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Neural Correlates Underlying Motor Map Plasticity And Skilled Motor Behavior

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The dissertation of Dhakshin Ramanathan is approved, and it is acceptable in quality and form for publication on microfilm.

Chair

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ABSTRACT OF THE DISSERTATION

Neural Correlates Underlying Motor Map Plasticity And Skilled Motor Behavior

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Neural plasticity - the capacity of the brain to change - has been described at almost every level of the nervous system. These include changes at the single neuron level in gene expression, protein phosphorylation and cellular distribution of proteins; changes in the morphology of spines and dendrites; changes in the synaptic signaling efficacy between different populations of neurons; and finally, large-scale changes in the organization or function of entire brain regions. This last type, denoted as cortical reorganization or map plasticity, is of special importance because it is believed to represent the large-scale integration of the many plastic changes that occur at the cellular and systems levels.

Large scale cortical reorganization has been studied primarily in sensory and motor areas of the brain, because of the ability to create detailed functional maps of these areas of the brain before and after different experimental manipulations. One of the first examples of such cortical reorganization was demonstrated in the somatosensory cortex following digit amputation in macaque monkeys [1]. Further studies demonstrated somatosensory cortical reorganization following other peripheral manipulations [2], skilled training on a tactile paradigm [3, 4], and recovery of function after a cortical injury [5].

It was first surmised that the neural correlates of map plasticity would be the same, regardless of whether such plasticity occurred following cortical injury, peripheral injury or behavioral training [6]. Thus, a finding describing the importance of acetylcholine for map plasticity following skilled motor learning [7] was taken as evidence that acetylcholine was necessary for all forms of cortical map plasticity.

In this dissertation, we challenged that assertion. Specifically, we have shown that the neural processes underlying cortical map plasticity vary depending on the experimental paradigm used to elicit it. Further, we demonstrate that certain aspects of this plasticity are specific to behavioral experience. We used the motor cortex of rats as a model system to study cortical reorganization following different types of injuries (both peripheral and central), as well as different types of behavioral experiences, including motor development during the juvenile period and skilled motor learning in adulthood.

We first studied the role acetylcholine plays in these different types of motor cortical plasticity. We found that the basal forebrain cholinergic system, the primary source of acetylcholine in the cortex, is required in adult animals for behaviorally driven forms of cortical plasticity, but not for plasticity that occurs spontaneously following nervous system injury. We also found that this cholinergic system is necessary for the normal development of the cortical motor system. We next proceeded to study whether cortical reorganization is ever associated with axonal plasticity of the corticospinal tract at the level of the spinal cord. As axonal plasticity of other fibers has been described following cortical lesions, these neurons were traced following a cortical injury and rehabilitation paradigm previously developed in the lab. We found no evidence of plasticity of the corticospinal tract system following either a brain injury alone, or a brain injury in conjunction with rehabilitation training. Map plasticity of the motor cortex occurs in many contexts, and is thus not by itself an indication of skilled motor behavior. In searching for a paradigm to study motor cortex plasticity that occurs primarily in the context of skilled motor behavior, we adopted a stimulation paradigm used by others to evoke higher-level encoding of motor movements. Using this long-term stimulation paradigm, we found a form of cortical plasticity that occurs only in the context of rehabilitation following a cortical lesion. Plasticity of these complex movement maps correlated with the functional recovery of the animals, validating their behavioral relevance.

We conclude that there are many different neural correlates underlying map plasticity of the motor cortex. In order to utilize this knowledge to enhance

recovery following injury, it is essential to understand which neural changes have behavioral and functional relevance.

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Chapter 1:
Introduction

In this introductory chapter, a thorough background of the motor cortex, motor control, and plasticity within the motor cortex will be presented. This will be followed by the experimental questions and specific aims this dissertation addressed.

