to underpin the unique structuring of motor element planning and execution [31]. DNFs, notably, are bistable or multistable attractor neural networks, that is, they are dynamical elements capable of being shifted into an activation mode by transitioning from lower attractor states to higher ones. Incoming sensoria generate peaks of activation that leverage the instabilities in the base states of these fields to induce transitions to the upper level ones [32]. Since motor plans must ultimately be transformed into effector actions it is likely that the upper states are also responsible for engaging the effectors and that sensorial input provides modal specific stimuli to insure the plan's performance by activating the transition from lower to higher level attractor states. Against this backdrop, and so providing overall coherence for the performative self, lie the lower attractor states that appear to act as the landscape for the three D body image, which contextualizes the respective effector events.

Understood systemically, global supervision implies that the whole of brain dynamical activity is accessible for a distributed oversight, which by extension means also that a smaller suite of behavioral repertoires are evoked for motor planning and motor activation. This oversight appears to involve the regulation of localized assemblies that are continually monitored by the global/self percept [15, 17]. The subordination of dynamical elements comprised of multiple neuronal units, in fact, is not unique to humans. *C. elegans* also exhibits a globally evoked dynamical operation in the form of coordinated sleep behaviors. Underlying this ability in humans and a number of other mammalian species, is a capacity for synchronizing activation of smaller subsets of dynamical elements, apparently regulated by a globally distributed oscillatory activity [33].

Governing how these assemblies become responsive, and thus regulating how dynamical units assemble into larger functional aggregates, are the relative free energy differences between units that regulate their assembly into the larger wholes along a descending free energy gradient [34]. In other words, higher order brain activity is organized naturally through energetic constraints that govern pattern assembly, an organizational strategy that is commonly used in biological systems in numerous other contexts. Such organized assemblies characterize numerous macromolecular events such as those of enzymatic catalysis. The combination of lower order monomers into combined polymer patterns yield novel cooperative effects that enhance function beyond a strict linear relation to the monomer alone, that is one greater than the sum of the parts. For example, cooperativity effects exhibited in hemoglobin multimers enhance oxygen binding to hemoglobin over and above a strict linear dependence on hemoglobin concentration, a feature critical to physiological performance.

Indeed, it is within the context of free energy efficiency that the selection of assemblies can be framed, that is, as a general principle determining brain responsivity. To permit information flow to exit attractor motifs, basins of attraction in attractors must be sufficiently shallow to overcome energy barriers dividing stabilized zones. This means that a key feature in creating and modifying assemblies is that of the intrinsic instabilities within the attractor space that can be accessed for egress. As with patterned assemblies that can form higher organizational layering, instabilities can form constituent features of stabilized zones at multiple levels that include motifs only, or higher levels that may be made of multiply clustered motifs including, for example, the exit from single attractor motifs that may occur at an instability creating a bifurcation, or in complex fields that may be composed of multiple single attractor states, such as, for example, dynamic neural fields, where field instabilities can be leveraged to generate a uniquely different field feature with a new output [12].

3. Modulating dynamical element stabilities across time scales: constructing the self percept during development

Inferences about neuroplastic changes to the self percept during dementias concern a construct that has itself been structured according to a pattern of organizational processes that together generate its mature form. Developmental events leading to the construct's mature expression can therefore be expected to inform not only mechanisms underlying its orderly developmental

assembly, but also how it is deconstructed in etiologies whose progression may be variable and irregular. Driving the developmental events and constitutive of their mature expression are again the twin needs of operational stability and flexibility that are the hallmarks of the dynamical systems used in complex neural operation. How are these needs met in development?

The phenomenologist Merleau Ponty [35] was among the first to suggest that repetitive interaction between the body and environment structured a habitual, posturally determined perception of the world. This observation is germane for suggesting several features of the performative self that are needed for its generation. First, it underscores the importance of the body as a stable reference point for interaction; thus, a fundamental goal of the developmental process is the achievement of this functional objective. Second, it is from the perspective of the body that the self engages the world; hence, it is necessary to also situate the body in spatiotemporal terms to understand how this engagement occurs. As suggested by Merleau Ponty this is a process likely to develop only through interactions requiring significant repetition, of the sort that has been identified in Hebbian-like mechanisms of synaptic activity induced strengthening.

Evidence for the creation of this 'stable' and coherent self image indicates that it is mediated by afferent input from various modalities of the body that is qualitatively greatest in development, but nevertheless also achieved experientially throughout the life of the individual. For example, neuroscientific studies of monocular deprivation in kittens show that ocular dominance columns fail to form when deprivation occurs during a critical phase of synaptic organization during development [36]. In effect the kittens become permanently blinded because of the absence of cortical network structures that would otherwise have been properly ordered by light stimulation during this critical phase. Similarly, experience also shapes neural order, as demonstrated in adult rats where navigational forays establish topographical map relating the spatial environment to bodily structure, a posturally determined aspect essential for planning future maneuvers [8, 9].

A significant aspect of such mapping is its reliance on dynamical systems mechanisms to create stable processes that persist and can be used over time. The Traves and Rolls memory model, for example, relies on the use of attractors to encapsulate mapped memories for such planning [10]. By means of such repeated, environmentally interactive events the neural architecture is developmentally shaped [37] into the global dynamical organization of the self.

3.1. Neuroplastic shaping of the self image through reciprocal brain body exchange

As Merleau-Ponty observed, interaction of the body with the environment is, in fact, the fundamental feature shaping the self percept during development, up to and within the boundaries of the genetic envelope [38], meaning that the synaptic architecture of the brain is not wholly prespecified in the genetic code but is, rather, a product of development, learning and experience, a premise invoked in the epigenetic model of development. Sensory reception, in particular, extends the point of perception to its bodily mooring and is the key determinant in shaping the dynamical operation of the brain. By modulating information content both quantitatively and qualitatively, the body shapes the brain's perception of the world. The choice of sensory modality and the degree to which it ultimately activates brain neural fields is thus

immediately influenced by the positioning of the body as it moves through the world [39]. For example, somatotopic zones that experience frequent contact are abundantly innervated, which seems to indicate that the body regulates the information delivered to the brain according to the degree of interaction that the body experiences; in other words, the body shapes the brain's perception according to the demands imposed upon it.

It is, in fact, by building upon the body's molding of the brain's perception through the reciprocal and perceptual dynamics of body brain neural exchange, that the unitary and performative dynamic of the self is generated, and wherein the individual is both perceived and actively engaged with the world [30]. Varela, Thompson, and Rosch propose, accordingly, a fundamental unity between perception and action

> *"By using the term action we mean to emphasize once again that sensory and motor processes, perception, and action, are fundamentally inseparable in the lived condition"*

which emphasizes the body's initiation of the brain's subsequent and reciprocal response, that is, a perception action loop that relates the perception of the individual to his actions. Esther Thelen proposes that the circuits participating in this cycle emerge from the activities that the loop generates [40]. Supporting these conclusions, the perceptual influence of body on brain has been demonstrated in studies on the perceptual and dynamical interaction with the environment in developing infants [41], acquired habitual motor abilities [42], biodynamic studies showing that movement and proprioception are intrinsically related to perception [43] and appears to relate to the need to construct a unitary construal of the body that can be used for action planning [8, 27].

Critical to the creation of this construct is the ability to relate the whole of ourselves to its performative role; thus, our holistic bodily perception is fundamental to the understanding of ourselves in an interactive context with the world, a developmental transition for this acquired ability that appears to take place around age three to four [8]. Existing evidence strongly suggests that before this time the self percept has not been sufficiently ordered to enable its use to guide the individual. This absence and the behavioral consequences that ensue nonetheless reveal its fundamental necessity to performance.

The objective of such development involves, necessarily, the recognition that we occupy a unique spatiotemporal location, that is, our recognition that we are the same individual in different places and at different times, as John Locke expressed. This holistic perception is achieved through a progressively entangled and dynamic framework that represents, as it were, a prototype and platform of peripherally initiated and increasingly complex integration of inwardly and outwardly directed exchange. Each interactive experience is recorded as a dynamic incident mapped to its respective body locus that yields a temporally successive series of individually contextualized events [7]. Building on this prototypical perceptual platform the performative and neural self construction extends inward and outward to its various peripheral sightings to assimilate this common representation, that is, a physical entity of which the representation is indicative. The performative self thus constitutes an ontological feature needed to confer individual unity that is employed not only performatively, but also for social and homeostatic viability [44].

3.2. Modulating performance elements during development

Key to autonomous behavior, further, is the ability to initiate and respond to environmental variation, meaning that the neural and bodily architecture must be made subject to suitable control. Thus, while the stable self percept forms the ground from which this performative dimension can emerge, action initiation must itself be open to a wide range of responses that are recognized as one's own; hence, another objective of the developmental process is the conferal of the ability to flexibly and selectively engage dynamical elements that can be accessed for a variety of neural trajectories.

Functionally meaningful events thus require that the selection and coordination of dynamical motor processes be amenable to self recruitment and capable of being disengaged when the motor plan has been executed. Importantly, functional behaviors must be reproducible, meaning that the coordinating network be sufficiently determinate that it can reliably account for metastable, multistable, and multifunctional patterns. Current work indicates that global brain networks, such as those likely to belong to the performative self, engage and disengage dynamically [15], that is, they are fundamentally required to be dynamical solutions. This sort of relation implies an operational configuration in which a global brain state entrains a localized network through the leveraging of local instabilities of the sort that have been described in large scale models of global dynamic density control [45] that are robust with persistent levels of activation that can assure delocalized and regionally distributed engagement [15]. However, it leaves open the question of the manner of eliciting local networks that govern various motor activities and of identifying them.

Developmental studies that implicate the need to evoke a coherent self image [27] as an a priori dimension of motor planning suggest that this elicitation of localized attractor assemblies occurs in relation to a sustained global brain state, against which they are framed; that is, they are recognized as constitutive actions emanating from the self. Critically, the activation of localized units in their mature stage must process through a stable trajectory, that is, there must be stable information flow that effects the motor plan. The origin of such information flow, however, cannot be wholly predetermined, but instead, as Merleau Ponty has observed and as can be seen from developmental sensory deprivation studies, is shaped by experience through empirically assessed corrective input to the flow path. The developmental process, accordingly, assembles the envelope within which the basic dynamical elements are structured through an initial activity dependent ordering, which is then later experientially refined via sequential, parallel, and hierarchically clustered patterns of activity strengthening. Accordingly, this developmental ordering suggests that the principle mechanism for evoking motor elements is through their developmentally defined, experientially sharpened, and regionally distributed, dynamical fields [31].

4. Alzheimer's dementia: the progressive loss of dynamical, global self systems

Although the etiological basis for AD has been studied from many different perspectives no single theory yet encompasses its neurological origin nor, correspondingly, have the many

therapeutic proposals resulting from these studies yet yielded tangible benefits. Studies of the impact of the disease on dynamical system elements, on the other hand, have yet to be included in the exploratory spectrum, a reflection of the field's emphasis on theoretical issues concerning the relation of its theoretical models to the physical structure that is the object of the disease. Increasingly, however, not only are dynamical elements considered to be fundamental features of brain operation but their role in global operation are specifically targeted by the disease. This is also to say that their exploration is likely to prove fruitful to the understanding of the cognitive dimension that is specifically impacted by the disease and that constitutes its hallmark. This chapter builds on these observations to propose that the global dynamical operation targeted by the disease is constituted by a construct subsumed within the percept of the self.

The evidence for the specific effect on the dynamical systems that underlie the self is three fold and is framed against the backdrop of the studies just mentioned that demonstrate the basic need to dynamically structure a coherent and stable self image that can make and execute motor plans for interacting with events in the world beyond the self: first, Alzheimer's specifically affects a major brain network closely implicated with the self, the Default Mode Network (DMN); second, there is a specific loss in the ability to maintain functional integration over distributed regional zones that are likely to be subordinated to self performance; and third, parametric indices of dynamical metastability, an index relating global dynamics to coordinative oversight, is significantly altered in AD patients.

We begin with the evidence linking the DMN to the self percept [46]. The DMN was first identified by nuclear imaging studies that showed that this region displayed consistently higher levels of activity during passive task conditions. This led to the hypothesis of its role in monitoring the external environment, body, and even emotion [47]. Additionally, the DMN activity is generally and persistently elevated relative to a number of other areas of the brain, a feature needed for guiding global activity. Task related increases in activity in other zones, moreover, coincide with a relative decrease in DMN activity suggesting a reciprocal relation between the two that is related to the performative context of the task [17]. It is likely therefore that the dynamical ground state of the DMN situates the self image in the context of effecting motor plans and tasks in order to contextualize them as ones own. Consistent with this interpretation its high metastability index reflects a sustained exploratory state where the self image can be accessed for task execution. In other words, the DMN appears to function as the center of a global and distributed network system [16] that assimilates the body image as a coherent and stable self reference that is persistently ready to engage the environment [27].

Secondly, and strikingly, fMRI observed activity patterns in the DMN substantially change during the progression of Alzheimer's. Posterior cingulate and right inferior temporal cortical activities, for example, decline whereas the activity of the bilateral inferior parietal cortex increases [48]. What is significant about these zones is that they form central connectivity hubs within the DMN that exhibit causally influential connections. Thus changes in connectivity strength between these zones appear to reflect a weakening of causally influential, functionally significant, relations between the principal nuclei of the DMN, with a corresponding and decreasing ability to sustain the self percept.

Physiologically, the impact of altered connectivity appears to relate to global dynamical activity that can be observed in altered oscillatory patterns of the electroencephalogram (EEG) [49] in the AD patient. Source based EEG maps, for example, exhibit Alzheimer specific modulation, with changes in cortical spectral power that are related to attenuated alpha and beta and increased theta and delta in the posterior cingulate cortex and intraparietal cortex. Global oscillatory profiles, notably, are invoked as mechanisms for conferring inter areal coupling between brain regions that enables synchronization of activity; that is, brain oscillations are a major means by which the brain coordinates activity across extended spatial distances. The use of globally distributed voltage waveforms as a synchronization mechanism affords the brain the capacity to regulate and control activation by the entrainment of dynamical systems such as dynamical neural fields that are, accordingly, made subordinate to the global self construct [15]. The reduction in connectivity observed in Alzheimer's and the corresponding loss of oscillatory control, seems to imply, therefore, that Alzheimer's incurs a loss of the ability to entrain localized dynamical elements that are subordinate to the action of the self percept. Consistent with this interpretation, regional coherence significantly declines for the posterior cingulate cortex (PCC) relative to the precuneus, with increasingly greater decoherence as the disease progresses [50].

Third, metastability parameters appear to exhibit performative time scales physiologically consistent with normal brain activity in the context of motor responsivity to environmental demands. Measurements of metastability are currently revealing that the disease progression significantly lowers this index [51]. Revealingly, access to the exploratory repertoire of the DMN is counterbalanced in normal cognition by the frontoparietal network which lowers metastability and synchronizes activity [17]. Together, these observations are likely to mean that AD etiological and symptomatic features relate to a loss of global dynamical mechanisms for self representation used to entrain localized, motor planning, dynamical fields.

5. Conclusion

Though later years of life are often portrayed as a time of self realization, it is also the period of life when health related disabilities are especially protracted. Among the most debilitating is the loss of self awareness and self control experienced in advanced Alzheimer's dementia. This realization has impelled numerous studies seeking to identify its etiological roots; its physiological basis, nonetheless, remains uncertain and an overarching framework has yet to be determined. Increasingly, the realization that brain activity is dynamically sustained offers a prospectus within which to frame Alzheimer's related behavioral manifestations. Dynamical systems constitute an operational ground not only as processional motifs but as functional units at progressively higher levels where their coherent assembly is used to guide global operation. This chapter proposes that the peculiarly debilitating and widely regarded hallmark aspects of AD are likely to relate to its ravaging of a global dynamical system that serves as the neural substrate for our sense of self.

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Chapter 7

Model Systems to Define Remyelination Therapies

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76318

Abstract

Demyelinating diseases of the central nervous system (CNS), such as multiple sclerosis (MS), are characterized by multiple focal *demyelinating* lesions, resulting in various functional deficits. The pathology of MS is defined by local loss of myelin sheaths in the brain and spinal cord associated with infiltration of peripheral immune cells. Classically, MS starts with a series of relapses and remissions, followed several years later by a more progressive form of the disease and a steady functional decline. Although the mechanism of disease initiation is poorly understood, disease progression is associated with immune system activation toward CNS antigens including myelin proteins. Animal models of MS have been critical in the development of MS therapies, with experimental allergic encephalitis (EAE) being the most common. This model has been instrumental in defining the role of T cells in disease progression and in the development of targeted therapies. Understanding the biology of myelin repair has, however, largely come from other model systems including local targeted demyelination in vivo, slice preparations, and in vitro. This has led to the identification of a diverse array of potential new targets to modulate disease progression. Development of these new avenues is the target of intensive ongoing research.

Keywords: remyelination, therapeutics, animal model, multiple sclerosis, oligodendrocytes, astrocytes, experimental allergic encephalitis (EAE)

1. Introduction

Myelin is the fatty insulation that surrounds axons, enhances axonal conduction rates, and protects axons from damage in the nervous system. In the central nervous system (CNS), the majority of myelin is a product of oligodendrocytes, and a single oligodendrocyte may

myelinate multiple segments of different axons. During development, oligodendrocytes are generated from precursors (OPCs—oligodendrocyte precursor cells) that arise in specific locations of the brain and spinal cord as a result of local inductive cues (discussed in more detail later). While oligodendrocytes and myelin are found throughout the CNS, the amount of myelin in white matter is substantially greater than that in gray matter. Indeed, the primary reason that white matter appears white is due to its high concentration of lipid-rich myelin. Because myelin plays a central role in modulating neuronal activity, its loss is frequently associated with functional deficits. Myelin loss or demyelination occurs in various different pathological conditions including developmental disorders such as the leukodystrophies, adult-onset insults such as stroke, and classical demyelinating diseases such as multiple sclerosis (MS) and related disorders. MS, the most common CNS demyelinating disease, was originally described over 100 years ago, and initial descriptions of the disease highlighted an illness of increasing functional deficits. Our understanding of the disease course and its progression has advanced over time, and it is now clear that MS is a more complex disorder in terms of clinical presentation and underlying pathogenesis [1–6].