ORGANIZATION OF THE MOTOR CORTEX

Before the modern scientific age, the basis of voluntary movement was unknown. For example, Aristotle wrote "The seat of the soul and the control of voluntary movement - in fact, of nervous functions in general, - are to be sought in the heart. The brain is an organ of minor importance." However, by the late 1800s scientists such as John Hughlings Jackson reaffirmed the brain as the seat of motor action: "The convolutions of the brain must contain nervous arrangements representing movements. There is nothing else they can represent except movements and impressions." [1]. Fritsch, Hitzig and soon after, Ferrier, were the first to experimentally validate the importance of the brain for motor actions: by passing current into the brain of dogs, movements on the contralateral hemisphere were evoked [1]. Sherrington was able to convincingly demonstrate that the motor cortex of mammals could be isolated to a small strip of cortex (the pre-Rolandic fissure), and that other areas evoking movements did so via interconnections with this part of cortex [2]. However, it was Penfield - through

his detailed stimulation studies in human patients - and Woolsey - replicating such studies at higher resolution in primates - who created the popular and widespread topographical maps of motor cortex represented in textbooks today (Fig 1, taken from *Neuroscience, 2nd Ed.*) [3-6].

Modern techniques to map the motor cortex rely on intra-cortical microstimulation, wherein a brief burst of current is applied to neurons in layer V and the muscle groups recruited at the minimal threshold are recorded across the entire motor cortex [7]. A typical example of a motor map evoked through ICMS in the rat is shown (Fig 2). Body parts evoked through stimulation of the motor cortex of rats include hindlimb, forelimb, neck, whisker and jaw. While the organization of these large body parts is fairly stereotypic across animals, within these motor areas there is greater variability. For example, within the forelimb motor area, the different parts of the forelimb - shoulder, elbow, wrist and digit movements – can be evoked at varying locations in different animals.

When first utilized, ICMS was believed to directly activate corticospinal tract neurons, resulting in a motor map that was simply a direct template of the corticospinal tract (CST) projection to the spinal cord [8]. However, it was soon discovered that the neural activation pattern following ICMS is not restricted to the current spread around the electrode. Instead, intracortical stimulation protocols activated many neurons through horizontal intracortical excitatory connections, and that this indirect activation is necessary to evoke muscle responses [9, 10]. Thus motor maps derived via ICMS are now believed to reflect both intra-cortical connectivity and corticospinal tract innervation patterns [11].

One indication that these maps reflect physiological changes within the cortex is that they are highly dynamic. Maps can change rapidly based on anesthetic level (unpublished observations), limb position and even the order locations within the cortex are mapped [12, 13]. Of course, it has been repeatedly shown that single units themselves are highly dynamic, changing with limb position and spontaneously over time [14, 15]. Thus, the dynamic nature of these maps may reflect the dynamic nature of the motor cortex itself, and highlights the complexity of computations the cortex engages in for motor control.

MOTOR CONTROL

Motor control describes how the nervous system plans and executes complex movements automatically, smoothly and with relatively little error. In 1889, Jackson wrote "To speak figuratively, the central nervous system knows nothing of muscles, it only knows movements…there are, we shall say, thirty muscles of the hand; these are represented in the nervous system in thousands of different combinations – that is, as very many movements" [16].
Amazingly, more than 100 years after this statement, there is still a debate as to whether the motor cortex represents "muscles" – i.e., low-level aspects of motor control - or "movements" - higher-level aspects of motor control (Fig. 3).

As with most such debates, the answer probably lies somewhere in the middle. There is much evidence to suggest that the motor cortex codes for both high-level as well as low-level aspects of motor control [17]. Studies using spike

triggered averaging in awake, behaving primates have shown that some neurons are associated very specifically to single muscle groups, while others are associated with many different muscle groups in a much more complex fashion [18]. In fact, many single unit studies have demonstrated neuronal activity associated with almost every aspect of movement control, from low-level factors like muscle groups and joint position, to higher level conceptual factors such as velocity, direction, force and even the end-point location [19-24].

It is possible that the type of motor strategy utilized (low vs. high level) is contextually dependent. For example, when one wants to reach out and grab a pen, only the end-point location is important; the nervous system needs to translate sensory information of that location backwards to a sequential and coordinated series of muscle group activations and inhibitions, a computation known as inverse processing [25]. While this movement could occur in any number of ways (as only the final location is important), in practice, these movements are executed fairly stereotypically in both humans and primates, suggesting certain algorithms encoding high-level aspects of movement, such as minimizing end-point variability, entropy or energy, are used by the cortex to generate these movements[19].

On the other hand, there are types of movements in which the entire motor process needs to be carefully controlled. Examples might include golfing, bowling or playing a musical instrument, where tiny variations during any part of the movement sequence will dramatically affect the desired outcome. These types of movement often require sustained practice, with error correction/learning playing

an important role in the final performance outcome. These types of movement suggest the cortex can also control very low-level aspects of movements, such as joint position, muscle force, etc, though of course there is much debate as to whether or not this is true [26, 27].

Normal methods of ICMS seem to evoke only the lowest level of computation, that of individual muscle groups. However, it was postulated that, by prolonging the duration of intra-cortical micro-stimulation, a more complex behavioral repertoire of movements could be elicited [28]. In fact, a large array of complex movements was mapped out across motor and premotor cortex and parts of parietal cortex of both old and new world monkey species [29-31]. These movements were complex in that they existed across multiple joints and involved multiple muscle groups. More interestingly, a single locus of stimulation always resulted in exactly the same end-point location of the limb, regardless of the initial start point, often evoking completely opposite muscle groups. Thus, it was found that intracortical microstimulation could evoke both low-level correlates of movement such as muscle group representation, as well as higher-level correlates of movements such as end-point location in space, simply by changing stimulation duration.

PLASTICITY FOLLOWING SKILLED MOTOR LEARNING

At its broadest, motor learning encompasses any motor adaptation that occurs to an animal's behavioral repertoire. This includes operant-conditioned

motor responses (learning to button press upon the correct stimulus, for example), rapid adaptations to novel forcefields (i.e. anti-gravity simulations or experimentally induced vestibular nerve lesions), and finally the acquisition of novel sequences of skilled movements (for example learning to play the piano, hit a golf ball or shoot a basketball) [25]. All of these different types of motor learning can be divided into similar component parts: a goal for the motor output; a plan for motor execution based on the desired goal; execution of said movement; a means to detect the amount of error between the goal and the actual movement; and the ability to modify future motor plans based on previous error.

Different motor areas – cerebellum, striatum and motor cortex – seem to play specific roles in this learning process. Selection and modification of motor goals, such as may happen during much of operant conditioning, probably involve cortico-striatal networks [32, 33]. Adaptations to changing forcefields require strong proprioceptive input, online error detection and correction based on prediction– all processes known to depend heavily on the cerebellum [34-36]. The storage of novel skilled movements or sequences of movements primarily involves the ability to modify and perhaps even instantiate new motor plans – a process believed to occur within motor and/or premotor cortex [37-40].

As it is difficult to engage in truly skilled motor behavior within the confines of an MRI machine, most neural measures of motor learning in humans focus on the learning of complex finger tapping patterns [37, 38, 41]. These studies show that skilled training results in an expanded cortical representation of the hand area in M1 [37, 42]. Interestingly, skilled musicians recruit far less of

their motor and premotor cortices during simple tasks than non-musicians, suggesting long-term skilled practice results in a more efficient coding as well [43, 44]. Disruption of neural activity within the motor cortex either before or directly after motor training results in a loss of the behavioral effects of practicing, without impairing behavior during the practice session itself [45, 46]. This suggests that plastic changes that occur within the motor cortex following a behavioral training session are essential for longer-term storage and/or retention of the movement sequence learned.

Similarly to these human reports, studies conducted in both primates and rats, have shown that skilled training of digits (primates) or distal forelimb (rats) result in an expanded representation of the trained body part within the motor cortex as measured by ICMS [40, 47]. This plasticity only occurs in the context of skilled motor training, as distinct from repetitive motor activity [39]. Further, this reorganization of the motor cortex is accompanied by an increase in synapses specific to that same area [48]. Interestingly, detailed time course analyses have demonstrated that both the changes in synaptic density and the reorganization of the motor cortex occur only in the later stages of motor learning and not in the early stages [39]. This may be because changes in synaptic efficacy via LTP occur in the early stages of motor learning [49, 50]. These results suggest that the synaptic and motor map reorganization that occur towards the end of the learning phase may instead reflect an instantiation of the behavior into a long-term representation within the motor cortex [39], though more research is needed to bear this out.