In classic cases, MS initially presents as a sudden-onset neurological deficit that resolves over a period of time. Subsequent attacks (relapses) are followed by periods of remission; however, over time, remission fails to result in a return to normal functionality and deficits slowly accumulate. Following this relapsing-remitting phase (relapsing-remitting MS), the disease enters a more chronic phase in which deficits accumulate in a progressive manner (progressive MS) (**Figure 1**). Not all patients follow this disease trajectory. In a distinct subset of patients, the

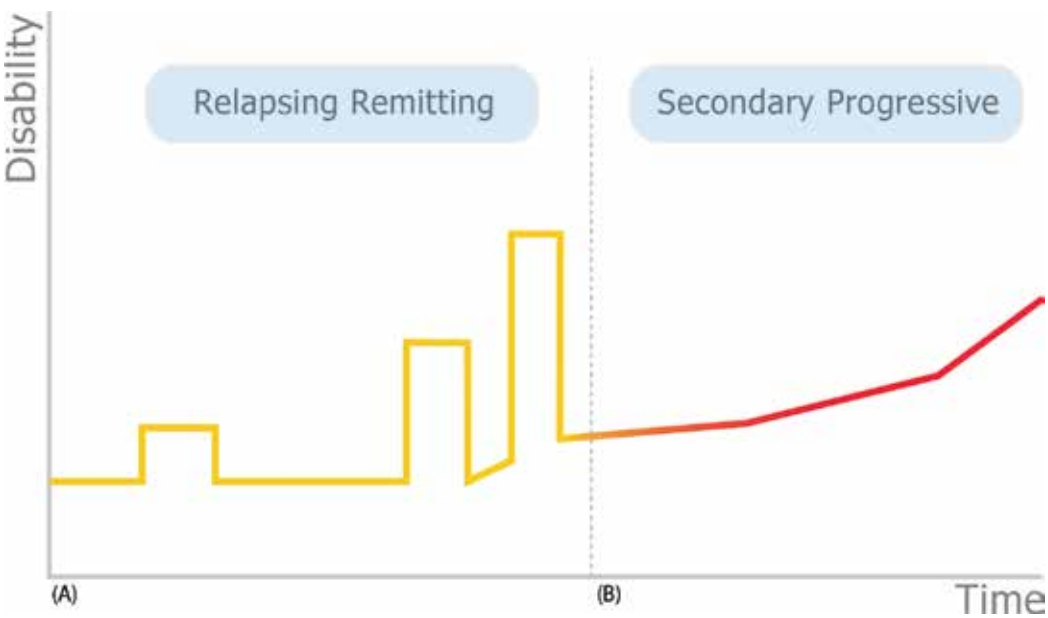

Figure 1. Graph showing typical disease activity in relapsing remitting MS, characterized by defined clinical attacks followed by full functional recovery (A) or incomplete recovery between attacks with residual deficits (B), eventually leading to worsening disability (C).

http://dx.doi.org/10.5772/intechopen.76318

disease presents initially as progressive functional impairment without obvious remissions—a condition known as primary progressive MS. Alternatively, some patients never progress beyond the relapsing remitting phase, and others only experience a single attack. In MS and numerous MS-related conditions, myelin loss is localized and multiple areas of demyelination or plaques may be present in a single patient. Each of the plaques appears to progress or resolve independently during the progression of the disease.

The variability in presentation and progression makes the accurate diagnosis of MS complicated [6]. This is further compounded by the lack of biomarkers that unambiguously identify MS, and consequently, a diagnosis of MS is dependent on several factors in an overall presentation rather than a single definitive test. Identifying factors include medical history and clinical examination, magnetic resonance imaging (MRI) imaging, and the presence of oligoclonal bands in CSF. While MRI and other imaging modalities are highly effective at identifying lesions in CNS white matter, they are not able to specifically characterize MS-associated demyelinated lesions, and other conditions such as inflammation may generate similar MRI findings. Recent advances in imaging modalities have enhanced the specificity of these approaches for demyelination, and it seems likely that more specific approaches will be implemented in the clinic in the near future. One such approach is using PET imaging to detect areas of myelin loss [7]. The development of selective tracers of myelin that can be visualized in a noninvasive manner is promising; however, the widespread application of this approach is likely to be limited by the short half-life of the probes and the necessity of a local cyclotron for their production.

While generally considered to be a disease of white matter, and myelin in particular, there is now strong data indicating that MS plaques also occur in gray matter, including synapses, and that altered synaptic transmission along with loss of gray matter may contribute to cognitive deficits and brain atrophy often associated with MS [8]. Wherever they occur, MS plaques are frequently associated with a core blood vessel and reactive astrocytes. The close association of blood vessels with MS plaques is indicative of the role of the immune system in the pathogenesis of MS, which is universally recognized as an autoimmune disease. Considerable evidence indicates that T cells that recognize myelin antigens enter the CNS and attack myelin and oligodendrocytes. This inflammatory insult recruits other cells including cells of the innate immune system such as macrophages and microglia that contribute to CNS damage. The majority of existing MS therapies are directed toward either suppressing the immune response or blocking the entry of T cells and other cells of the peripheral immune system into the CNS. While such approaches have been quite effective at modulating the severity and interval of relapses in relapsing remitting MS, it has become clear that they fail to block overall progression of the disease and brain atrophy, and neurodegeneration is only slightly improved. Such observations have led to a concerted effort to identify therapies for MS that are targeted toward promoting myelin repair or inhibiting damage within the CNS but have been somewhat hampered by the lack of understanding of the causes of MS.

The chronic nature of MS and the likelihood that the disease has been ongoing for an extended interval prior to becoming symptomatic make it extremely difficult to identify the initial pathogenic signal. One attractive hypothesis is that a potential trigger for MS is a response to

a prior infection or other environmental signal [9]. This notion is supported by the findings that MS patients have elevated immunological responses to various pathogens, which may account for some aspects of the epidemiology of MS. A wide range of pathogens including spirochetes, chlamydia, and a range of viruses have been linked to MS [1]. It seems likely that the role of such pathogens is to enhance susceptibility to MS rather than directly induce disease. One of the strongest links in MS is viral infection [10], and a number of different viruses have been implicated including Epstein-Barr, human herpes virus 6, and human endogenous retroviruses [10–12]. Precisely how the viral infection contributes to MS development has not been clarified, but it may reflect the initial stimulation of immune cells to viral antigens or the induction of oligodendrocyte death as a result of viral infection.

One aspect of MS that has been extensively studied is its genetic linkage [13], and instead of a gain or loss of function of one individual gene, there are a range of genetic associations linked to MS. In particular, MHC class 11 molecules such as HLA-DR and HLA-DQ alleles are considered risk factors for the disease [14]. Given the immunological nature of the disease, the association with immunomodulatory genes is expected; however, the mechanisms by which these genetic changes increase disease susceptibility are still unclear. For example, certain MHC molecules can promote the development of an autoimmune response following a sub-acute challenge from a structurally similar antigen. The contribution of genetic or epigenetic changes in cells of the oligodendrocyte lineage or myelin that contribute to MS susceptibility remains to be clarified.

To understand the biology of MS requires a clear understanding of the cellular and molecular mechanisms that mediate myelination and myelin maintenance, and much of our understanding of the control of myelination comes from studies in development. Oligodendrocytes, the myelinating cells of the CNS [15–18], are generated from precursor cells (OPC or oligodendrocyte precursor cells) that arise in distinct location of the embryonic CNS in response to selective inductive cues and subsequently disperse throughout the CNS [17, 19–21]. The early commitment of neural stem cells to the oligodendrocyte lineage depends on environmental cues that include sonic hedgehog and the subsequent induction of transcriptional signaling pathways that promote the appearance of OPCs, their proliferation, and subsequent migration. One of the major mitogens and potential growth factors that support the expansion of the oligodendrocyte lineage is platelet-derived growth factor alpha (PDGFα). The receptor PDGFαR is expressed predominantly by OPCs in vivo and allows for the unambiguous identification of OPCs in the setting of demyelination and repair. In the spinal cord, OPCs originate at the ventral midline during embryonic development and subsequently disperse widely through gray and white matter. This migration is guided by a number of different signals including Netrin 1 and Wnts and appears to track with the vasculature. Prior to myelination, OPCs differentiate to oligodendrocytes, a process that includes the cessation of proliferation and the induction of additional transcription factors including Myrf that are essential for oligodendrocyte maturation. The differentiation of oligodendrocytes is clearly environmentally regulated. For example, myelin debris has been shown to inhibit the differentiation of oligodendrocytes and may be an important factor in the control of remyelination where delayed myelin clearance may inhibit repair [22, 23]. Once oligodendrocytes mature, there is a defined time window during which they extend multiple processes to contact adjacent axons and initiate myelination. Less is known about the molecular interactions that orchestrate the initiation of

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myelination. Several parameters such as axonal size and electrical activity have been implicated as important in the early stages of myelination. In addition, several factors have been suggested to inhibit the onset of myelination, and these include LINGO-1 and the expression of PSA-NCAM on axonal surfaces. Following differentiation and maturation, oligodendrocytes begin to generate myelin sheaths. An individual oligodendrocyte initially generates an excess number of myelin sheaths, some of which grow and are stabilized, while others shrink and are subsequently lost. What regulates the growth and retraction of myelin sheaths is not well understood, but recent studies suggest that it may be regulated by axonal activity.

Myelin is a specialized plasma membrane that provides a fatty insulation around axons and allows the rapid conduction of electrical impulses by increasing conduction velocity, reducing the threshold for firing, and providing axonal protection [24]. Myelin sheaths are discontinuous and are linked by Nodes of Ranvier (**Figure 2**) that have a characteristic morphology and are areas of high concentration of ion channels that support electrical impulse propagation. Nodes of Ranvier appear to be particularly sensitive to damage, and their disruption results in perturbation of axonal conduction. The region between two nodes is known as the internode, and it is made up of a number of specific proteins [25], including the major proteins myelin basic protein (MBP) and proteolipid protein (PLP) as well as other minor proteins such as myelin-associated glycoprotein (MAG), myelin oligodendrocyte protein (MOG), 2'3' cyclic nucleotide 3'phosphdiesterase (CNP), and myelin-associated oligodendrocyte basic protein (MOBP) [26].

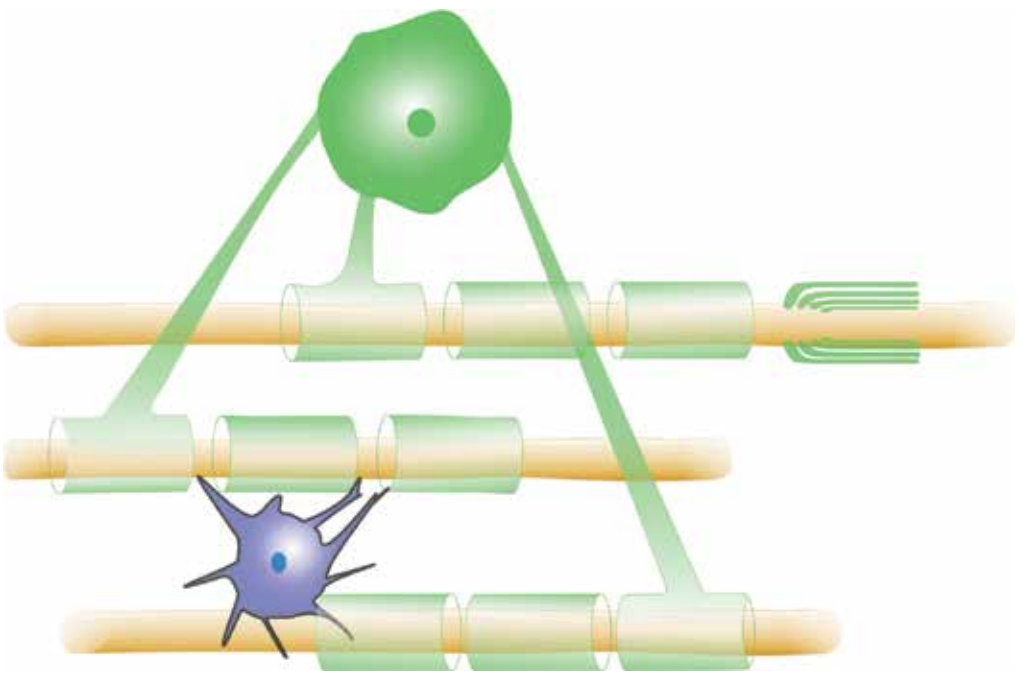

Figure 2. CNS myelin. Schematic representation of an oligodendrocyte ensheathing several axons in the CNS. Segments of myelin sheaths are separated by Nodes of Ranvier, which are contacted by astrocyte foot processes.

Each of these myelin components is presumably important for normal myelin function. For example, MBP mutant animals such as *shiverer* fail to form compact myelin and have limited life span [27], while animals lacking PLP develop normally but manifest axonal pathology later in life [28, 29].

The normal development of myelin is also dependent on additional CNS cell types: axonal processes are targets for myelination, and astrocytes are important in the development and survival of cells of the oligodendrocyte lineage [30]. Astrocytes are a heterogeneous cell population that have been proposed to perform multiple functions that support development and maintenance of the brain and spinal cord. During development, astrocytes guide the migration of neurons from their germinal zones to their final destination [31] and act as substrates for long-distance axonal growth to their targets. In the adult, astrocytes are important for the removal of neurotransmitters, control of the ionic environment, and maintenance of the blood-brain barrier as well as either supporting or inhibiting regeneration through the formation of glial scars [32–34] that are comprised of astrocyte processes and extracellular matrix. A similar glial scar is formed around chronic demyelinating lesions and has been suggested to block myelin repair [35], although recent studies indicate that the astrocyte response is beneficial in certain animal models.

Histological studies provide an evidence of neuronal damage in MS, including axonal loss in areas of demyelination [29, 36] or even frank brain atrophy due to widespread loss of neuronal cell bodies and their axons [37]. It is unclear whether the axonal loss is secondary to myelin loss or independent of it via direct antigenic targeting. The role of astrocytes in MS disease pathogenesis is less well defined. For example, in areas of demyelination, a reactive astrocytic response is commonly characterized by elevated expression of glial fibrillary acidic protein (GFAP) that may be either protective or pathogenic [38]. Disruption of the blood-brain barrier is also important in the formation of demyelinating lesions in MS, and astrocytes have been proposed to play an important role in the maintenance of the blood-brain barrier in the adult CNS. The best evidence for an astrocytic role in demyelination comes from the studies of the MS variant known as neuromyelitis optica (NMO) that preferentially presents in the optic nerve and spinal cord. In a significant subset of NMO patients, demyelination is thought to result from the binding of pathogenic antibodies against aquaporin 4, a molecule expressed on the end feet of astrocytes around blood vessels. Antibody binding results in astrocyte death and subsequent demyelination, although the molecular linkages in this cascade are unknown.

The cellular complexity and heterogeneity of MS-like diseases represent a significant challenge in developing effective animal models that accurately mimic disease progression, and this has led to the generation of a number of different models, each of which highlights distinct components of the disease [39]. Some of the most powerful and best-studied models of MS are those that utilize selective stimulation of the peripheral immune system as the major driver of CNS pathogenesis, and these are discussed in more detail later.

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2. Animal models of demyelinating diseases: strengths and weaknesses

2.1. Immunological models for CNS demyelination

Multiple sclerosis is characterized by the engagement of the immune system, and this has been primarily modeled through approaches collectively known as experimental allergic encephalitis (EAE) [40, 41]. In general, EAE is an inflammation-mediated demyelinating disease that is induced in host animals through immunization with CNS tissue resulting in a host of functional deficits that correlate with immune cell infiltration into the CNS. The functional deficit is then scored on a 1–5 scale: 1 presents with a flaccid tail, 2 with hindlimb weakness, 3 with hindlimb paralysis, 4 with forelimb and hindlimb paralysis, and 5 death. In most studies, the scale is expanded to between 2.5 and 3.5, allowing for better definition of functional changes.

Initial development of EAE involved injection of spinal cord homogenates into rabbits resulting in hindlimb paralysis and other functional deficits. Subsequently, immunization of monkeys with spinal cord homogenate derived from rabbit CNS [42] showed the pathological accumulation of cells around blood vessels of the brain and spinal cord. Variability in individual animal responses limited initial studies; however, this has largely been resolved through the use of immune stimulants such as complete Freund's adjuvant (CFA) combined with pertussis toxin. This model has been refined through identification of effective protein antigens. These antigens are predominantly myelin-associated proteins including myelin basic protein (MBP), myelin-oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP) [41]. Minor myelin components are also capable of generating disease suggesting that most myelin components can act as effective priming antigens. The identification of specific myelin protein peptides that provoke a reproducible and consistent disease following immunization into genetically defined host populations has resulted in several major models of EAE that are now commonly used. These include the induction of EAE in the *SJL* mouse genotype following immunization with the $PLP_{139\text{-}151}$ peptide, which generates a relapsing remitting disease mimicking some characteristics of relapsing remitting MS. An alternative model utilizes C57/Bl6 mice immunized with the MOG $peptide_{35\text{-}55}$. This model is often used to recapitulate more advanced stages of MS because it generates a more chronic disease course. Other less common models include the induction of EAE in PL/J mice following immunization with MBP or MOBP and immunization of Biozzi ABH mice with MOG protein that models selective aspects of MS.

Several major themes have emerged from studies on the mechanisms of disease in murine EAE. One common finding is a primary role for T cells in disease development. Adoptive transfer clearly demonstrated that T cells specific for MBP antigen were capable of transferring disease to naïve hosts [43]. The functional deficits in this model were transient, resolving within 1–2 weeks, and were not characterized by extensive demyelination suggesting the pathology in MS reflects multiple pathogenic processes. One strong candidate that contributes

to EAE and MS pathology is B cells [44]. B cells play multiple roles in immune-mediated pathology in the CNS. On the one hand, they facilitate activation and expansion of T cell populations within the CNS and enhance the recruitment of other immune cells into the CNS.

On the other hand, B cells produce antibodies directed against the different myelin antigens. For example, some MS lesions are characterized by an overexpression of anti-myelin antibodies, with MOG as a potential antigenic target [45]. Understanding the roles of B cells in the underlying pathogenesis of MS and other neuro-inflammatory diseases now seems to be at the forefront of research development after demonstrating that two B-cell inhibitors, Rituximab and Ocrelizumab, were shown to be highly effective in some MS patients, including those with primary progressive MS [46–48].

The role of the innate immune system in demyelinating pathologies is also an area of current focus. Microglial cells are also known to undergo reactive changes, whereby they aid in myelin clearance, but also could potentially participate in antigen presentation along with dendritic cells. One hypothesis is that pathological mechanisms vary by the stage of disease. Relapsing remitting disease, for example, may be largely driven by influx from the peripheral adaptive immune cells, whereas secondary and primary progressive forms of the disease are largely driven by the innate immune system.

Myelin components are not the only antigenic targets in MS. For example, axon-specific proteins, such as the neurofilament triplet, and node of Ranvier components, such as Contactin/TAG-1 and S100, have also been associated with EAE and MS [49]. It is unclear, however, whether the aforementioned proteins are primary disease targets or their involvement is secondary to myelin loss. It is likely, however, that as the disease progresses, the ongoing destruction of neural tissue expands the pathological basis of the disease resulting in more widespread damage and worsening functional deficits.

EAE models have been invaluable in elucidating critical aspects of MS biology and other demyelinating CNS diseases and have been reviewed in detail [40, 41]. One of the major advantages is that EAE utilizes well-defined antigenic targets and can be adaptable to numerous genetic animal models. This has allowed the identification of several well-defined networks resulting in T cell activation and trafficking, as well as shed light into the role of T cell subsets in disease progression. What is important is that these disease models still serve as primary tools not only for disease modeling but also for validating and identifying new therapeutic targets.

Another aspect of MS pathology that has started to gain ground is the effect on long-term synaptic plasticity, which is the physiological mechanism responsible for learning and memory and also is a key determinant of clinical recovery after cortical injury. It has now become clear that MS is frequently associated with cognitive and behavioral changes, which have been detected in the early stages of the disease, and are certainly more common than previously thought [50]. These changes are likely the result of synaptic impairment or altered synaptic plasticity. Among the different brain regions, the hippocampus is the most vulnerable. Despite its obvious importance, very few studies have been directed at understanding the hippocampal synaptic plasticity after EAE and not all are in agreement with what effect EAE has on hippocampal long-term synaptic plasticity. There is, however, sufficient evidence to indicate that

activated microglia are responsible, and that changes in synaptic plasticity are rather dynamic, effectively mirroring the stages of the disease and severity of inflammation. Along those lines, it has also been suggested that enhanced cortical plasticity is predictive of functional recovery after a relapse [51].

An important variant of immune-mediated models of demyelination is the generation of local rather than systemic lesions [52]. This has been achieved by sensitizing host animals with sub-threshold levels of encephalogenic peptides and subsequently delivering a local injection of a pro-inflammatory cytokine to stimulate local demyelination. For example, injection of $_{1–125}$MOG peptide and incomplete Freund's adjuvant into Lewis rats results in an immune response but no overt clinical symptoms. Subsequent local injection of tumor necrosis factor alpha (TNF-α) or interferon-gamma (INF-γ) results in localized infiltration of immune cells, local demyelination, and axonal damage. Such studies revealed a rapid local functional deficit reflecting immune-mediated damage. This was followed by some functional recovery, although axonal damage remained. There are several strengths to this model including the ability to assess long-term consequences of a localized immune response and the capability to develop novel therapies to modulate initial immunological insult and promote long-term functional recovery. Such a model has several weaknesses including the localized nature of the insult and the method of induction of inflammatory stimuli. Local injection of cytokines results in damage to the blood-brain barrier and the stimulation of a robust astroglial response making mechanistic interpretation of the outcome of these studies difficult. There are a number of important differences between MS and EAE. EAE is generated through injection of selected antigens, while the trigger for MS is unclear. To date, there has been no description of a spontaneously occurring form of MS in animals. Second, the inclusion of unrelated antigens when inducing EAE has led to developing disease mechanisms and therapies that have otherwise failed clinical trials

Many of the current therapies used in the treatment of MS have emerged from studies of EAE, and it is not surprising that they are targeted toward regulation of immune cell responses. Such recent treatments include Fingolimod (FTY720) directed against the sphingosine-1-phosphate receptor that regulates T and B cell responses appears to directly stimulate remyelination in the CNS [53, 54], and Natalizumab directed toward adhesion molecules on lymphocytes blocks the entrance of those cells in the parenchyma of the CNS [55]. Such therapies, while modulating relapse activity, have generated unexpected side effects in the setting of clinical applications that have in certain cases limited their utilization. Furthermore, long-term studies suggest that while such therapies are effective at modulating inflammatory responses, they are less effective at controlling the disease activity or promoting recovery in the CNS. Current studies are becoming increasingly focused on developing approaches to promote myelin repair in the CNS, and EAE is not particularly suited to identification of repair mechanism.

2.2. Demyelination induced by gliotoxins

One of the major drawbacks of the aforementioned immune-mediated models of demyelination is that both pathological and repair processes occur simultaneously, which complicates the interpretation of potential repair strategies. To define the pathways mediating myelin repair, a variety of alternative models are available, and these include both focal and systemic

glial toxin treatments. These models, while they do not recapitulate the complex etiology and pathogenesis of MS, have two major strengths. First, the onset of the insult can be tightly regulated in time and space; second, the epochs of demyelination and remyelination are largely separate, allowing for the characterization of molecular cues regulating each aspect of lesion generation and repair.

The most common model utilizes the generation of focal areas of demyelination induced by direct injection of chemicals that selectively ablate oligodendrocytes and their myelin. Many different demyelinating agents have been used, although the most common include lysolecithin, ethidium bromide, and antibodies against the major sphingolipid component of myelin, galactocerebroside.

Lysolecithin (L-α-Lysophosphatidylcholine or LPC) when injected into white matter as a 1% solution induces focal demyelination [56, 57]. Common locations for LPC-induced lesions include spinal cord white matter, the midline of the corpus callosum, and caudal cerebellar peduncle. Injection of LPC results in a rapid loss of myelin and oligodendrocytes. Compared to other models, LPC lacks absolute cellular specificity, and there is a reduction in astrocytes and some axonal loss in the lesion. One powerful feature of LPC lesions is their ability to recover. In general, demyelination occurs rapidly, and the lesion area is largely devoid of myelin 2–3 days after lesion generation. Oligodendrocyte precursor cells repopulate the lesion sites around 5 days and subsequently proliferate and differentiate into oligodendrocytes, with remyelination taking place between 7 and 14 days in rodents on average. The latter varies with the lesion site and animal age. By 30 days post-lesion, remyelination is essentially complete (**Figure 3**). These observations have led to the identification of several distinct molecular mechanisms, such as Notch and Wnt pathways, retinoid X receptor gamma signaling, growth factors such as hepatocyte growth factor and neuregulin, hormones including progesterone, cell cycle proteins such as cyclin-dependent kinases, chemokine receptors such as CXCR2, the NOGO receptor LINGO-1, and death receptor 6 (DR6) signaling. In white matter tracts containing large-caliber axons, the remyelinated axons have thinner myelin sheaths than the originals (**Figure 3**).

An alternative glial toxin, ethidium bromide results in cell loss due to its DNA-intercalating properties; therefore, all nucleated cells are affected in this model. Ethidium bromide is injected directly into white matter tracts, and the lesions tend to be larger than LPC lesions and have been utilized to assay the effects of age, sex, growth factors, and the role of microglia/macrophage activation on remyelination. As expected, ethidium bromide injections cause a more widespread loss of astrocytes, oligodendrocytes, and OPCs while sparing axons. This is followed by the influx of macrophages in and around the lesion and the development of reactive astrocytosis, which aims to seal off the lesion site [58]. In contrast to LPC-induced lesions, a significant amount of remyelination in ethidium bromide-induced lesions in the spinal cord is accomplished by Schwann cells. It was initially assumed that such Schwann cells were derived from peripheral nerves or spinal nerve roots adjacent to the lesion; however, fate mapping studies suggest that OPCs generate Schwann cells in the absence of astrocytes [59] raising the possibility that astrocyte regulate the fate of OPCs. Given the more widespread loss of neural cells, ethidium bromide lesions are less commonly used for the identification of remyelinating therapies.

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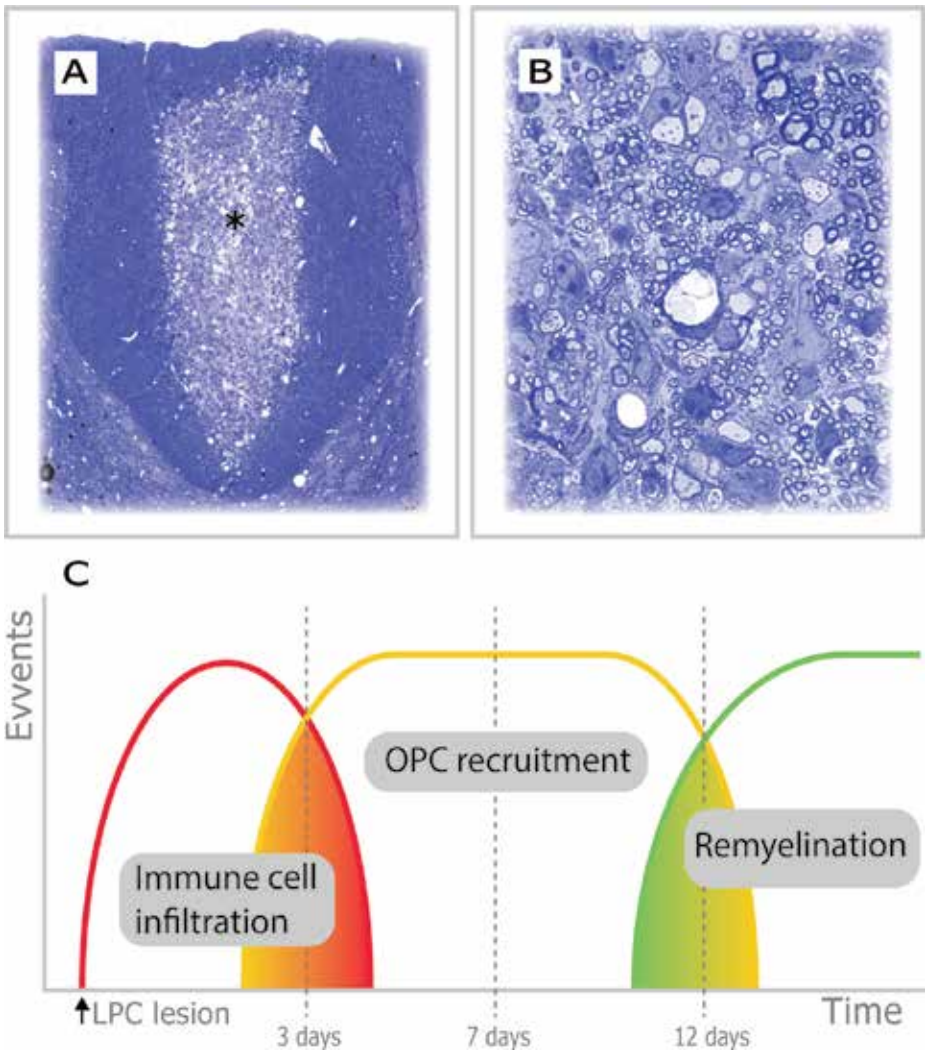

Figure 3. A) Representative image of dorsal spinal column cross section, stained with Toluidine blue, showing an LPC-induced demyelinating lesions denoted by the asterisk. B) Representative high magnification image of an LPC lesion during remyelination. C) Graph depicting typical disease progression in a characteristic LPC lesion; including immune cell infiltra-tion around 3 days, followed by OPC recruitment peaking at 7 days, and then the onset of remyleination at apporximately 12 days post injection.

To provide enhanced cellular specificity, cell type-specific surface antibodies have been used to target the complement cascade and induce selective cell lysis [60]. This model has been effective using antibodies to galactocerebroside (GalC), the major myelin sphingolipid to eliminate mature oligodendrocytes. Initial studies demonstrated that a single intraspinal injection of complement proteins plus anti-GalC resulted in demyelination and partial loss of oligodendrocytes. Analysis of the mechanism of myelin repair suggested that it was the result of recruitment of OPCs and not Schwann cells or mature oligodendrocytes [61].

A major strength of the local toxin models is that they provide a localized region of reproducible synchronized demyelination allowing for analysis of remyelination in the absence of concurrent demyelination. The timing of remyelination differs between the models, although all undergo spontaneous repair. Another advantage of using these models is their adaptability; lesions can be generated in animals of any age, at any accessible location, and from different genetic backgrounds. The disadvantage is that the mechanism of cell death is non-physiologic, and so whether this truly models naturally occurring lesion development, disease progression, and clinical phenotype is unclear. One particular aspect where these models have proven beneficial is the development of myelin-promoting therapies, as opposed to those modulating immune responses. For example, using an LPC-induced demyelination model, LINGO-1 was identified as a potential therapeutic target, whereby anti-LINGO-1 antibodies promoted OPC differentiation and subsequent remyelination [62, 63].

LINGO-1 knockout mice show precocious myelination, suggesting that LINGO-1 antagonists might be useful to accelerate myelin repair. Using both the LPC and cuprizone models (see below) of demyelination, anti-LINGO-1 antibody treatments significantly increase the speed of remyelination, suggesting a new therapeutic option for MS patients. The anti-LINGO-1 Li81 antibody is the first MS therapy directly targeting remyelination and is currently in MS clinical trials.

A second commonly used approach for glial toxin-induced demyelination is systemic oral delivery of toxins that preferentially target oligodendrocytes. Systemic delivery of a glial toxin in a noninvasive manner has a number of advantages. For example, it overcomes the complexity associated with direct injections into the CNS and provides a larger demyelinating area allowing for easier molecular analysis. The most frequently utilized systemic toxin is cuprizone.

Ingestion of the copper chelator cuprizone (biscyclohexanone oxaldihydrazone) results in demyelination of specific brain regions, which is thought to reflect mitochondrial stress and an innate immune response [64]. Cuprizone-induced demyelination results from loss of oligodendrocytes rather than direct insults to myelin sheaths, and mice aged 6–9 weeks given 0.2–0.3% cuprizone treatment of for 5–6 weeks develop acute demyelination of the corpus callosum and other rostral white matter regions. Interestingly, the spinal cord is less susceptible, which could be in part due to a differential sensitivity by spinal oligodendrocytes to cuprizone, and/or nonuniform penetration in different CNS tissues. Oligodendrocyte apoptosis is also associated with extensive reactive astrogliosis and microglial activation. Acute demyelination is followed by spontaneous remyelination that occurs following removal of cuprizone from the diet. When cuprizone treatment is prolonged to 12 weeks or longer, remyelination is very sparse, resulting in a model of chronic demyelination.

The extended time course of disease induction and repair makes the cuprizone model useful for studying the biological processes related to both demyelination and remyelination in the CNS. The cuprizone model has been extensively used to examine the potential of various compounds to stimulate myelin repair [65, 66]. Because the time course of cuprizone treatment is so long, demyelination is progressive and remyelination begins while demyelination is still taking place. Combining cuprizone with rapamycin, which blocks mTor signaling,

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decreases the efficiency of remyelination, making it easier to analyze and quantify repair processes. The cuprizone model is easier to use compared to other models in that the toxin is included in regular mouse chow that is fed to the animals each day. There are, however, a number of concerns with this model. First, cuprizone is generally limited to mice, and there is a clear genetic linkage to the susceptibility for cuprizone toxicity. Likewise, there are differences in susceptibility between gender and age that are poorly understood [67]; however, proof-of-principle studies demonstrate that signals known from in vitro studies to stimulate oligodendrocyte differentiation such as thyroid hormone (T3) promote remyelination in the cuprizone model, making it useful for therapeutic discovery.

2.3. Cell death models of demyelination

A number of studies have begun to suggest that demyelination may be a primary result of oligodendrocyte death, with activation of the immune system as a secondary event. Whether in the complex setting of disease damage to oligodendrocytes is direct or indirect likely depends on the immediate pathological conditions. An alternative cellular target that may trigger oligodendrocyte damage and demyelination is myelinated axons. Axonal damage and loss are frequently seen in MS lesions [36] and models of immune-mediated demyelination, although it is unclear whether axonal degeneration follows myelin loss or whether demyelination is a consequence of axonal degeneration. To distinguish between these possibilities, animal models in which oligodendrocytes are directly targeted for cell death are being developed to assess whether the loss of oligodendrocytes results in demyelination, how effectively and rapidly remyelination occurs, and whether localized demyelination results in axonal damage. Information from such studies will help define new mechanisms of CNS pathology and novel targets for therapeutic intervention. Currently, there are three major ways for selectively inducing oligodendrocyte cell death in the adult vertebrate CNS.

One approach to drive selective death of neural cells involves the selective expression of a toxic molecule targeted to specific cell types [68]. For example, extensive loss of oligodendrocytes has been achieved through the targeted expression of the alpha subunit of the diphtheria toxin (DT). Diphtheria toxin (DT) is composed of two subunits (alpha and beta), each having different functions. The beta subunit interacts with cell receptors to facilitate the entry of the toxin into the cell, whereas the alpha subunit is the cytotoxic component that acts intracellularly.

The cytotoxicity of DT results from inhibition of protein translation and cell death. In the absence of its beta subunit, DT is unable to penetrate cells, limiting the nonspecific induction of cell death in neighboring cells. Targeting the expression of the DT to oligodendrocytes is achieved using Cre/LoxP technology using a major myelin protein promoter, and its activation is through tamoxifen-induced removal of transcriptional stop sequences resulting in death of oligodendrocytes. One interesting finding from these studies is that extensive loss of oligodendrocyte cell bodies is not correlated with rapid myelin loss. After a post-treatment delay of approximately 3 weeks, the mice displayed progressive motor deficits associated with significant myelin degradation and vacuolization. A second unexpected outcome of these studies was that the widespread loss of oligodendrocytes did not trigger a rapid immune response.

While remyelination was extensive, and the animals appeared to recover completely with longer survival times, recovery was compromised and there was an infiltration of T cells into the CNS. Adoptive transplantation of these T cells into naïve hosts was sufficient to transfer disease. It is likely that the initial insult served to prime the immune system, which eventually led to an autoimmune response and subsequent CNS demyelination [69].

The DT model also differs from MS in a number of key ways. As discussed above, MS is a spontaneous disease, and the lesions develop in a variable manner in both time and space. MS is also not toxin-induced, although there might be a role for pathogens in initial disease stages. The cell death model, on the other hand, depends on the use of a toxin that effectively terminates protein translation, causing cell ablation, and subsequent recruitment of phagocytic cells. Another key difference between the two is that the clearance of myelin in MS following oligodendrocyte loss is rather rapid and is driven by both resident and peripheral immune cells. In contrast, myelin clearance is clearly delayed in the DT model, which would indicate that it is either inhibited or nonexistent. A major concern for the DT model is the complete nature of oligodendrocyte loss, which differs significantly from the focal loss of oligodendrocytes in MS.

In a related model, the specificity of the toxic insult is targeted through receptor expression in a null background [70]. For example, expression of the DT receptor (DTR) under the control of an oligodendrocyte-specific promoter results in cell type sensitivity to diphtheria toxin. Exposure to DT results in the induction of cell death by inhibiting protein synthesis. The clinical phenotype includes ataxia, limb paralysis, and tail spasticity that appear around 10 days post-injection and progressively develop. Perturbations in somatosensory evoked potentials together with histological markers of neurodegeneration, and abnormal Nodes of Ranvier indicate dysfunctional neural networks. The pathology differs between the models; while the DT mice display severe demyelination, the DTR mice show little demyelination. This may reflect that in the DTR model, there is a more extensive engagement of axonal damage leading to death before demyelination develops.