PLASTICITY FOLLOWING PERIPHERAL INJURIES

Map plasticity within the motor cortex was first described following a peripheral motor nerve lesion [51, 52]. In these studies, a lesion of the facial nerve of rats abolished all ICMS evoked whisker movements from the motor cortex. Within hours of this lesion, stimulation within the same area began to evoke movements of cortically adjacent body parts, and this reorganization remained when assessed several weeks after the initial lesion [52, 53]. This finding extended earlier work demonstrating somatosensory plasticity following peripheral de-afferentation in primates [54], suggesting such plasticity was common to many areas of cortex. Indeed, further studies demonstrated similar cortical reorganization in auditory and visual cortices following micro-lesions to the cochlea or retina respectively [55-57]. In all of these cortical regions, both rapid as well as longer-term plasticity have been described, suggesting a common neural mechanism underlying peripheral-lesion induced plasticity across cortical regions.

PLASTICITY FOLLOWING CNS INJURY

One of the motivations of studying neural plasticity is the potential clinical application of enhancing functional recovery following brain injuries. Strokes are the most common traumatic brain injury [58]. Much work has been done to

understand how the brain responds following such ischemic injury, and in what ways that response is comparable to phenomenon observed following peripheral injury or normal motor learning [59-62].

Using fMRI, studies in rats have demonstrated several phases of neural plasticity following ischemic injury [63]. Immediately post-injury, peri-lesional areas of the sensorimotor cortex show a decrease in neural activity associated with movements [63]. This has been associated both with the after-effects of hypoxia as well increased inhibition within those brain regions that may reduce excitotoxic and/or reperfusion injury. Several days following this immediate down-regulation of activity, much of the cortical activation for movement shifts to the contralesional cortex [63, 64]. Similarly, ipsilateral lesions of motor cortex result in enhanced plasticity within contra-lesional motor cortex, as well as enhanced skilled motor acquisition of the forelimb contralateral to the intact hemisphere [65]. It has been postulated that this shift in cortical activation to contralesional cortex, while perhaps beneficial for behavioral ability in the short-term, in the long-term leads to learned disuse of the ipsilesional cortex and a reduction in peri-lesional plasticity and total functional recovery [66]. Ideally, therefore, cortical activation is shifted back to perilesional cortex [63, 64] as subjects recover. The degree to which perilesional cortex is reactivated strongly correlates with the functional recovery attained [64].

Results from experiments investigating plasticity in ICMS-evoked maps generally agree with the fMRI data described above. Following a small lesion isolated to the forelimb area of M1 in motor cortex, much of the remaining

perilesional cortex becomes silent [47]. However, rehabilitation training resulting in functional recovery causes that peri-lesional cortex to become reactivated [47]. With larger lesions of the entire forelimb area, rehabilitation training leads to ectopic reorganization and expansion of forelimb representations at distant sites [67, 68].

ACETYLCHOLINE AND CORTICAL PLASTICITY

The predominate source of acetylcholine in the cortex is derived from neurons originates from the nucleus basalis, a structure located within the basal forebrain [69]. This basal forebrain cholinergic system plays a key role in regulating many different forms of plasticity in many different cortical regions [70, 71]. Iontophoresis of acetylcholine paired with visual stimulation results in long-lasting modifications to the receptive fields of those neurons [72], and map plasticity within the visual cortex [73]. Pairing of nucleus basalis stimulation with tones results in cortical map reorganization of the auditory cortex specific to the paired tone [74]; further, the receptive field changes that occur following such pairing mimic those seen after pairing with behaviorally relevant paradigms [75-78].

Studies in which the basal forebrain cholinergic system was lesioned demonstrate the necessity of this system for many forms of plasticity. Cholinergic lesions [79], resulted in impaired somatosensory barrel plasticity following many different whisker pairing and clipping paradigms [80-83]. Lesions of the basal

forebrain cholinergic system impair skilled motor learning and motor recovery following cortical injury, and abolish the associated cortical plasticity that normally occurs following such paradigms [68, 84]. The consistent finding across many different paradigms demonstrating the necessity of acetylcholine for cortical plasticity led to the notion that the basal forebrain cholinergic system was a required substrate for cortical plasticity in general. That speculation, however, was brought into question by a recent study demonstrating that cortical plasticity following partial cochlear lesions would occur even following destruction of the basal forebrain cholinergic system [85, 86]. This finding suggested that a new hypothesis regarding the role of acetylcholine in cortical plasticity was warranted.