A potential strength of the DTR model is that it may provide a model system to examine the mechanisms and develop targeted therapies against axonal damage in demyelinating diseases since axonopathy is a frequent pathological finding in MS.

During CNS development, many cell types including oligodendrocytes are produced in excess and the additional cells are eliminated through apoptosis-mediated cell death. Cell type-specific induction of apoptosis through activation of an inducible caspase 9 construct driven off a selective promoter has been used to specifically eliminate lymphocytes and oligodendrocytes [71]. Induction of oligodendrocyte apoptosis in the adult CNS results in rapid demyelination and local activation of microglia in the absence of T cell infiltration [72, 73]. During development, activation of oligodendrocyte apoptosis in the first postnatal week inhibits myelination, which subsequently recovers but has increased susceptibility to adult insults [72]. The role of oligodendrocyte apoptosis in early stages of MS is not well defined; however, apoptotic oligodendrocytes have been reported in the early lesions [74], suggesting this may contribute to MS plaque formation. Similarly, activated microglia but an absence of peripheral immune cells has been described in some early lesions.

Overall, while models of selective oligodendrocyte death have provided important insights into the response of the neural cells and the pathway of myelin loss, they have not yet been used to identify new pathways of pathology or illuminate new targets for therapeutic interventions. Whether they will provide a useful platform for the development of therapies for distinct subsets of MS awaits further refinement and analysis.

2.4. In vitro discovery platforms for therapeutic development

Over the past decade, there has been significant development of new platforms for remyelination drug discovery. These include the use of isolated purified cell preparations, rodent IPS cells that provide an unlimited supply of cells, human cell line-derived neural cells, human IPS cells, and in silico model systems. Each of these platforms has its own advantages and disadvantages. In general, such in vitro approaches have been relatively powerful in identifying pathways that regulate myelin formation from mature oligodendrocytes but have been less effective at identifying signaling pathways that regulate the proliferation and survival of oligodendrocytes and their precursors.

With the development of culture models for CNS neural cells and the ability to unambiguously identify distinct cell populations, the ability to identify molecular signaling that promoted the development of oligodendrocytes was feasible. Early studies utilized mixed cultures derived from either white matter such as the optic nerve, mixed gray and white matter such as the spinal cord or predominantly grey matter such as cerebral cortex. Addition of selected growth factors or other signaling molecules that resulted in an increase in mature oligodendrocytes was considered potential therapy. There are two major concerns with this approach. First, the cellular target(s) of the added molecules is unclear, since the culture contains not only cells of the oligodendrocyte lineage but also astrocytes, neurons, and innate immune cells of the CNS, any of which might mediate the response. The second concern is that increased numbers of mature oligodendrocyte may result from either enhanced progenitor proliferation, reduced cell death, or increased cell differentiation, and distinguishing between these mechanisms has proven challenging. To refine the cellular target(s) of potential therapeutics, purified cell cultures have been utilized. Purification of rodent or murine OPCs either through differential antibody binding (panning) or FACS sorting allows for assessment of the direct response of the cell population to therapeutic exposure. Such approaches have been used recently to identify signaling mechanisms that promote the appearance of mature oligodendrocytes [75–78]. One concept that has gained significant support in recent years is the notion that the rate-limiting step in remyelination is the differentiation and maturation of oligodendrocytes to myelinating cells. Several more refined approaches have been developed to identify factors that directly regulate oligodendrocyte maturation. These include the use of purified OPCs initially derived from human material. The emergence of IPS technology combined with identification of molecular environments that promote the survival of human cells has facilitated the identification of several small molecules that mediate oligodendrocyte maturation such as retinoic receptors, benztropine and miconazole. In the majority of such screens, the readout has been enhanced by expression of myelin proteins such as MBP. While this has proven useful, the ultimate goal of remyelinating therapies is the generation of new

myelin. Recent studies have used a biophysical approach to identify signals that promote the formation of myelin on artificial substrates. When grown in the presence of inert fibers of the appropriate dimensions, oligodendrocytes will begin to enwrap them as if they were immature axons. Molecules that enhance that process are considered strong candidate to promote remyelination in the CNS, and molecules including Clemastine an anti-histamine drug have been identified in similar assays.

While the reductionist approaches provide important insights into isolated cellular responses of the oligodendrocyte lineage, they lack any physiological setting. As a result, it is unclear whether signals that modulate oligodendrocyte maturation in isolation will promote myelin repair in the developing or diseased CNS. One model to address this concern is the use of slice cultures. Slices of the CNS grown on the air/medium interface develop robust myelination. The most successful slices are those derived from cerebellum and coronal sections through the corpus callosum. Treatment of such slices with LPC results in rapid demyelination and allows for analysis of drug-induced repair in an efficient and physiological environment. In most studies, multiple different models are used to determine the efficacy individual compounds to promote remyelination.

3. Conclusions and comments

There is a broad range of animal models that address distinct aspects of multiple sclerosis and other demyelinating diseases. Each of the models has specific strengths and weaknesses in furthering our understanding of the pathogenic processes that mediate demyelination and in identifying new opportunities for the effective promotion of myelin repair. EAE models have led to the development of many therapeutic targets aimed at halting disease progression. More recently, other models such as those targeting oligodendrocyte cell death have been instrumental in fine-tuning our understanding of the pathology of demyelination/remyelination in MS and other similar diseases. Each of the model systems discussed in this review deserves particular credit, as it has helped solve a different piece of the puzzle. For example, while EAE models have unraveled many of the immunological bases of the CNS demyelination, particularly the role of T cells in MS, the use of glial toxins such as LPC or ethidium bromide has emerged as extremely useful in reshaping our understanding of the environmental and cell-based mechanisms of remyelination, and the models of oligodendrocyte death provide insights into factors driving the pathology. It seems likely that new models will be forthcoming that more effectively address the role of cells other than those of the immune and oligodendrocyte lineage. Understanding the role of microglia and astrocytes, as well as further clarity around the mechanism of vascular components in disease progression, will allow new therapeutic avenues to be developed in future studies.

Acknowledgements

This work was supported by NIH grant NS 30800 to R.H. Miller.

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Chapter 8

Dendritic Spine Modifications in Brain Physiology

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73326

Abstract

An essential feature for an organism to survive is to adapt and learn. Studies in the past decades have revealed that synaptic plasticity is a key cellular mechanism underlying learning and memory functions in the adult, and the refinement of neural connections during development. Memory and refinement of connections can last for a long period of time, and hence requires the corresponding structural changes to take place. Alterations in dendritic spine morphology (enlargement or shrinkage) and/or spine density (increase or decrease) have been shown to occur with synaptic modifications, and have been proposed to enable persistent, long-term modifications of synapses. In this chapter, we will review the basics of spine plasticity and its functional contributions to synaptic modification, with focus on modifications of spine morphology (enlargement and shrinkage).

Keywords: spine formation, synaptic plasticity, AMPA receptor trafficking, cytoskeleton, long-term potentiation, long-term depression

1. Introduction

Dendritic spines are small protruding structures from the dendrites, around 1 μm in diameter. Spines are the primary site of excitatory inputs onto neurons and about 90% of excitatory synapses occur on spines of the excitatory neurons in the adult cortex [1].

Based on the size, spine head size and spine neck length, they can be roughly divided into three distinct types: mushroom, thin and stubby spine. Mushroom type spines have large spine heads and narrow spine necks, thin spines have small spine heads and thin spine necks, while stubby spines bear no distinction between spine heads and necks [2, 3]. In reality, the distribution of spines is not in these distinct sets but in a continuous distribution.

A major component of dendritic spines is cytoskeleton, which is critical to the structure and function of spines. Cytoskeletons consist of actin filaments and microtubules. Actin filaments

are highly enriched in spine heads, while microtubules are found mostly in the dendritic shafts [4, 5]. Actin filaments inside spines are of two pools, G-actin and F-actin. G-actin is a monomer form of actin and F-actin is the polymer form, while they are found in the tip and base of spines, respectively [6]. Actin filaments bind to the scaffold proteins such as PSD-95 and Shanks, which anchor receptors and signaling molecules at the synapses [7]. Actin filaments are dynamic since actin monomer dissociates from the pointed end while new actin monomers are recruited into the barbed end. Certain actin-binding proteins regulate this dynamic process [8].

Spines are dynamic features in that they are in a constant motion (termed morphing), and their sizes fluctuate around a mean value [9, 10]. Dendritic filopodia are highly motile and flexible, and their lifetime is on the order of minutes to hours [11, 12]. This high motility may allow filopodia to explore the space around them in search for potential presynaptic inputs to form connections [13]. To understand this dynamic nature and to monitor these changes accurately, two-photon imaging has become an invaluable tool. With two-photon imaging, spine morphology and dynamics can be studied in much more details using time-lapse and repetitive imaging. This approach has revealed spine modifications under physiological or pathological conditions or events [14–16] and has greatly advanced our understanding of spine function and allowed in-depth study on the underlying structure–function relationship. During brain development dendritic spines are dynamic in their genesis and elimination, while in adolescence spines show much higher elimination than formation which results in a net spine loss or pruning. In contrast, the rate of spine genesis and elimination in adult is much lower and about equal, and this balance maintains the stability of spine density [17, 18].

Spine is considered as a unique calcium compartment, because the transfer of electrical charge is limited by the spine neck. The length of spine neck controls the degree of interaction between spines and their parent dendrite. In general, short spines and parent dendrites show similar responses to glutamate, while long spines exhibit faster and larger responses [19]. Spine plasticity is evidenced by their rapid (on the order of seconds) and persistent (for months to years) changes in response to physiological or pathological stimuli. Large spines has been suggested to be the site of stable long-term memory storage [1] while filopodia are considered by most to be an immature form of spine. Filopodia may transform into mature spines or are eliminated [20]. Hence, we define spine plasticity in two forms: alterations in their morphology/size and alterations in their density. We note that both forms of plasticity reflect modification of synaptic connections. In neurodegenerative and psychiatric diseases, spine density and spine morphology are altered, and changes in spine density and morphology may at least partially account for altered brain functions in these diseases [1, 21–24]. Therefore, better understanding of spine pathology may provide better therapeutic intervention.

In this chapter, we will discuss signaling mechanisms underlying the formation and maintenance of spines, plasticity of spine morphology and its relationship to modification of synaptic strength.

2. Development of dendritic spines during brain development

The relative sequence of synapse and spine genesis during brain development is still in debate. Some evidences suggest that spine genesis lags behind synapse genesis. Fiala et al.

showed that axonal fibers made synaptic contacts with long filopodia, which subsequently were transformed into mature spines [25]. Durand et al. reported during the first postnatal week in rats, synapses on the excitatory neurons are functional and plastic in the absence of dendritic spines [26]. Hence synaptic function and plasticity can take place without spines. After induction of long-term enhancement of synaptic connections in area CA1, new spines appeared on the postsynaptic dendrite [27]. Maletic-Savatic found that with axonal inputs activation, these small filopodia-like protrusions enlarged and became dendritic spines [28]. For those filopodia that do not connect with axonal inputs, they did not turn into mature spines and were absorbed back into dendrites [12]. Interestingly, increase of spine synapse might inhibit the mobility of nearby filopodia on the same dendrite and diminish the formation of synapses [29]. These results indicate that synapse formation or strengthening promotes the formation or maturation of spines, and lend support for the notion that synapse genesis occurs prior to spine genesis.

Do spines form from filopodia, or from existing synapses on the dendritic shafts? In mature cultures, some stable spines could emerge without going through the dynamic filopodia stage [12]. The series sample analysis in young hippocampal area CA1 also revealed that most of synapses are on dendritic shafts, with rare synapses on stubby and mushroom spines [3]. Despite all this, the transformation from dendritic shaft synapses to spines has not been supported by direct observations [30]. On the other hand, several *in vitro* studies revealed that during the initial 1–2 weeks in culture, the long and headless filopodia bear no synaptic contacts associated with the presynaptic axons. Over the subsequent 4 weeks, these dynamic filopodia turned into stable, mushroom-like spines [29, 31, 32]. Fiala et al. also found that in the hippocampal CA1, synapses were present on both filopodia and dendrites. From PN1 to PN12, the number of shaft synapses and filopodia synapses was decreased, while the number of stubby and spine synapses was significantly increased [25]. Thus, it is likely that during early development, shaft synapses are the dominant form of synaptic contact. With development, and likely the need for increasing contact area, spine synapse replace shaft synapses to become the major form of synapses, at least in the adult cortex. Recently, shaft synapses are shown to define the locations where dendritic spines are formed [30] (**Figure 1**), providing more evidence for the transition from shaft synapse to spine synapse as a major process in synapse formation and maturation.

The initial surge of spine genesis leads the generation of more spines than what eventually is retained in the adult brain, and pruning of excessive spines after spine genesis allows a better adaptation to the environment [33]. This pruning process could be evoked by low-frequency glutamatergic stimulation and requires activation of NMDA receptors [34–36].

Figure 1. Dendritic spines are derived from filopodia with the assistance of shaft synapses. (1) presynaptic axon forms synapses with dendritic shaft. (2) a dendritic protrusion occurs adjacent to the dendritic shaft synapse. (3) the dendritic protrusion contacts with the presynaptic axon and eventually a mushroom dendritic spine is generated. Modified from [30].

In young adolescent mice (1-month old), within a 2 week period of time, 13–20% of total spines were eliminated with 5–8% formed in the barrel, motor and frontal cortices, and this imbalance led to a significant spine loss in many brain regions. However, in the adult mice (4–6 months old), 3–5% of spines were eliminated and formed in 2 weeks [18]. Most evidences support that dendritic spines are stable in the adulthood [37, 38]. Grutzendler et al. reported that spines in the primary visual cortex of young adolescence have a turnover rate of 27% per month but this rate dropped to only 4% in the adult [15]. In contrast, Trachtenberg et al. found that adult spines are highly dynamic with about 20% turnover per day in the mouse barrel cortex [16]. This discrepancy is likely due to differences in the methodology in that the use of cranial window in the latter study triggered inflammatory responses in the brain which resulted in elevated turnover rates. It should be pointed out that spine turnover rate differs in various brain regions. For example, Holtmaat et al. reported that spines turn over more slowly (both generation and elimination) in the visual cortex than in the somatosensory cortex, with the fraction of transient spines (lifetime ≤ 4 days) also lower in visual cortex [17].

3. Signaling events during spine formation and maintenance

Motility of dendritic spines is regulated by the dynamic balance between G-actin and F-actin [39]. F-actin consists of two pools, a large dynamic pool in the tip of the spine head and a small stable pool in the base of spine [6] [40]. With LTP induction, the stable F-actin is severed into short segments and reorganized to expand the spine [41]. Thus, the dynamics of actin cytoskeleton controls dendritic spine morphological remodeling and plenty of signaling molecules participate in this process [42–44].

Spine morphology is regulated by actin binding and cytoskeleton proteins. Drebrin was the first identified to modify dendritic spines since overexpression of drebrin in cultured neurons increased the length of spines [45]. Spines in the drebrin knockout mice exhibited normal morphology but altered plasticity [46]. Takahashi et al. reported that drebrin entered filopodia and formed an actin filament cluster to recruit postsynaptic components (including scaffolding protein PSD95), and this process enables the transition from filopodia to mature spines. Based on this observation, filopodia are classified into two types, an immature diffuse-type and a mature cluster-type. A filopodium with a drebrin cluster, whose maximum intensity was higher than twice the average intensity of the filopodium, was classified as a cluster-type filopodium. Otherwise, it was classified as a diffuse-type filopodium. The cluster-type filopodia were likely to be converted to mature spines [47]. In addition, overexpression of drebrin in neurons caused F-actin to accumulate in the growth cone, whereas knockdown of drebrin reduced F-actin level [48]. Drebrin binds to F-actin to generate thick bundles of F-actin [49], and drebrin also competes with other actin binding proteins such as ADF/cofilin which depolymerizes F-actin [50, 51].

Besides drebrin, other actin-binding proteins including myosin II, Abi-1 and spinophilin regulate actin polymerization in the dendritic spines. Myosin II belongs to the family of molecular motors which is highly expressed in dendritic spines, and regulates dendritic spine morphology and

synaptic plasticity [52]. Blockade of myosin II with shRNA suppressed the formation of mushroom-like spines and increased the presence of filopodia [53]. Abi-1 is a member of the c-Abl tyrosine interactor (Abi) protein family, which interacts with scaffolding proteins and F-actin in the spines [54]. Knocking down of Abi-1 by RNAi shifted spines to an immature form [55]. Spinophilin has an actin-binding domain at its N terminus and can bundle F-actin filaments [56]. Knockout of spinophilin in mice increased the presence of filopodia [57].

Actin polymerization is regulated by actin binding proteins, whose active and inactive states are regulated by small GTPases. Of the Rho family of small GTPases, three are most actively involved in spine morphogenesis, RhoA, Rac1 and Cdc42. These three GTPases are distinguished by two opposite activities: RhoA inhibits whereas Rac1 and Cdc42 promote spine growth. Tashiro et al. reported that in hippocampal neurons, Rac1 increased spine density but reduced spine length, while RhoA decreased both spine density and spine length [58]. Interestingly, RhoA and Cdc42 play opposite roles in stress fiber formation by controlling the phosphorylation of myosin light chain. RhoA inhibits myosin phosphatase via the Rho kinase while Rac1 and Cdc42 activate it via the serine/threonine kinase PAK [59, 60]. Thus, Rac1 and RhoA might have opposite effects on the same target proteins and hence opposite effects in regulating spine density. Similarly, Nakayama et al. found that Rac1 is essential for the maintenance of dendritic spines while enhanced RhoA activity led to significant simplification of dendrites [61].

Receptor tyrosine kinases also regulate spine morphology. Among them, the erythropoietin-producing hepatocellular carcinoma (Eph) receptors have unique activity on synapse. They consist of type A and type B receptor subclasses based on their binding capability to Ephrin A and Ephrin B ligands. Moeller et al. reported that activation of EphB2 in the cultured hippocampal neurons led to shortening of filopodia [62]. Furthermore, activation of EphB likely phosphorylates guanine exchange factors (GEFs) such as kalirin7, which further stimulates Rho family GTPases Rac1 and Cdc42 [63]. Opposite to EphB2, activation of EphA4 by its ligand, ephrin-A3, located in the perisynaptic processes of astrocytes, decreased spine length and density. Loss of EphA4 led to spine elongation and disorganization [64]. Similarly, in the hippocampus of ephrin-A3-null mice, EphA4 phosphorylation was decreased and abnormal spine elongation was observed [65]. Thus, either loss of EphA4 or ephrin-A3 induces identical dendritic spine deficits.