TIME COURSE OF PLASTICITY

ICMS-derived motor maps reorganize following skilled motor learning, peripheral motor nerve injuries and central nervous system injuries. However, the time course of plasticity following these various experimental paradigms of plasticity varies quite extensively, and is suggestive of different neural correlates that may be associated with them (Table 1). Peripheral and central nervous system injuries lead to some forms of plasticity that occur extremely rapidly (within hours) following the injury, often with no behavior required on the part of the subject [51]. On the other hand, reorganization following skilled motor training occurs only after substantial motor training repeated over time [84]. Rehabilitation training following a central nervous system injury results in

plasticity on the order of weeks rather than days [68]. This differing time course may suggest the types of neural plasticity that may accompany these different forms of plasticity.

In sum, outstanding questions regarding motor map plasticity include: what is the role of the basal forebrain cholinergic system in modulating cortical plasticity; what is the relationship of motor map plasticity and motor behavior; and what aspects of motor map plasticity can be used to enhance functional recovery following nervous system injury?

EXPERIMENT PROPOSAL

In this thesis, we have investigated the neural processes underlying cortical reorganization of the motor cortex within many different experimental paradigms and at multiple different levels of organization. As discussed, there are three well recognized antecedents to cortical reorganization: injury, behavioral training, and the interaction resulting from behavioral training following injury. In this thesis, we will provide evidence to support the following hypothesis: **cortical map plasticity can occur through different neural mechanisms depending on the experimental manipulation, and only some of these correlate with behavioral outcomes.** Three general questions relating to this hypothesis were addressed in this dissertation:

1. **What is the role of the basal forebrain cholinergic system in regulating cortical map plasticity?** The basal forebrain cholinergic system is required for certain forms of map plasticity but not others. By comparing the requirements of this cholinergic system in multiple plasticity paradigms, we aim to understand in what circumstances it is and in what circumstances it is not required.

2. **What are the behaviorally relevant aspects of motor cortical plasticity?** While much evidence shows that cortical plasticity occurs following behavioral training, either with or without nervous system injury, such plasticity also occurs following peripheral and central nervous system injuries without any further behavioral training. Thus, cortical plasticity is a better measure of changes within the motor cortex, as opposed to a specific measure of behaviorally relevant change. We will investigate if there are measures of motor cortical plasticity that more directly correlate with behavioral outcomes.

3. **Is axonal plasticity associated with behavioral recovery following motor cortical injury?** It is important to understand the neural correlates underlying cortical plasticity following recovery after brain injury. Many researchers have described axonal plasticity of cortico-striatal and intracortical neurons following such brain lesions. However, no one has yet investigated whether corticospinal tract plasticity occurs at the level of

the spinal cord in association with functional recovery after brain injury. We will investigate this aspect of cortical plasticity in a previously characterized paradigm.

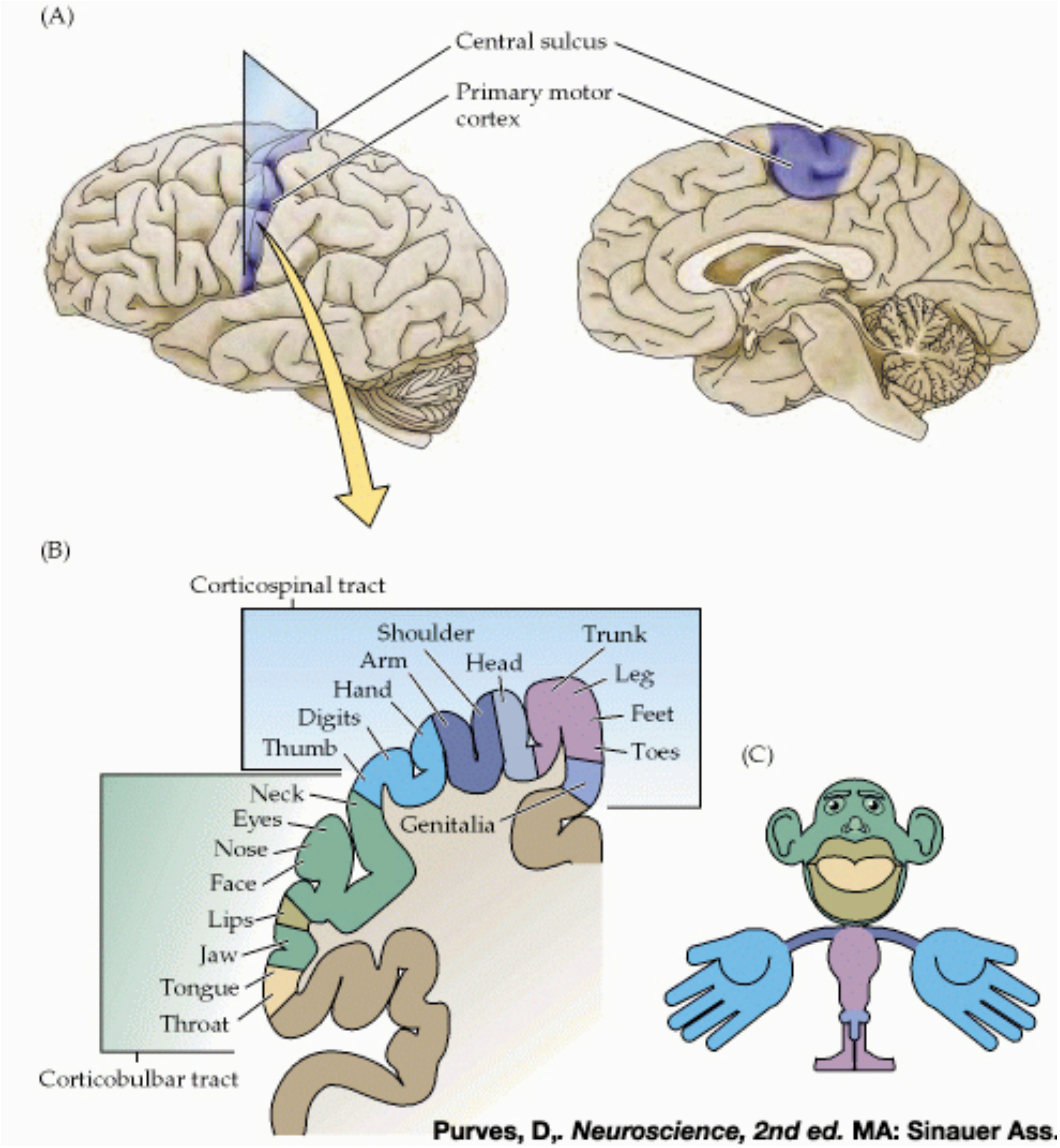

Figure 1.1 – Textbook Depiction of Motor Map

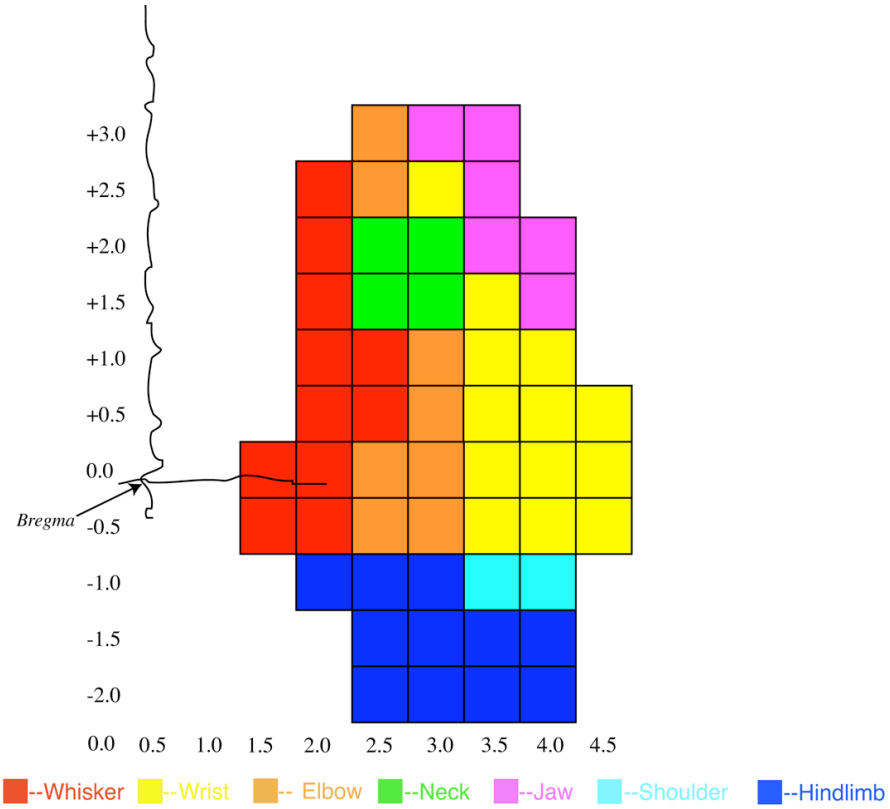

Figure 1.2: Motor Map Generated Using Intracortical Microstimulation in Rat Motor Cortex. Within rat motor cortex are two characteristic forelimb areas, distinguished primarily by anatomic location. The "primary" forelimb area is the caudal forelimb area (CFA). This area is separated by neck and jaw from the rostral forelimb area (RFA