In summary, we have reviewed three types of important and representative signaling molecules in spine function. The first signaling pathway is mediated by actin binding proteins, the second is the family of small GTPases (including Rac1, RhoA and Cdc42, which determine the activity states of actin binding proteins), and the third is receptor tyrosine kinases. The absence or malfunctioning of the above three signaling pathways leads to altered spine morphogenesis and function.

4. Spine plasticity

Spine plasticity may be exhibited in two forms—changes in spine density and spine dimension. Change in spine density reflects modification of connection density between the presynaptic and postsynaptic neurons, which happens most commonly during brain development (increase, decrease/pruning) and aging/degeneration (decrease). Changes in spine

morphology/dimension, especially the size of spine head, have been widely reported, and are believed to be associated with changes in the strength of synapses that reside on these altered spines. In this chapter, we will focus on alterations of spine dimension.

4.1. Changes in spine morphology

Due to the heterogeneity of spine size/morphology, the most convincing way to demonstrate that spine morphology is altered is to compare the same set of spines before and after a manipulation, such as synaptic plasticity-inducing stimuli in brain slices or learning *in vivo*. By using time-lapse two-photon imaging on the same set of spines, Yang et al. found new spines were formed after *in vivo* experience in the form of sensory or motor, and a fraction of these newly appeared spines persisted for months after the experience. More importantly, of the appearance of these new spines is specifically related to the *in vivo* experience or training [66]. In addition, Hayashi-Takagi et al. showed that motor learning on rotarod led to an enhanced *Arc* signaling, together with an expansion of a subset of spines in the motor cortex. By expressing a photoactivatable GTPase Rac1 in spines, they further showed that prolonged photo-activation of Rac1 resulted in reversal of spine expansion and loss of motor memory. This is a striking demonstration that potentiated synapses and enlarged spines are likely the underlying biological substrates of stored memory and formed memory can be erased by reversing these changes [67].

Many studies have examined spine modifications with the induction of long-term potentiation (LTP) and long-term depression (LTD), since this procedure allows the examination of the same set spines associated with fast, large and long-lasting changes in synaptic strength. These two forms of synaptic plasticity refer to the increase and decrease in synaptic strength respectively, and are generally regarded as the cellular basis of synaptic modifications underlying developmental remodeling of neuronal connections, and learning and memory function in the adult brain [68]. When studied in brain slices (acute or organotypic culture), LTP or LTD is induced by stimulation of the presynaptic inputs with distinct patterns. In some studies, changes in the synaptic strength were also monitored, and thus changes in synaptic function and spine morphology can be related to each other in the same set of spines/synapse, or even a single synapse/spine [34, 69–71]. In general, spines exhibit the capacity of bi-directional changes in that spine enlargement is observed with LTP while spine shrinkage with LTD [72–74] (**Figure 2**). Either uncaging of caged glutamate onto a single spine [75] or electrical stimulation of a population of synapses [70, 71] had confirmed the above observations. Uncaging of glutamate directly enhances postsynaptic AMPAR function, and since it bypasses presynaptic release, and thus has provided the unambiguous evidence that postsynaptic changes can underlie the expression of LTP [69, 75]. These observations further indicate that morphological and functional changes are likely driven by the same stimuli or process (see below).

In general, there is a good correlation between the strength of a given synapse (measured by electrophysiological responses) and the size of spine. Electrophysiological responses are further determined by the number/density of AMPA receptors at a given synapse. Takumi et al. found a linear relationship between AMPAR density and the diameter of PSD [76]. Matsuzaki et al.

http://dx.doi.org/10.5772/intechopen.73326

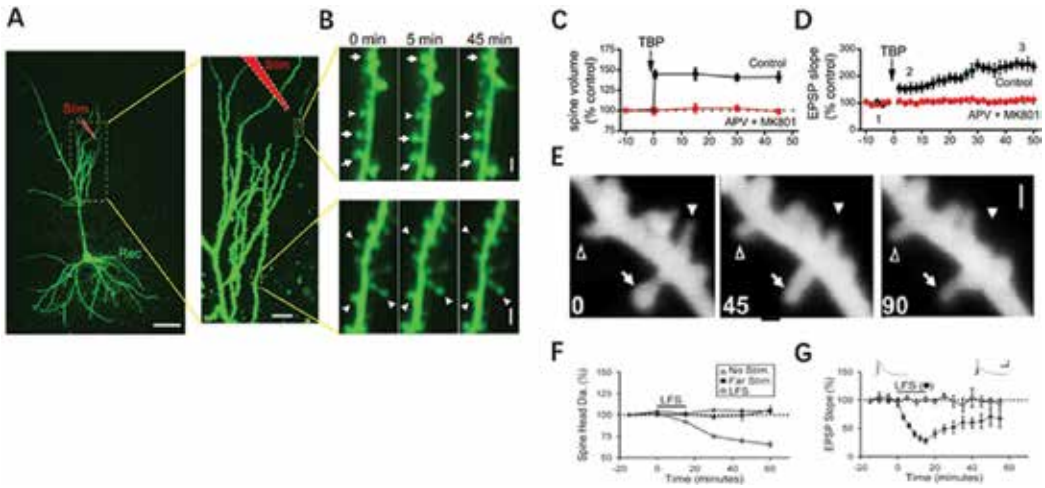

Figure 2. Bi-directional modification of spine size by synaptic plasticity. (A) Recording, synaptic stimulation and fluorescence imaging of the same set of synapses and spines in acute hippocampal slices. (B) Spine enlargement associated with LTP induction by theta burst stimulation, this enlargement is persistent and long-lasting. In addition, enlargement was restricted to spines close (upper) but not far away from the stimulation site (lower). (C) Change in spine volume before and after LTP induction and its requirement of activation of NMDARs since it was prevented by bath application of NMDAR antagonists APV and MK-801. (D) Increase in synaptic strength (EPSPs) as a result of LTP induction. LTP occurred in two phases, an initial rapid increase (indicated by 2 on the plot) and a slower gradual increase (indicated by 3). LTP was also sensitive to NMDAR blockade. (E) Spine shrinkage associated with LTD, it is persistent and long-lasting. (F) Shrinkage of spines occurred to those spines close to, but not to those far away from the stimulating electrode, or those had not received any stimulation. Compared to the almost instantaneous enlargement of spine heads after LTP, spine shrinkage develops slowly and takes much longer to reach a plateau. (G) Low frequency stimulation led to depression of EPSPs. Taken from [34, 71].

reported that the number of AMPAR in spines is of a large range. In addition, mushroom spines are enriched with AMPARs, compared to the low distribution of them in the thin spines and filopodia. These observations support a strong relationship between number of AMPAR and volume of spines [77]. In addition, it provides direct evidence that mushroom spines are functionally mature while thin spines and filopodia are not.

4.2. Synaptic plasticity

Synapses can change their strength by the activity patterns that they receive, and this modification allows synaptic strength to be adjusted to better suit the need for adaptation. Originally put forwarded by Donald Hebb and later adopted as "fire together, wire together" model, the current model of synaptic modification states that neurons sharing spike activity have increased connections between these two partners [78]. After the discovery of LTP in 1973, this activity-driven increase in synaptic strength has been extensively studied, both in acute brain slices and *in vivo* [68]; and has been used widely as a tool to induce synaptic modification in order to study the associated processes. Various molecules have been identified to be required for the induction and expression of LTP [68]. In general, Ca2+ entry or elevated intracellular Ca2+ concentration is required to convert electrical activity into intracellular signaling that determines the direction of synaptic changes. Usually a large but transient elevation in

Ca2+ concentration induces LTP while a small but much longer elevation in Ca2+ concentration results in LTD [79]. This Ca2+ entry can be through opening NMDA receptors, voltage-gated Ca2+ channels or metabotropic glutamate receptors [68]. Increase in postsynaptic kinase activity is usually required for LTP while phosphatase activity required for LTD. After a long debate, it is now generally agreed that synaptic modifications are expressed in the postsynaptic neurons, except in a few specific cases (such as mossy fiber LTP in the hippocampal CA3 region) [80, 81]. Postsynaptic changes involve the translocation of AMPARs and their phosphorylation state, in that LTP is associated with translocation of AMPARs to synapse and/or increased phosphorylation of AMPARs while LTD with endocytosis of AMPARs and/or dephosphorylation of AMPARs [82–85].

4.3. Relationship between spine and synapse plasticity

Since both increase in the synaptic strength and enlargement of dendritic spine occur with LTP, an obvious question is whether changes in synaptic physiology/function are casually related to changes in spine morphology/structure. More specifically, are these two processes driven by the same initial process? Does the occurrence or persistence of one process require the occurrence/presence of the other? It is now well established that influx of Ca2+ through synaptic NMDARs during LTP induction drives AMPAR phosphorylation and/or insertion [68], and polymerization of action filaments inside spines which drives enlargement of spine heads [74] (**Figure 3**). Thus, the initial changes in function and structure are driven by the same signaling process. This initial increase in synaptic response and spine volume occurs very rapidly (less than 1 min) [71].

Dendritic spine heads accumulate F-actin during the rapid expansion phase of synaptic modification. Potentiation of single synapse/spine with uncaging of glutamate led to a significant expansion of the spine head and a shortening and widening of the spine neck [86, 87]. Spine expansion takes place rapidly after LTP, as fast as it can be measured (~ 20 sec after LTP induction) [71]. F-actin concentration inside the spine head rises, together with the entry of actin-severing, actin-depolymerizing/−polymerizing, actin-capping proteins, while actin-stabilizing proteins leave the spines [6, 39, 86, 88–90]. Actin-depolymerizing agent, cofilin, is highly elevated in spines during this initial process [89]. Interestingly, unlike the expression of LTP, this initial spine expansion did not require postsynaptic exocytosis or PKA signaling [71], suggesting the involvement of different signaling pathways in spine enlargement than that supports LTP. After this initial rapid expansion, the next phase of events lasts up to 1 h, with spine head volume decreased from the initial increase, but still larger than the pre-LTP baseline. In addition, the total actin concentration in the spine may drop to the baseline level [89].

LTD is associated with the shrinkage of spines and removal of synaptic AMPARs via internalization [34, 36, 39]. During the induction of LTD with low frequency synaptic stimulation, Ca2+ influx through the activated NMDARs is required for both LTD and spine shrinkage [34, 70]. Ca2+ entry through synaptic NMDARs leads to the activation of calcineurin which is also required for both LTD and spine shrinkage, while activation of protein phosphatase 2A is required for LTD expression but not spine shrinkage, while elevated cofilin activity is required for spine shrinkage but not LTD [34, 39, 70, 91]. Consistent with the above conclusion, Sdrulla

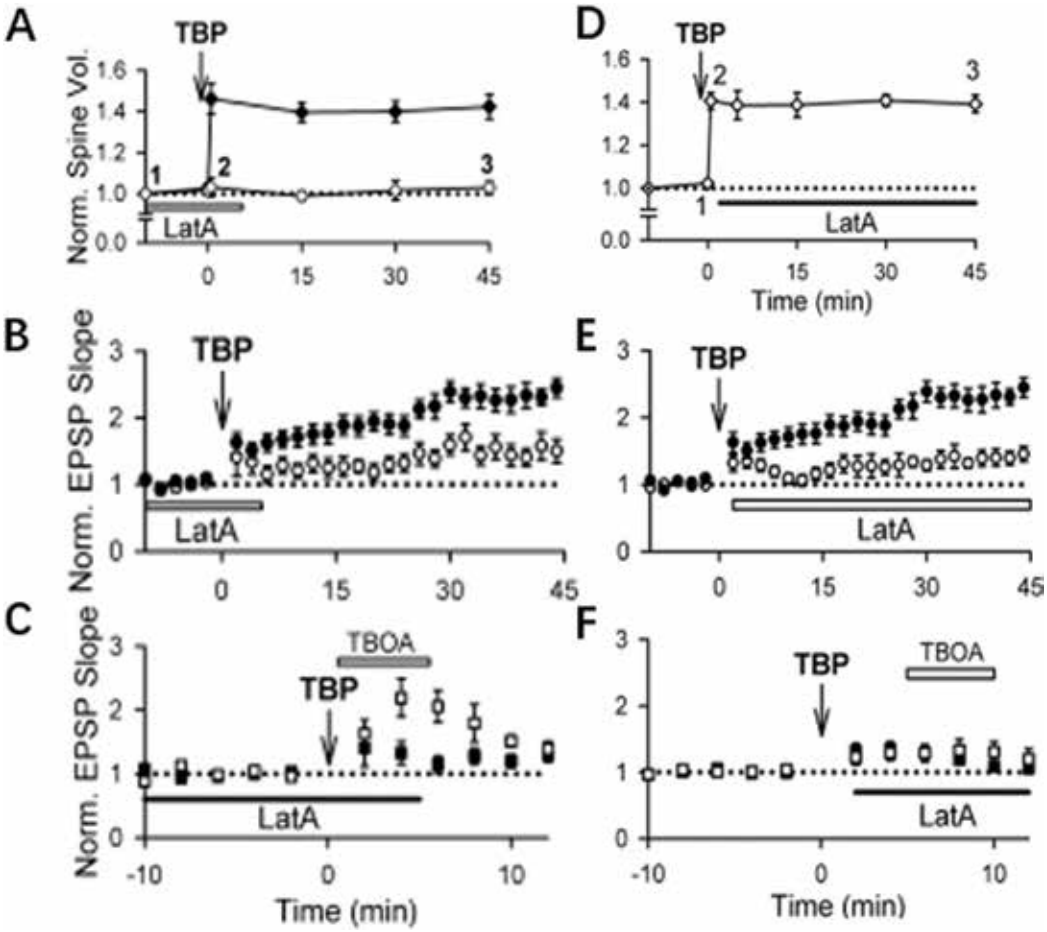

Figure 3. Time-dependent reversal of synaptic and spine modification. (A) Spine shrinkage induced by low frequency stimulation (LFS) can be readily reversed by subsequent high frequency stimulation (HFS). (B) Spine enlargement induced by HFS is also readily reversed by subsequent LFS. (C) Spine enlargement induced by TBP can be reversed by LFS only if LFS is given within a time window of about 15 min after TBP. (D) this critical reversal window also holds for reversing TBP-induced LTP. (E) LFS given outside this reversal window does not affect spine size. (F) LFS given outside the reversal window does not reverse LTP either. Taken from [34, 71].

and Linden demonstrated that LTD expression and spine changes in cerebellar Purkinje cells could be induced independently of each other, and induction of one did not affect the other [92]. Wang et al. reported that trafficking of AMPARs to and away from PSDs was activity-independent and not associated with alterations in spine size. The significance of this finding requires further investigation [70].

One interesting and important feature of synaptic modification is its reversibility. This reversibility is defined by reversal of synaptic modification after its induction [34, 93]. There are a few aspects to this reversal: (1) reversal applies to both LTP and LTD, and spine enlargement and spine shrinkage [34, 71] (**Figure 4**). More specifically, low frequency stimulation reverses LTP and spine enlargement, while high frequency stimulation reverses LTD

and spine shrinkage. (2) There is a critical time window only during which reversal can occur [34, 71, 93, 94] (**Figure 4**). In hippocampal slices, the window for LTP reversal is about 15–30 min [71]. (3) The typical stimuli that can induce reversal are not capable altering basal synaptic strength or spine dimension [71, 93, 95, 96].

Although it is generally believed that expression of LTP requires the addition of synaptic AMPARs, some evidences suggest that these newly added AMPARs are not delivered directly into the PSDs inside spines, but rather they are either delivered to regions outside synapses (i.e., the perisynaptic regions; [82] or onto dendritic shaft [83]. These AMPARs

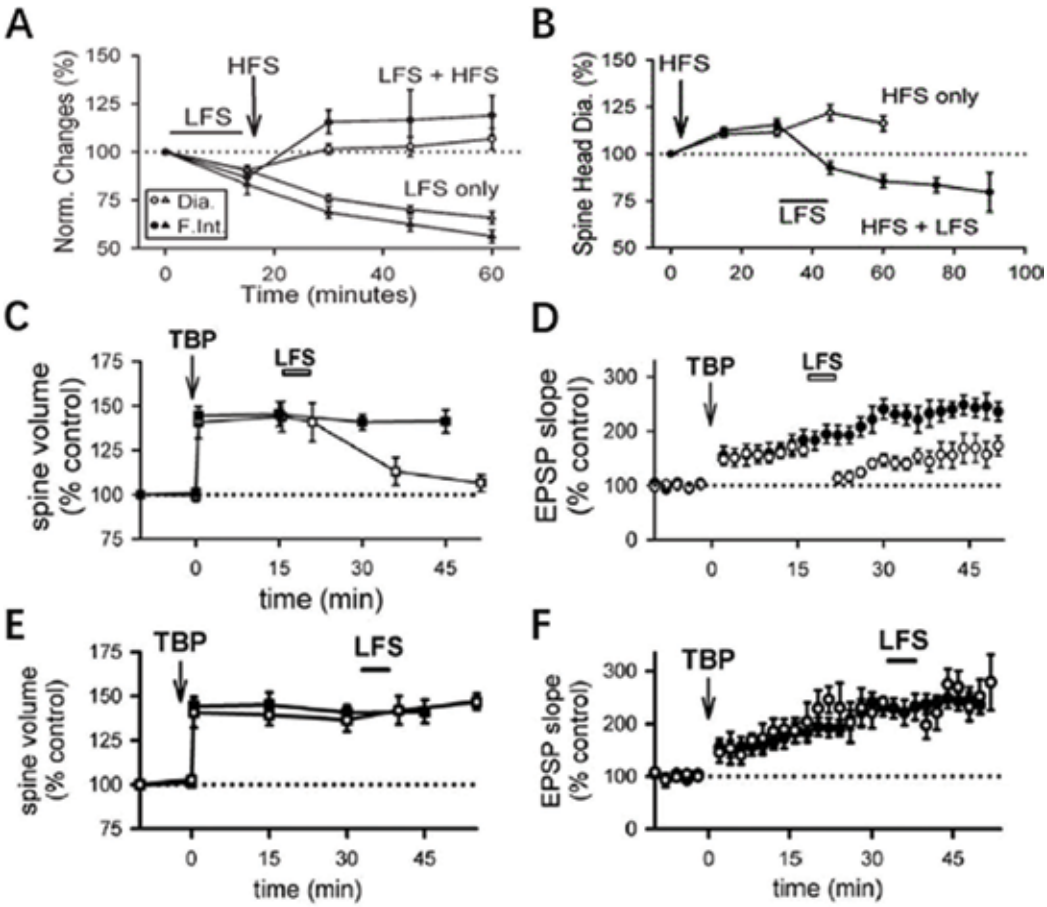

Figure 4. Actin polymerization is required for spine expansion and maintenance of perisynaptic AMPA receptors. (A) Actin depolymerizing agent Latrunculin A (LatA) added before and during TBP abolished spine expansion. (B) Bath perfusion of LatA before and during TBP impaired enhancement of synaptic response (EPSPs). (C) Bath perfusion of LatA 2 min after TBP did not disrupt the delivery of AMPARs to perisynaptic site, as revealed by the increased in responses to application of glutamate transporter blocker TBOA (open symbols). (D) Bath perfusion of LatA 2 min after TBP did not affect spine enlargement, indicating that persistent spine enlargement does not require persistent actin polymerization. (E) Bath perfusion of LatA 2 min after TBP prevented the occurrence of gradual increase in EPSP enhancement which requires the synaptic addition of new AMPARs. (F) Addition of LatA 2 min after TBP removed the newly delivered perisynaptic AMPARs as revealed by the absence of increase in response to TBOA. Taken from [83].

then move laterally into spines/PSDs. Thus, with LTP induction, two rapid processes (within 30 sec) occurs independent of each other: spine expansion which requires actin polymerization and activation of NMDARs but not postsynaptic PKA activation, and delivery of AMPARs to the perisynaptic regions which requires activation of NMDARs and postsynaptic PKA signaling but not actin polymerization. The next 15 min or so (reversal time window) determines whether LTP and spine expansion can be stabilized into a long-term change. During this period, translocation of the newly delivered perisynaptic AMPARs stabilized spine expansion, while removal of these receptors led to collapse of enlarged spines [71]. On the other hand, reversal of spine enlargement also removed these perisynaptic AMPARs. Hence, there is a mutual interaction between perisynaptic AMPARs and spine enlargement in that the presence of one is required to sustain the other (**Figure 3**). Yang et al. found that postsynaptic PKC activity is required for the translocation of perisynaptic AMPARs to synapse, and in the absence of PKC activity, these AMPARs remain perisynaptic. Importantly, as long as the perisynaptic AMPARs are present, both LTP and spine expansion exist in a labile state in that they can either be reverted to the baseline state (no plasticity), or they can enter a stabilized state of persistent increase in synaptic strength and spine size (persistent plasticity). Low frequency synaptic stimulation given within a 15 min "grace period" post-LTP induction reversed spine expansion and removed AMPARs from the perisynaptic regions and hence blocked the conversion of short-term plasticity to a long-term one (**Figure 5**) [71, 82].