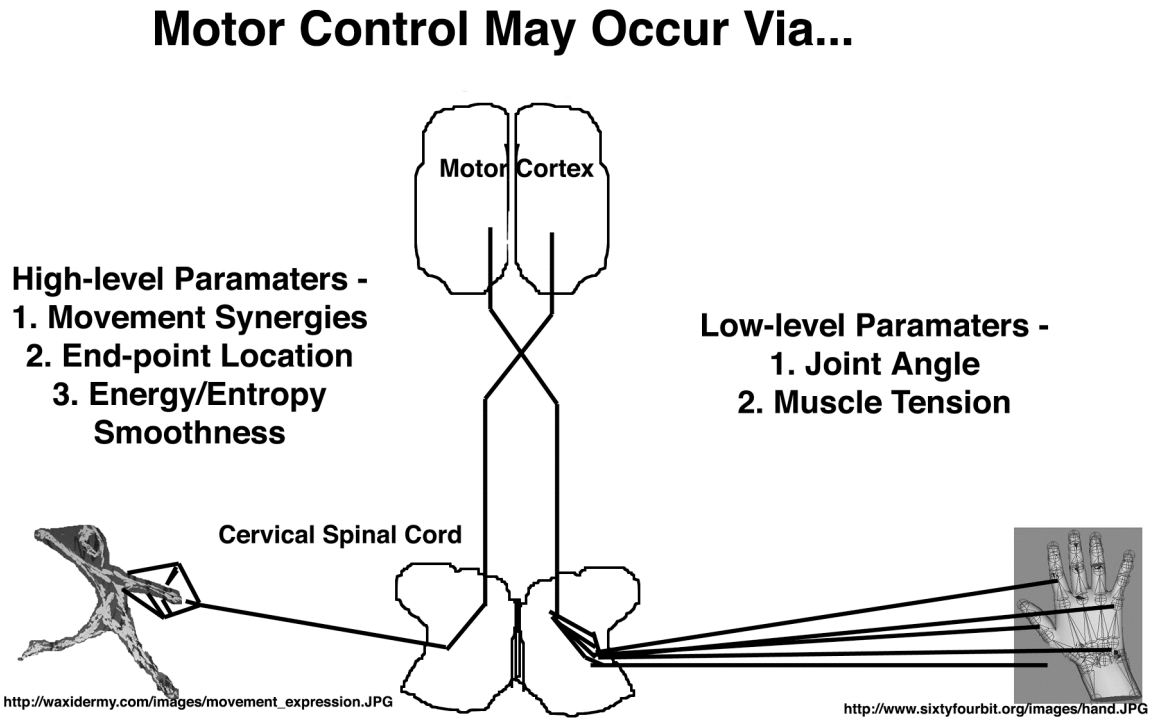

Figure 1.3: Motor Cortex May Utilize Different Strategies for Motor Control. The above diagram illustrates how the motor cortex may utilize low-level control over individual muscles and joints, or high-level control over entire movements, movement synergies, and other aspects of movement including smoothness, energy or entroy.