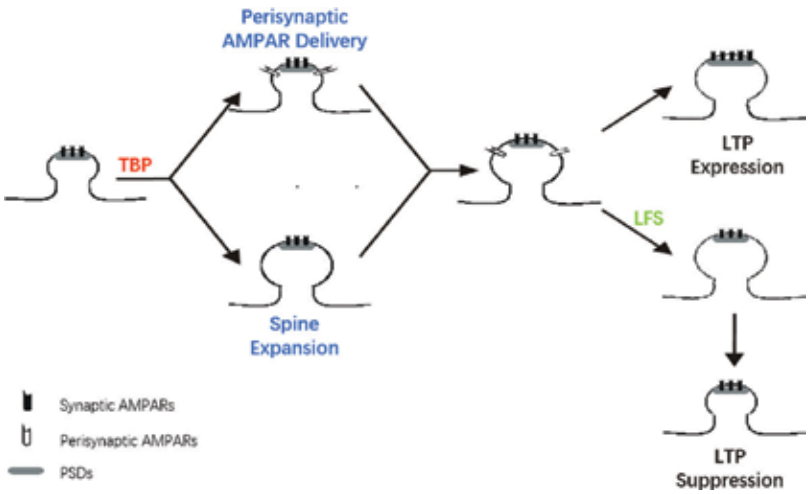

Figure 5. Two-step model for coordinated expression of synaptic potentiation and spine enlargement. TBP triggers two initial processes, spine enlargement and perisynaptic delivery of AMPARs. These two processes occur simultaneously and mostly independent of each other. In the absence of any disturbance, perisynaptic AMPARs translocate into synapse, which stabilizes both AMPARs and spine enlargement, and this leads to persistent potentiation of synaptic responses and spine enlargement. On the other hand, when low frequency synaptic stimulation (LFS) is given within this "grace period" when both processes are in a liable state, it readily removes perisynaptic AMPARs. As a result, synaptic potentiation is aborted and spine enlargement collapses, and no LTP and spine enlargement. This model shows the interaction between functional and structural aspects of synapse modification is critical to the persistency and long-lasting occurrence of synaptic modification.

The above results are consist with a model (**Figure 5**) in which (1) the expression of functional plasticity (LTP) and structural plasticity (spine enlargement) are initially two independent processes only share the activation of NMDARs; (2) these two processes then enter an interactive state that the continuous presence of one is required for the persistence of the other; (3) the above state is liable in that interference (such as low frequency stimulation) can revert both changes back to the baseline; (4) once both processes are stabilized, synapse modification has entered a state resistant to reversal. We like to note that during the "grace period" coordinated changes in synaptic plasticity (function and structure) are cross-checked to ensure that they do occur together, and in the situation only one such process occurs (perhaps can be viewed as a mistake), the other process will be aborted albeit in process. This double-proof mechanism is essential to ensure that only appropriate changes are allowed to be sustained, and may thus be especially important in face of the highly dynamic nature of synaptic modifications, such as those occurring during early neural development [94].

Sustained reduction in synaptic strength may eventually lead to the loss of synaptic connections, and this loss is manifested as a reduction in spine density. Spine loss appears to be a protracted process and hence it is difficult to study. Even if monitoring changes in the same set of synapses/spines, it is usually more difficult to exclude the possibility that the reduced synaptic function and spine number is caused by deterioration of the health of the preparation, or by some other unknown or uncontrolled processes that occur randomly during the long period (>hours) between LTD induction and spine loss. Nonetheless, a few studies have examined this process. By using organotypical slices and monitoring both presynaptic boutons and spines, Becker et al. showed that LTD induction increased the turnover rate of presynaptic boutons and resulted in decreased synaptic contacts between the pre- and post-synaptic sites. Although presynaptic boutons and postsynaptic spines disappear at much greater rate after LTD, there is no particular pattern to follow, since disappearance of either presynaptic boutons or spines could occur prior to the other [97]. Therefore, the above observations suggest that the mismatch between presynaptic and postsynaptic sites is more likely a key factor in the elimination of synapse, while the exact sequence might not play much role.

5. Conclusions

Dendritic spines are small protrusions on the dendritic shaft as major excitatory inputs site on the excitatory neurons in the adult cortex. Spines play critical roles in the excitatory synaptic transmission and plasticity. Genesis of spines occurs during brain development, and is subjected to activity-dependent modulation to determine their fates, either to transit to mature spines or be eliminated. Spines are the site where physiological/functional and morphological/structural modifications meet and integrate, during both physiological (such as memory formation) and pathological (such as neurodegeneration) processes. Interestingly, early changes in functional and structural aspects of synapse modification occur independently, but they subsequently interact with each other to sustain changes in both. This highly interactive nature ensures that the end result is a coherent modification of synapse function

and structure. Extensive progress has been made on our understanding of the structure and function of spine which vastly has advanced our understanding of neuronal and synaptic communication and plasticity. In addition, changes in spine density and dimension may serve as a marker of pathological processes and hence have potential therapeutic/diagnostic values.

Acknowledgements

This work was supported by grants from Peking University Shenzhen Graduate School and grants from Shenzhen government (ZDSYS201504301539161 and KQTD2015032709315529).

Competing interests

The authors declare that they have no competing interests.

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Chapter 9

Sleep Disorders in Multiple Sclerosis

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72831

Abstract

Patients with multiple sclerosis (MS) have multiple causes of poor sleep and potential triggers may relate to MS-related symptoms, co-morbidities and adverse effects from medication. Sleep disorders may occur independently of demographic factors, gender and clinical condition. The real frequency of sleep disturbances in MS and their impact on the patients' quality of life are unknown. The prevalence of sleep problems in the population with MS ranges between 47 and 62% and is more frequent in women, as well as having a higher risk of mortality. High psychological burden has been associated with poor sleep and with increased risk of co-morbid conditions such as heart disease, obesity, dyslipidemia and diabetes, which may have a profound impact on long-term health. The poor sleeping patients with MS were more likely to report fatigue and sleepiness. Insomnia is present in mood disorders, restless leg syndrome (RLS), pain, nocturia and obstructive sleep apnea (OSA), in patients with MS. All the symptoms are intermixed, and it is not possible to discern the precipitating factor or the perpetuating factor. Clinicians should routinely ask about sleep when forming a comprehensive care plan for patients with MS. Sleep specialty referrals should be considered for management of conditions that require polysomnography (PSG) diagnosis.

Keywords: sleep disorders, insomnia, sleepiness, fatigue, respiratory disorders during sleep, cognitive impairment, cognitive behavioral therapy, multiple sclerosis

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system that is increasingly prevalent in young adults. Patients with MS have multiple causes of poor sleep, and potential triggers may be related to MS-related symptoms, co-morbidities and adverse effects from drug therapy.

Major sleep disorders have been reported to be associated with numerous co-morbid conditions, including heart disease, obesity, stroke and diabetes [1].

The poor sleepers among patients with MS are more likely to report fatigue (one of the most frequent symptoms in MS) and pain (a co-morbid condition to MS-related fatigue but is confounded by depression and medication for treating pain or pain-induced sleep disorder) [1].

All the symptoms are intermixed, and it is not possible to discern the precipitating factor and the perpetuating factor. In this respect, sleepiness and fatigue may converge in some situations [1].

Insomnia is present in mood disorders (depression and anxiety), restless leg syndrome (RLS), pain, nocturia and others [1]. RLS is also an important cause of pain in patients with MS. Sleep disorders may occur independently of demographic factors such as gender and clinical-demographic factors. High psychological burden has been said to be independently associated with poor sleep patients with increased risk of co-morbid conditions such as heart disease, obesity and diabetes, which may have a profound impact on long-term health. The reverse situation is also possible.

The frequency of sleep disturbances in MS and their impact on the patients' quality of life are unknown. The prevalence of sleep problems in the MS population ranges from 47 to 62%, with a higher prevalence in women [2–5]. Sex hormones and genetic mechanisms, psychosocial factors, certain physical factors that disrupt sleep, such as pain or bladder dysfunction may contribute to sleep differences between women and men [5].

Obstructive sleep apnea (OSA), RLS and chronic insomnia in particular are frequent problems in the MS population, and play a key role in the development of debilitating fatigue and other poor functional outcomes in MS. Yet, despite their impact, sleep disorders in MS remain critically under-recognized in most clinical settings. A recommended approach to the fatigued patient with MS is also highlighted [6].

Sleep disorders during the course of MS may be secondary to numerous symptoms arising from the disease itself or can be primary with a common biological link. In either of the two cases, a bidirectional relationship exists between these co-morbid conditions [7].

Sleep disturbances have been associated with increased risk of mortality, cardiac disease, obesity and diabetes [8] and can contribute to the depression, pain and fatigue symptoms that are commonly seen in MS patients, which are often disabling [3, 9].

Sleep disorders are under-recognized in persons with MS. These sleep disorders can contribute significantly to fatigue, other daytime dysfunction and poor quality of life. A systematic, practical approach that takes into account clinical features of MS is recommended to enhance recognition of these conditions and facilitate appropriate treatment. Clinicians caring for patients with MS should routinely screen for sleep disturbances and initiate diagnostic workups, if clinically indicated.

Sleep specialty referrals should be considered for management of conditions that require polysomnography (PSG) diagnosis, for complex patients who present a diagnostic challenge and for patients who do not respond to first-line treatments. Clinicians should also routinely ask about sleep when forming a comprehensive care plan for patients with MS.

http://dx.doi.org/10.5772/intechopen.72831

2. Effect or influence of multiple sclerosis in sleep disorders

2.1. Insomnia

Sleeplessness or insomnia is an inability to fall asleep or to stay asleep as long as desired. Insomnia is described as a complaint of prolonged sleep-onset latency, disturbance of sleep maintenance or the experience of non-refreshing sleep [10]. Episodic insomnia can usually be traced to an acute psychological stressor or an environmental change. Chronic insomnia may be related to a combination of factors including depression, poor sleep hygiene, learned sleeplessness, sleep-disordered breathing, nocturia, drugs or extrinsic factors such as noise [6, 11].

Patients with MS face a high risk of insomnia of around 40% [6] compared to roughly 10–15% in the general population [12]. Awakening too early in the morning is the most common symptom (58%) [13].

Primary symptoms of MS that can condition the onset of insomnia are neurogenic bladder (nocturia), spasticity, sexual dysfunction, neuropathic pain, paroxysmal phenomena, depression and anxiety [7].

Insomnia affects daytime activities, because of fatigue, mood disturbances (depression and anxiety), attention, concentration and memory impairment [7]. Higher fatigue scores have also been found to correlate with insomnia, especially middle insomnia [13].

2.1.1. Diagnostic approach

Patients with insomnia may complain of difficulty falling asleep, difficulty staying asleep or waking up sooner than desired [10]. There are screening tools to identify such patients.

The Pittsburgh Sleep Quality Index (PSQI) measures seven domains: subjective sleep quality, latency, duration, habitual efficiency, disturbances, use of sleep medication and daytime dysfunction over the last month. Each of these seven domains is self-rated by the individuals. The score of each question is based on a scale from 0 to 3, in which a score of 3 demonstrates the negative extreme on the Likert Scale. A global sum of 5 or greater indicates a poor sleeper (sensitivity of almost 100% for insomnia) [14, 15].

Insomnia Severity Index (ISI): a seven item questionnaire designed to assess the nature, severity, and impact of insomnia in adults. Scores >15 reflect moderate clinical insomnia. It is also a useful tool to monitor the effects of insomnia interventions [6].

Athens Insomnia Scale (AIS): a psychometric instrument designed for quantifying sleep difficulty. It consists of eight items: the first five pertain to sleep induction, awakenings during the night, final awakening, total sleep duration and sleep quality; while the last three refer to well-being, functioning capacity and sleepiness during the day. Either the entire eight-item scale (AIS-8) or the brief five-item version (AIS-5), which contains only the first five items, can be used. The score of each question is based on a scale from 0 to 3, where a score of 3 indicates the negative or normal extreme on the Likert Scale. A cut-off score of ≥6 on the AIS is used to establish the diagnosis of insomnia [16].

2.1.2. Management

After detecting insomnia, amelioration of any precipitating causes of insomnia is a cardinal step in its management. Medications or substances that may contribute to insomnia should be reduced or discontinued, if possible, there should be a check on which drugs the patient is taking (medications used to alleviate MS-related symptoms, including over-the-counter medications).

Selective serotonin reuptake inhibitors, while helpful for depressive symptoms, may worsen insomnia.

Stimulants and wake-promoting agents, which are commonly used for fatigue, may interfere with sleep initiation if taken during the late afternoon or early evening hours.

Antihistamines, which are used as sleep aids by up to 25% of patients with MS, have the potential to worsen RLS, and thereby worsen sleep-onset insomnia [6].

Co-morbid symptoms must be identified and treated: neuropathic pain (tricyclic antidepressants and the α-2-δ ligand pregabalin), spasticity (baclofen or tizanidine) and urinary urgency (anticholinergics) [6].

Cognitive behavioral therapy (CBT) is an innovative psychotherapy approach. CBT treatment could reduce anxiety and depression by changing thoughts and beliefs and consequently reduce the symptoms of insomnia [6, 17].

Pharmacological therapies can be considered if more conservative strategies have been exhausted or are not fully effective: benzodiazepines, benzodiazepine agonists, melatonin receptor agonists and orexin receptor antagonists.

2.2. Hypersomnia

Excessive daytime sleepiness (EDS)/excessive daytime drowsiness disrupt daily performance.

Hypersomnia may be due to acute thalamus injuries, mental disorders, especially depressive symptoms, sleep deprivation or as a consequence of received treatments.

2.2.1. Diagnostic approach

The Epworth Sleepiness Scale (ESS) is a screening tool that assesses sleepiness and has eight items. ESS values equal to or greater than 10 indicate excessive daytime sleepiness (EDS), and in this case, patients should undergo polygraphy or PSG (screening for sleep apnea) [18].

All fatigued patients should be asked about sleepiness and fill in the Epworth Sleepiness Scale (ESS) as these are not always associated with sleepiness.

Magnetic resonance imaging (MRI) should be performed because of the need to identify structural lesions in the brain.

2.2.2. Management

After detecting hypersomnia, the physician should check for any medications involved and withdraw them if possible, treat acute lesions of multiple sclerosis with corticosteroids,

indicate adequate sleep hygiene and assess whether specific treatment is needed to improve daily performance.

2.3. Restless leg syndrome

The four main criteria for diagnosis of RLS are: (1) unpleasant sensations in the legs; (2) worsening of the symptoms during rest; (3) relief of the symptoms by movement and (4) exacerbation of the symptoms in the evening or at night [19].

The periodic limb movement disorder (PLMD) includes repetitive periodic shaking episodes lasting between 0.5 and 5 seconds that occur during sleep every 20–40 seconds; mainly in the legs, but sometimes in the arms.

RLS and PLMD are motor disorders of sleep considered separate clinical entities, both conditions have the potential to cause disrupted sleep, share similar pathogenesis and have an increased prevalence among persons with MS. PLMD also frequently occur in the absence of RLS.

Most RLS patients (80–90%) have periodic leg movements PLM during sleep. They can cause arousals or micro-arousals leading to non-refreshing sleep, daytime sleepiness and fatigue. The prevalence of RLS in the general population ranges from 1 to 12% [20]. The prevalence of RLS in MS patients is two to five times higher than in the general population [21–24].

Differentiating RLS from other sensory and motor symptoms of MS can be difficult, as MS patients frequently suffer from spasms, dysesthesia, paraesthesia and spasticity in the legs, which worsen with immobility [25].

Predictive factors for RLS in MS patients include: older age, longer disease duration, progressive primary forms, greater disability as measured by the Expanded Disability Status Scale (EDSS), especially in the pyramidal and sensory subscales and shaking of the legs before onset of sleep [26]. Furthermore, RLS symptoms are more severe when associated with MS than when not associated with MS.

MS patients with RLS have more cervical cord lesions than those patients without RLS. These lesions possibly disrupt the ascending and descending pathways with cerebrospinal disconnection leading to these symptoms [10].

Primary RLS is a genetic form of RLS with autosomal dominant transmission [27]. Four genes have been associated with this syndrome [28] but no crossing over of those involved in MS [10].

Pathogenesis of RLS and PRLS shows dysfunction of downstream dopaminergic pathways, namely diencephalospinal and reticulospinal pathways, that project to the spinal cord. These pathways, *via* dopaminergic transmission, are responsible for the suppression of sensory inputs and motor excitability, and are susceptible to damage from diseases affecting the spinal cord. Impaired iron metabolism is also thought to contribute to the pathogenesis of RLS, as iron is a cofactor for a rate-limiting step in the synthesis of dopamine [6, 10].

Certain medications used in the management of persons with MS, such as antiemetic drugs, antipsychotic dopamine antagonists, antidepressants and antihistamines can also cause or worsen RLS [6, 10].

2.3.1. Diagnostic approach

Many descriptors can be used by patients to describe the restless sensation, including creeping, crawling, itching, burning, tightening, tingling or pain.

Symptoms of leg tightness relieved by voluntary movement suggest RLS, whereas involuntary spasms, even if a circadian component is endorsed, suggest spasticity. Rhythmic involuntary movements triggered by stretching or certain leg positions suggest clonus.

The Restless Legs Syndrome Diagnostic Index (RLS-DI) is a 10-item questionnaire. Scores range from −22 (no RLS) to +20 (definite RLS). A score of +11 yields 93.0% sensitivity and 96.1% specificity to accurately diagnose RLS [6].

Diagnosis of PLMD requires overnight PSG to assess for the presence of leg movements [6, 10].

The RLS Rating Scale is a useful tool to track treatment response and is a 10-item self-administered scale. Scores of 11–20 reflect moderate RLS.

2.3.2. Management

Iron supplementation should be implemented for a ferritin level of less than 50 ng/ml.

Reduction or discontinuation of medications and substances that can cause or worsen RLS or PLMD (dopamine antagonists, lithium, selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, antihistamines, tricyclic antidepressants, alcohol, tobacco and caffeine) is recommended.

Dopamine agonists (pramipexole, ropinirole and rotigotine), and the α-2-δ ligand gabapentin and anticonvulsants are first-line treatments.

Benzodiazepines and opioid agents (oxycodone and methadone) are second line treatments.

Treatment for refractory RLS, or augmentation in response to dopaminergic therapy, is also likely to be optimized by sleep specialty care [6].

2.4. Respiratory disorders during sleep

Sleep-disordered breathing is characterized by episodes of nocturnal hypopnea and apnea resulting in a reduction or a cessation of airflow in the upper airway.

Patients with sleep-disordered breathing may complain of "fatigue," decreased concentration, mood changes, erectile dysfunction, nocturia and mood changes, all these complaints are similar to those experienced in MS [29].

2.4.1. Apnea and hypopnea

Apneas and hypopneas may be caused by a collapse of the tissues and muscles in the pharynx (obstructive apnea/hypopnea) or a failure in the medullary respiratory signal (central apnea/hypopnea) [10].

Maintenance of upper airway patency during sleep requires an increase in pharyngeal tone that is primarily mediated by efferent motor output from cranial nerves X and XII to the palatal and genioglossus muscles, respectively. This process is largely influenced by afferent sensory input from pressure receptors in the upper airway, peripheral chemoreceptors in the aortic and carotid bodies, and brainstem respiratory generators. Pathophysiological processes that disrupt these tightly regulated brainstem pathways have the potential to impair nocturnal respiration. The medullary reticular formation is responsible for controlling automatic breathing during sleep [10].

Causative factors include obesity, craniofacial abnormalities, enlarged tonsils, congestive heart disease and degenerative central nervous system (CNS) disorders, to name a few [10].

Such apnea and hypopnea episodes may lead to nocturnal hypoxemia, frequent awakenings and daytime somnolence. When the apneas are associated with respiratory effort, the term obstructive apnea is used, and central apnea is used when there is a lack of respiratory effort [10].

Central sleep apnea is diagnosed when more of 50% of the events are central in patients with both central and obstructive apneas.

2.4.2. Obstructive sleep apnea

Obstructive sleep apnea is characterized by repeated episodes of upper airway obstruction and hypoxia during sleep [6].

The incidence of OSA in patients with MS is 2–21% and is one of the most common respiratory disorders [10].

Patients with MS who have a diagnosis of OSA and those at an elevated risk of OSA have increased fatigue and diminished quality life compared with undiagnosed or low-risk patients [6].

Sleepiness is primarily a result of acutely or chronically reduced sleep time, or poor sleep quality. Apnea severity may correlate with impaired cognition in MS [6].

2.4.2.1. Diagnostic approach

Questions must be asked about symptoms of snoring, pauses in breathing witnessed by a bed partner, gasping or choking upon awakening, non-restorative sleep, excessive daytime hypersomnolence or fatigue, cognitive disturbances and nighttime awakenings, any of which may arise in part from underlying OSA [6].

Dysarthria or dysphagia, obesity, increased neck circumference, crowded oropharyngeal inlet, retrognathia, or micrognathia are common physical exam findings [6].

The STOP-Bang questionnaire is a screening tool consisting of eight questions and measures that form the acronym snoring, tired, observed apnea, Blood Pressure-Body Mass Index, age, neck circumference and gender. Scores of 3 or higher indicate an elevated risk of OSA [6].

A full-night PSG is necessary to demonstrate the presence of obstructive respiratory events during sleep to confirm the diagnosis of OSA. These events may be partial (hypopneas) or

complete (apneas), but must demonstrate evidence of a reduction in airflow during sleep, despite continued effort to breathe [6].

2.4.2.2. Management

Management strategies for sleep-disordered breathing should take into account the patient's primary apnea subtype, apnea severity, co-morbidities and behaviors, and other MS-specific symptoms or limitations. Guidance by a sleep medicine physician is often helpful [6]. Discontinuation of medication, such as opiates, antispasmodics or CNS depressants medications [6].

Positive airway pressure (PAP) therapy is delivered by a mechanical device and mask to splint the upper airway open during sleep. Supplemental oxygen, bi-level PAP and adaptive servo ventilation are other improvements that these devices have [6].

Oral appliances work by repositioning the mandible in the anterior and inferior position [6]. By improving nocturnal oxygen saturation and sleep quality, PAP therapy effectively reduces fatigue and can be effective in the treatment of depression. This is especially important given the link between fatigue and depression in MS [6, 18].

Disease-modifying therapy use, in particular emerged as a strong predictor of *reduced* apnea severity, raising interesting possibilities about the role of local and/or systemic inflammation in OSA [6].

2.4.3. Central sleep apnea

Central sleep apnea (CSA) is rare, and the prevalence is unclear in the general population. CSA involves repeated complete or partial reduction of airflow, caused by an intermittent lack of respiratory effort by failure in the medullary respiratory signal [10].

While the prevalence of CSA is less than that of OSA, patients with CNS disorders that affect pontine and medullary respiratory generators, including MS, may be at increased risk for this condition as well even nocturnal death (Ondine's curse) [6, 10].

CNS and brain stem-related nocturnal respiratory abnormalities such as central sleep apnea, paroxysmal hyperventilation, hypoventilation, respiratory muscle weakness and respiratory arrest have all been described and should be considered in this patient population in the evaluation of symptoms of daytime somnolence, increased fatigue and non-refreshing sleep [11].

2.4.3.1. Diagnostic approach

In patients with symptoms of daytime somnolence, increased fatigue and non-refreshing sleep, the physician must ask about nocturnal respiratory abnormalities [11] and look for evidence of reduction in airflow in the absence of respiratory effort in an overnight PSG.

In patients with both central and obstructive apneas, central sleep apnea is diagnosed when more than 50% of the events are central. The coexistence of OSA or CSA and MS has been described by several authors [2, 10, 30].

2.4.3.2. Management

In the cases of central sleep apnea not symptomatic or central sleep apnea during sleep-wake transition (20% of central sleep apnea cases resolve spontaneously) observation is recommended.

In other cases, PAP treatment, adaptive servo ventilation, oxygen, added dead space, carbon dioxide inhalation and overdrive atrial pacing are needed.

2.4.4. Nocturnal urinary disorders

Sleep disturbance is associated with outcomes such as increased risk of falls and mortality. Nocturia may both precipitate poor sleep and perpetuate insomnia (awakenings associated with nocturia may themselves be perpetuating factors) [31].

Overactive bladder (OAB) syndrome is a condition that accompanies urgency (a significant factor for sleep disruption), with or without incontinence, frequently with increased daytime frequency and nocturia [32]).

Nocturia is defined by the International Continence Society as the complaint that an individual has to wake at night one or more times to void. It reflects the relationship between the amount of urine produced while asleep, and the storage by the bladder of urine received. Nocturia is a symptom rather than a disease and causative categories have been proposed and is the most common storage symptom in the general population [32].

Nocturia can occur as part of lower urinary tract dysfunction (LUTD), notably in overactive bladder syndrome (OAB). Nocturia can also occur in association with other forms of LUTD, such as bladder outlet obstruction or chronic pelvic pain syndrome [33].

Nocturia is due to nocturnal polyuria, a decreased nocturnal bladder capacity or a mixture of the two. Various duplicating factors for nocturia have been reported, including pathological conditions such as diabetes, LUTD, cardiovascular disease, primary sleep disorders and sleep apnea [32].

Nocturia is a feature of systemic conditions affecting water and salt balance, leading to excessive production of urine at all times (global polyuria) or primarily at night (nocturnal polyuria), so that nocturia can be a systemic symptom such as cardiovascular, endocrine and renal disease can affect water and salt homeostasis, leading to an increased rate of urine production [33].

Nocturia can significantly influence quality of life, efficiency, vigor and awareness of health, primarily due to sleep disruption.

2.4.4.1. Diagnostic approach

Asking how many times the patient wakes up at night because of nocturia, whether urinary urgency exists and the characteristics of urination, quantity of fluid intakes, physical exercise, medication being taken, etc. is also necessary *for the diagnosis of urinary problems*.

The use of specific questionnaires such as the PSQ, ISI and ASI for the diagnosis of insomnia or the ESS for the diurnal hypersomnia helps the physician to approach the functional repercussion of the problem.

The study occasionally needs to be completed with ultrasound studies, urodynamics, MRI and urinary sediment to identify LUTD or OAB problems.

2.4.4.2. Management

Interventions targeting nocturia may potentially improve sleep quality [31].

Hygienic measures such as reduced fluid intake at the end of the evening and frequently going to the bathroom during the day can help. Adequate treatment of co-morbid conditions such as diabetes mellitus, congestive heart failure or sleep apnea requires direct intervention for improvement nocturia.

Anticholinergics, mirabegron, a-blockers, 5-a reductase inhibitors, oral phosphodiester-ase-5 inhibitors, desmopressin, diuretics, sleep-promoting agents and phytotherapy are used to treat urinary problems [33]. Half of MS patients with moderate to severe overactive bladder symptoms are treated with an anticholinergic medication [18].

Antimuscarinic drugs (Solifenacin), the most appropriate treatment for OAB, inhibited bladder stimulation may originate a decrease of drive to the brain stem, improve urination urgency and frequency and effectively reduce involuntary contractions and increase bladder capacity in patients with storage symptoms. The night time dosing of antimuscarinic drugs may improve tolerance compared to daytime dosing [32].

Antidiuretic therapy using clinician-directed dose titration has been reported to be more effective than placebo in terms of reduced nocturnal voiding frequency and duration of undisturbed sleep [33].

Nocturia severity improvement contributes to overall improvements in health-related quality of life [33].

The impact of treatment for nocturia in MS fatigue is unknown [18]. Non-pharmacological therapies such as cognitive behavioral therapy for nocturia (CBT-N) act on the abovementioned perpetuating factors. Sleep restriction entails reducing the excessive time in bed (a common occurrence in insomnia) and thereby improves sleep efficiency.

3. Effect of the quality of sleep in multiple sclerosis

Sleep disturbances have been associated with increased risk of mortality, cardiac disease, obesity and diabetes [8] and can contribute to the depression, pain and fatigue symptoms that are commonly seen in MS patients, which are often disabling [3, 9, 10].

3.1. Fatigue

Fatigue is defined as a subjective lack of physical or mental energy perceived by the patients or their caregivers which interferes with desired activities of daily living and it is the most frequent symptom in MS [18].

Between 80 and 97% [6] of patients report chronic fatigue, and more than 33% of patients rate this symptom as the most disabling [34–36].

Fatigue may occur at any stage of the disease and can even precede MS onset by several years. Fatigue affects the social and professional capabilities of patients, is a major reason for early retirement, reduced employment and is considered to be one of the main causes of impaired quality of life among MS patients, regardless of depression or disability [18].

Fatigue starts early in the morning and increases during the day. The perception of fatigue is exacerbated with environmental temperature and humidity [25], with age, [36] greater EDSS, mental or physical activity, infections and food ingestion [37].

Fatigue also deteriorates cognitive domains, such as information processing, memory and attention, [35] and it has significant socioeconomic consequences, including loss of work hours and in some instances, loss of employment, as well as family relationships and leisure time [36].

Fatigue is a symptom in MS patients and may have multi-factorial causes such as immunologic abnormalities (pro-inflammatory cytokines such as INF-α), endocrine influences (cortisol and dehydroepiandrosterone (DHEA)), axonal loss, altered patterns of cerebral activation, sleep disorders (RLS, chronic insomnia, sleep-disordered breathing and altered sleep microstructure), depression and medications used to treat MS symptoms or immunomodulatory and immunosuppressive treatments [7, 38, 39].

"Primary" fatigue is related to the pathological changes of the disease itself, and results from a spectrum where one pole is the inability to generate the force required to perform the task due to a failure of force production at the muscle level "*peripheral fatigue*"; and the other pole is the inability to sustain the required neural drive to muscle because of supraspinal, spinal and even peripheral nerve contribution "*central fatigue.*"

"Central fatigue" can be the result of both cognitive and physical exertion and can reflect either a subjective sensation (fatigue) or an objective change in performance (fatigability) [37]. Dopamine imbalance plays a major role in developing fatigue. Central fatigue is a failure of the non-motor functions of the basal ganglia.

The subjective feeling of fatigue is related to inflammation and increased levels of cytokines such as interleukin-1 (IL-1), IL-6 and TNF-alpha [40].

"Secondary" fatigue attributed to mimicking symptoms, co-morbid sleep, irritable bowel syndrome, migraine, mood disorders, depression and anxiety and medication side effects [36, 37]. Persons with secondary fatigue report greater levels of fatigue than those with isolated primary fatigue [36].

There is a great variability in MS lesions from extensive areas of destruction during MS attacks, healing processes of and neuroplasticity. The clinical manifestations of fatigue do not seem to exclusively depend on the structural damage, but rather on the balance between restorative and inflammatory/degenerative processes and the rupture of the neural network [37].

In this respect, there is evidence that supports these hypotheses, linking fatigue with structural or functional abnormalities (atrophy in the thalamus, corpus callosum, cortical gray matter

regions: superior frontal and inferior parietal gyrus, parietal lobe) within various brain networks (the cortico-subcortical circuit as a substrate for MS fatigue and the involvement of a "fronto-striatal network"), greater activation of the premotor area ipsilateral to the movement with functional MRI (fMRI), decreased N-acetylaspartate-creatine ratio (NAA/Cr) as a marker of axonal dysfunction. Resting-state fMRI studies show changes in functional connectivity (FC) of the basal ganglia including reward processing and motivation. In addition to motor functions, the abovementioned aspects are involved in the pathophysiology of fatigue [18].

3.1.1. Diagnostic approach

Patients with MS report being fatigued very often, sometimes it is just the feeling of lack of energy but in others it interferes with their work or their daily life. There are tools that help quantify the degree of fatigue which are described below.

Severity Scale (FSS): is a self-administered questionnaire with nine items (questions) investigating the severity of fatigue in different situations during the previous week. Grading of each item ranges from 1 to 7, where 1 indicates strong disagreement and 7 strong agreement and the final score represents the mean value of the 9 items. A total score of less than 36 suggests that you may not be suffering from fatigue [24].

Modified Fatigue Impact Scale (MFIS): The full-length MFIS consists of 21 items (total score 0–84, 38 as a cutoff to discriminate fatigued from non-fatigued individuals) while the abbreviated version has 5 items (0–20.). The abbreviated version can be used if time is limited but the full-length version has the advantage of generating physical, cognitive and psychosocial functioning subscales. The MFIS is one of the components of the MS quality life inventory [37].

MS patients, regardless of their fatigue level, have a significantly high frequency of RLS, higher Epworth sleepiness scale (ESS) scores, and higher PSQI scores. The time in bed, wake time after sleep onset %, total arousal index, limb movement arousal index and periodic limb movement arousal index are abnormal. The sleep efficiency index and sleep continuity index are lower in fatigued MS patients than non-fatigued MS patients. The PSQI results also suggest more disrupted sleep in fatigued MS patients. For all of the reasons above, quality of sleep studies should be performed with fatigued MS patients.

Once the patient has been identified with fatigue, it is necessary to investigate whether other co-morbidities are present (depression, anxiety, sleep disturbance, diabetes, heart disease, obesity, anemia, thyroid disease and nocturnal urinary disorders), what factors influence perpetuating fatigue and what situations can be modified in their lifestyle [6].

3.1.2. Management

Interventions targeting fatigue may potentially improve sleep quality and quality of life [31].

Pharmacological interventions are also reviewed and if there is evidence that a drug is involved in fatigue, it should be suppressed or the dose decreased [18]. Disease-modifying treatments (DMTs) are generally used to reduce relapses and progression and they occasionally cause an increase in fatigue, and in these circumstances it is important to change the medication for another DMTs [40].

Hygienic measures such as energy conservation programs, specific rehabilitation interventions physical (endurance, resistance, aerobic and combined training), aquatic therapy, cooling therapies, Tai chi, stretching, mindfulness-based interventions, yoga, acupuncture, progressive muscle relaxation and sleep hygiene advice (dependent on the nature of the sleep disorder) are more effective than pharmacological interventions [41].

Adequate treatment of co-morbid conditions such as diabetes mellitus, congestive heart failure, obesity, sleep apnea and other sleep disorders, depression and anxiety with pharmacological, psychological, behavioral and educational interventions is recommended [40, 41]. Pharmacological interventions for fatigue that are effective for reducing fatigue in patients with MS include amantadine, pemoline, prokarin (1 pilot study, side effects not reported), modafinil and pemoline combined with aspirin are efficacious for reducing fatigue in patients with multiple sclerosis. Carnitine has a discreet effectiveness. In general, the risk benefit of the drugs used for fatigue makes their recommendation be evaluated in each patient, highlighting them to the amantadine [37, 41]. Aminopyridines and coenzyme Q10 have an effect on fatigue by improving nerve conduction.

Nowadays, non-invasive brain stimulation (NIBS) techniques are gaining interest in the treatment of MS fatigue [37].

Promotion of health behaviors such as quitting smoking, physical activity (a high level of physical activity was borderline significantly associated with a decrease in co-morbidity) [42] and healthy eating may prevent some co-morbidities which were slow to show improvement in fatigue after the intervention, but they are effective [36].

3.2. Cognition

Cognitive impairment is a frequent feature of MS affecting up to 65% of patients [43] at both the earlier and later stages of the disease [44] and it tends to worsen over time [45].

MS negatively affects several aspects of cognitive functions, including attention, information processing [46], learning and memory, executive function and visuospatial abilities [47], having an important impact on quality of life [48], employment status [49], daily functioning, independence [50] and participation in social activities [51, 52].

Several factors have a negative influence on cognition in MS patients, such as depression [53], fatigue and sleep disturbances. Proper sleep is important for memory consolidation [54], and sleep deprivation has been related to impaired functioning in various cognitive domains [55].

Sleep disturbance causes a decrease in sustained attention [56], interferes with information processing and executive functioning [52]. Sleep disturbed patients reported higher levels of subjective cognitive problems compared to patients with normal sleep [52].

OSA and sleep disturbance are significantly associated with diminished visual memory, verbal memory, executive function (as reflected by response inhibition), attention, processing speed and working memory [52].

Excessive daytime sleepiness can lead to poor attention, poor memory, mood disturbances and increased risk of accidents [29].

In subjects with insomnia, a functional magnetic resonance imaging (fMRI) showed hypoactivation of the medial and inferior prefrontal areas during a cognitive task, in relation to the control subjects, which returned to normal values after treatment. Insomnia or superficial sleep produces less activation of the hippocampus and less connectivity is observed in the thalamus than in the control subjects. Damage to the hippocampus and thalamus (e.g., lesions and atrophy) in MS is associated with worse cognition. In controls, both regions may be related to sleep and cognition [52].

MS patients performed worse on all cognitive tests compared to controls. MS patients had less normalized gray matter (GM) volume, normalized white matter (WM) volume, hippocampal volume and thalamic volume. The hippocampus and thalamus showed increased functional connectivity (FC) in patients compared to controls, but lower FC was observed in patients with sleep disturbances (32%) [52].

3.2.1. Diagnostic approach

Neuropsychological manifestations can even be detected in patients during early stages of the disease. The Brief Repeatable Battery-Neuropsychology (BRB-N) [57] test was developed as a short and sensitive test to identify disturbances of cognitive domains in MS patients. The BRB-N has become the most widely used neuropsychological battery for MS, [58] and it is now being applied in clinical trials to monitor cognitive changes.

Different cognitive impairment criteria have been used: <1.0 SD, <1.5 SD and <2.0 SD in one, two or three subtests of the battery, respectively [59, 60].

3.2.2. Management

Strategies to optimize sleep could improve cognitive function in patients with MS.

In the case of insomnia, relaxation techniques such as autogenic training or progressive muscle relaxation can help the patient fall asleep earlier and have a longer sleep. But they do not improve sleep, so it has no sleep recovery effect. Behavioral therapies can improve sleep, but not prolong it. A combination of relaxation techniques and behavior therapy could be the most appropriate therapy for certain sleep disorders.

The general strategies for insomnia treatment include aspects of sleep hygiene such as extensions of night time in bed and frequent naps during the day. Pharmacological treatment is usually administered with stimulants such as amphetamines, methylphenidates, pemoline and modafinil [61].

As regards sleep hygiene, it is often necessary to make some lifestyle changes such as dinner should not be too late, nor too spicy or copious, maintain a regular sleep schedule, do not spend too much time in bed other than bedtime, do not drink caffeinated beverages such as coffee, black tea or cola, or caffeine medications, 4–6 hours before bedtime, do not smoke before going to bed or during the night, try to get enough rest and darken the bedroom, ventilate the bedroom, the temperature should not exceed 18°, do not do any physically demanding sport immediately before sleep because otherwise it will stimulate too much circulation, do not drink alcohol before going to bed or avoid sleeping too much during the day.

Patients with fatigue should organize daily routine and workloads. The physician also needs to improve the efficiency of information processing and working memory in these patients with fatigue [40].

Anxiety, depression, difficulty in sleeping and fatigue may have an impact on cognitive problems. If a person with MS experiences these symptoms and has problems with memory and cognition, they need to be provided with assessment and treatment (occupational therapist and neuropsychologist).

The concept of mental toughness (MT) has recently been recognized for its psychological importance not just in coping with stress but also for its association with increased physical activity (PA), and for its impact on both stress and objective sleep quality. MT consists of four key factors such as control (of own life and emotions), commitment, challenge and confidence (in own abilities and in other people); thus covering a range of cognitive-emotional processes closely involved in coping with stress, emotions, unexpected events and social setting [62].

3.3. Depression

Patients who suffer from problematic sleep and/or fatigue (with or without anxiety) may be more likely to experience higher depressive symptoms [63].

Depression is a mental illness that causes feelings of sadness and loss of hope, changes in sleeping and eating habits, loss of interest in your usual activities and pains that have no physical explanation.

3.3.1. Diagnostic approach

A trans-diagnostic approach to symptoms may be more effective than targeting each symptom separately, such as depression treatment or pain treatment alone. Trans-diagnostic models explain how multiple co-morbid symptoms or disorders develop rather than create disorder or symptom specific models [63].

3.3.2. Management

A trans-diagnostic treatment is an intervention that targets a range of diagnoses or problems through the use of treatment strategies targeting psychological processes that are common across disorders. It may be useful to consider all five factors such as depression, pain, anxiety, sleep and fatigue in designing a treatment plan. Treatments for the constellation of biopsychosocial concerns affecting many people living with MS.

The beneficial effects on depression of CBT targeting insomnia highlight a need for a comprehensive assessment of multiple concerns such as depression, anxiety, sleep problems or fatigue when treating people with MS who report higher levels of pain [63].

3.4. Trigger for an acute multiple sclerosis exacerbation

The mechanism by which sleep disorders trigger an acute MS relapse might be multi-factorial. Normal sleeping plays an important role in maintaining the normal function of the immune

system. Various studies have shown that sleep disorders are associated with elevated serum levels of pro-inflammatory cytokines and markers of oxidative stress [15].

The circadian regulation of cytokine output produces a daily rhythm in the inflammatory profile, with a pro-inflammatory state occurring at night. Disrupted sleep can interfere with this pattern leading to prolonged periods of inflammation throughout the day, thereby exacerbating symptoms. Furthermore, the circadian rhythmicity of key components of the immune system has been shown to be dysregulated in MS patients [64].

The central circadian pacemaker, located in the hypothalamic suprachiasmatic nuclei, is responsible for regulating the timing and expression of various circadian rhythms [65].

Sleep dysfunction and disruption in the circadian system alter the synchrony between these transcriptional and translational feedback loops, resulting in increased cellular permeability, which is thought to be an important underlying mechanism for initiating the inflammatory cascades causing a disease flare. In addition, the presence of pro-inflammatory cytokines has been proven to suppress the activity of circadian genes [65].

Melatonin is produced by the pineal gland that regulates circadian and seasonal rhythms. Secretion of melatonin is suppressed during daylight and enhanced during the night, promotes sleep by reducing sleep latency, decreasing wake time and increasing overall sleep quality [65].

Melatonin promotes anti-inflammatory states: it inhibits nitric oxide production, nuclear factor-κB activation and tumor necrosis factor- α, it reduces COX-2 expression and matrix metalloproteinase activity (modulating apoptosis) [65].

Circadian sleep disorders are common in MS patients and could be linked to a disruption in melatonin production, which is important in sleep-wake cycle regulation. Melatonin helps dampen the overactive immune system and low levels are associated with relapse [64].

According to studies on an animal model, sleep deprivation is associated with an accelerated autoantibody production rate and increases oxidative stress (toxic effect on oligodendrocytes causing oligodendrocyte death and myelin damage). Chronic sleep deprivation breaks down blood-brain barrier (BBB) thereby increasing permeability [15].

Sleep disorders also result in an elevated serum concentration of interleukin-6 (IL-6), which further activates polyclonal B cells and triggers an autoimmune reaction. The serum concentration of IL-6 is significantly associated with the number of relapses in female patients with relapsing-remitting multiple sclerosis (RRMS) [15]. In the study of Sahraian et al. [15], the group in relapse had worse scores of global PSIQI for the previous month than remission group (87.5% were poor quality sleepers). Age, gender, EDSS and disease duration did not associate with sleep quality in either group.

4. Co-morbidity condition

Co-morbidities have been shown to affect MS progression, time to initiation of the disease-modifying therapy (DMT), as well as treatment compliance, which may be related to the increased mortality of these patients as compared to the general population.

Co-morbidities can negatively impact sleep in MS patients, which can, in turn, lead to a worsening of symptoms, especially fatigue and pain.

Patients with sleep disorders are at risk of co-occurrence of other problems like vascular diseases, obesity and diabetes that would threaten the health of patients in the long term [17].

Circadian disruptions occur in shift workers and appear to contribute to hypertension, diabetes, breast cancer, lung cancer and elevated prostate-specific antigen. Shift work entails changes in diet, exercise and tobacco use, which can confound circadian rhythm and sleep disturbance studies [65].

4.1. Narcolepsy

Narcolepsy is classified as a chronic sleep disorder associated with sleep attacks and other features attributed to abnormalities of rapid eye movement sleep, such as hypnagogic/hypnopompic hallucinations, cataplexy, sleep paralysis and disrupted nocturnal sleep. The usual PSG features include a mean sleep latency of less than or equal to 8 minutes and two or more sleep onset rapid eye movement periods [6]. There is a high variability in the prevalence across different geographic areas, which is thought to be related to differences between the populations and current study methods [10].

Narcolepsy is estimated to affect 0.02–0.05% of the general population, the overall prevalence of narcolepsy among persons with MS is unknown [6, 10].

There are two subtypes of primary narcolepsy which are described below.

Narcolepsy type 1(immune-mediated loss of hypocretin-secreting cells in the lateral hypothalamus) [6, 10] is characterized by the presence of cataplexy (a reliable clinical marker for hypocretin deficiency) and hypocretin deficiency in CSF (<110 pg./dl).

Narcolepsy type 2: normal hypocretin levels [6, 10].

The secondary causes of narcolepsy show that MS is the fourth most common cause of narcolepsy after inherited disorders, CNS tumors and brain injury, and it has been found that 12% of the cases of secondary narcolepsy were due to MS [6, 10, 66].

In terms of genetics, 95% of narcoleptic patients and 50–60% of MS patients are positive for DR2 haplotype. The human leukocyte antigen (HLA) DQB1*0602, a known genetic risk factor for narcolepsy, also influences the presence and severity of MS. Therefore, both diseases are closely related to the same genes of the human leukocyte antigen (HLA) system, which is the basis for labeling for most autoimmune diseases. This relationship suggests that similar autoimmune factors may be at work in the development of each disorder and might be partially responsible for symptoms of fatigue and sleepiness [6, 10, 67].

The aforementioned findings merit further attention given the potential impact of sleep disorders on the health and quality of life of MS patients [10].

4.1.1. Diagnostic approach

A diagnosis of narcolepsy requires PSG and CSF hypocretin assays (only performed at a few academic institutions).

Narcolepsy cannot be established in the presence of concomitant OSA, insufficient sleep, shift work or another circadian sleep disorder [10].

In such cases, adequate treatment of concomitant sleep disorders must be confirmed prior to the multiple sleep latency testing.

The usual PSG features include a mean sleep latency of less than or equal to 8 minutes and two or more sleep onset rapid eye movement periods [6].

It is necessary to perform MRI studies to rule out secondary causes of narcolepsy [6].

4.1.2. Management

Patients with suspected narcolepsy are usually referred for diagnosis and management by sleep specialists: wake-promoting agents or stimulants may be used to increase wakefulness and vigilance.

Sodium oxybate (an endogenous metabolite of gamma-aminobutyric acid (GABA) may be used in selected cases.

REM-suppressing antidepressants may be useful for cataplexy and sleep paralysis.

In cases of secondary narcolepsy when new hypothalamic lesions are identified, a trial of high-dose steroids should be considered [6].

4.2. Overweight and obesity

Being overweight is having more body fat than is optimally healthy. The degree to which a person is overweight is generally described by the body mass index (BMI). Overweight is defined as a BMI above or equal to 25 and below 30.

Obesity is defined as a BMI over 30. The prevalence of overweight and obesity in patients with multiple sclerosis ranges from 19 to 55%. These differences are due to the distinct prevalence in the general population, differences in geographic origin, population type (military veterans or hospital users) and/or age group. It is notable that the American population has the highest numbers of obesity. Besides which, it is worth mentioning the different methodology used, including overweight with obesity in some studies [42, 68].

Spanish data (NARCOMS study) have shown that overweight people with MS had lower general and mental health scores compared to those with normal weight and found no differences in other quality of life scales of the SF-36 [69].

Depression levels were higher in the overweight versus normal weight MS Spanish patients. This finding is due to pathophysiological mechanisms common to both depression and obesity, given that chronic low-grade pro-inflammatory states can generate various abnormalities in different neural networks [69].

BMI was significantly related to levels of disability, with obese participants 1.4 times more likely to have moderate/severe disability while controlling for age, gender, time since diagnosis and number of co-morbidities. As the BMI increases, the number of co-morbidities

increases with higher odds for disability and prior relapse and lower health-related quality of life [42].

Central obesity, as defined by increased waist circumference, absolute waist circumference >102 cm in men and >88 cm in women or waist-hip ratio (the circumference of the waist divided by that of the hips) of >0.9 for men and >0.85 for women, is often indicative of metabolic syndrome, and is suggested to be a more potent risk factor (cardiovascular disease, Alzheimer's diseases and type 2 diabetes) than body mass index alone.

4.2.1. Diagnostic approach

Weight, height, BMI = weight (kg)/height (m^2), waist circumference and waist-hip ratio.

4.2.2. Management

Patients who are overweight and obese are usually referred for diagnosis and management by a multidisciplinary team such as specialist nutritionist, physiotherapists, surgeons and neuropsychologists.

Gradual weight loss and gentle physical exercise and stretching are recommended. Small meals and small amounts of food, low in animal fats and fresh fruits. Bariatric surgery may be necessary in severe cases of obesity.

Co-morbidities have been shown to be associated with increased hospitalization, rate of progression to disability, decreased quality of life and increased mortality risk which is why they have to be properly treated [42].

Adverse health behavior including being overweight and obese, smoking and sedentary behavior are common in people with MS [42]. These behaviors can be modified and may significantly change the level of health. Health professionals should be focused on achieving these behavioral changes in patients with MS.

5. Influence of the treatment of multiple sclerosis in sleep

The therapeutic approach to multiple sclerosis involves pharmacological, rehabilitative, psychological, lifestyle modifying interventions, etc. These can be used independently or coordinated with each other with a holistic view. This approach involves changes in the structure of sleep, which are not always beneficial.

5.1. Treatment of relapses

Therapeutic options to treat MS relapses include oral glucocorticosteroids [70, 71] or their intravenous administration at a high dose as first line and therapeutic plasma exchange (TPE) and intravenous immunoglobulin (IVIG) as second line treatments in glucocorticosteroids unresponsive patients [72], corticotrophin injection and Acthar [73].

The action mechanisms of glucocorticosteroids in the immune system are pleiotropic, induced apoptosis of peripheral blood leucocytes and down-regulation of T-cell activity delayed for 7–10 days after a 5-day course of administration [72].

TPE is the removal of circulating antibodies, cytokines, immune complexes and complementary factors, all of which are assumed to be involved in immune-mediated neuroinflammation.

IVIG reduces or prevents the activation of inflammatory cells and alters antibody responses.

Optimal treatment of relapses increases the chance of limiting or avoiding residual deficits which have been related to the progression of disability in MS [72].

Sleep disturbance (insomnia) might be one of the side effects of corticosteroid therapy during an acute exacerbation in MS. Benzodiazepines are useful during these periods [74].

5.2. Disease-modifying therapies

Interferons are DMTs that produce major alterations of sleep, mainly by the flulike reaction, fever, headache, alteration of the mood and fatigue. It is imperative to treat these effects to improve the patient's quality of life including finding what time is best to administer the treatment. The monoclonal antibody Natalizumab could reduce fatigue [37].

5.3. Symptomatic treatment

Specific treatment of symptoms of MS manifestations occasionally interferes with sleep quality, leading to insomnia or drowsiness. The treatments the patient is receiving need to be reviewed in the event of any sleep disturbance.

Selective serotonin reuptake inhibitors, while helpful for depressive symptoms, may worsen insomnia. Stimulants and wake-promoting agents, which are commonly used for fatigue, may interfere with sleep initiation if taken during the late afternoon or early evening hours. Antihistamines, which are used as sleep aids by up to 25% of patients with MS, have the potential to worsen RLS, and thereby worsen sleep-onset insomnia.

Patients suffering from fatigue symptoms are often treated with antidepressants due to the strong association between depression and fatigue. Modafinil, amantadine and aminopyridine are known as fatigue treatment options, although the physician must monitor the real effect on sleep and adjust the administration schedules so as not to mask the effect on fatigue.

Medications used to alleviate MS-related symptoms, including over-the-counter medications, also have the potential to interfere with sleep. Given the high frequency use of these medications in this population, the physician should carefully consider screening for these medications and assessing possible effects on sleep.

5.3.1. Management

The first approach includes reviewing the list of drugs being taken by the patient and adjusting doses or suspending them if necessary to avoid interference with other situations of the patient. In this respect, the multidisciplinary approach to the patient is important.

6. Conclusions

Sleep disorders in patients with MS are frequently underdiagnosed. Clinicians caring for patients with MS should routinely screen for sleep disturbances.

All the symptoms are related, many of them share the same pathophysiology where it is not possible to identify the precipitating factor and the perpetuating factor. Sleep disturbances increase the risk of mortality, co-morbidities (cardiac disease, obesity and diabetes) and can contribute to the depression, pain, cognitive impairment and fatigue symptoms which are disabling and worsen the prognosis of multiple sclerosis.

The therapeutic approach to sleep disorders in MS involves pharmacological, rehabilitative, physical, psychological, educational and lifestyle modification interventions. These can be used independently in combination, with combined therapies being more effective.

The list of drugs being taken by the patient should always be reviewed and doses should be adjusted or suspended if necessary to avoid interference with sleeps disorders.

Conflict of interest

Montserrat González Platas and María Yaiza Perez Martín report no conflicts of interest concerning the manuscript.

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This book provides comprehensive and up-to-date insights into emerging research trends on neuroplasticity with current or future treatments for neurodevelopment and neurodegenerative diseases. The authors discuss structural and functional changes associated with cortical remapping, sensory substitution, synaptic and non-synaptic compensatory plasticity due to brain damage, brain training, chronic pain, meditation, music, exercise and related states. Key features include pathogenesis, and existing and new therapies together with a pharmacological and non-pharmacological approach in clinical treatment and management. The authors are established experts that contributed significantly to a better understanding of the etiology of neuroplasticity. This book is recommended to healthcare providers, clinical scientists, students and patients.

Published in London, UK

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ISBN 978-1-83881-286-7