Table 1.1: Comparison of Various Paradigms Eliciting Map Plasticity

	Peripheral / Central Nervous System Injury	**Skilled Training**	**Skilled Training Following CNS Injury**
Time Course of Plasticity:	Minutes - Hours	Days - Weeks	Weeks - Months
Behavioral Requirement:	No	Yes	Yes
Cholinergic Dependence:	No	Yes	Yes
Hypothesized Neural Correlates:	Dis-inhibition within motor cortex	Synaptic plasticity (LTP, new spines)	Axonal and dendritic plasticity, synaptic plasticity, LTP

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Chapter 2:
The Basal Forebrain Cholinergic System is Selectively Required for Behaviorally Mediated Cortical Map Plasticity

ABSTRACT

Prior studies have indicated that basal forebrain cholinergic mechanisms are essential for mediating cortical map plasticity associated with skilled motor learning. Other studies, however, have demonstrated that cholinergic mechanisms are not required for map plasticity following the ablation of peripheral sensory receptors. The present study sought to resolve this apparent discrepancy by testing the hypothesis that the basal forebrain cholinergic system is specifically required for mediating plasticity associated with behavioral experience but is not essential for plasticity occurring in the absence of behavioral experience. The present findings support the proposed hypothesis by demonstrating that selective lesions of the basal forebrain cholinergic system do not disrupt cortical reorganization that occurs independent of behavioral experience, such as that following peripheral nerve lesions. Further, when animals undergo a peripheral nerve lesion followed by skilled motor training, cholinergic lesions selectively block only the plasticity associated with skilled motor training. These findings suggest that the basal forebrain cholinergic system mediates specific forms of plasticity that are associated with complex cortical processing underlying behavioral experience.

INTRODUCTION

Extensive evidence has implicated the basal forebrain cholinergic system in many forms of cortical plasticity. Pairing a sensory event with either stimulation of the basal forebrain, or with an acute application of acetylcholine, selectively induces cortical plasticity associated specifically with the paired stimulus [1-3]. Further, specific lesions of the basal forebrain cholinergic system (BFCS) prevent cortical plasticity elicited in many experimental paradigms [4-8]. Taken together, these results have led to the prevailing view that acetylcholine plays an essential role in mediating cortical map plasticity. This hypothesis was directly challenged by a recent study clearly demonstrating that cortical map plasticity could occur within auditory cortex following cochlear injury, even following complete destruction of the basal forebrain cholinergic system [9]. Despite some caveats [10], their study indicated that a revised theory regarding the role of basal forebrain cholinergic mechanisms in modulating cortical map plasticity is needed.

We hypothesize that acetylcholine is necessary only for behaviorally driven cortical map plasticity; that is, plasticity that arises as a result of attentionally-demanding stimuli requiring higher order cognitive processing. In this model, acetylcholine does not gate all forms of plasticity, but rather plays a necessary role in modulating the cognitive strategies and underlying neural activity resulting in map plasticity following the acquisition of skilled behaviors. This theory is supported by numerous electrophysiological and behavioral studies demonstrating that acetylcholine plays a role specifically in higher-order

cognitive tasks mediating selective attention, learning and memory [11-17]. Further, little evidence implicates acetylcholine in mediating neural changes independently of behavior.

To test this revised hypothesis regarding cholinergic mechanisms in cortical plasticity, we used the motor cortex as a test system, since plasticity in this cortical domain has been demonstrated in both behavioral and non-behavioral contexts. Early studies demonstrated plasticity within the motor cortex in the absence of behavioral activity [18]. Following transactions of the facial motor nerve [19], extensive reorganization of motor representations occur where adjacent areas within the motor cortex, such as eye and forelimb, expand into the area previously occupied by whisker cortex. This phenomenon occurs almost immediately following the lesion (within 2 hours) and persists for at least several weeks after the initial injury [20, 21]. Further, this plasticity occurs even while animals remain under anesthesia, indicating that attentional or other cognitive faculties are not required.

In addition to non-behaviorally mediated forms of cortical plasticity elicited by acute, lesion-evoked changes in neuronal activity, other studies have demonstrated that motor map plasticity can occur in association with skilled behavioral acquisition. Studies in both primates and rats have shown that skilled reach training with the distal forelimb results in the reorganization of cortical motor representations, with a significant expansion of the trained body part within [22, 23]. This form of motor map plasticity has also been demonstrated in the

context of rehabilitation training and functional recovery following following brain injury in rodents, monkeys and humans [8, 24-27].

In the present study, we assessed the role of the basal forebrain cholinergic system in mediating both behavioral and non-behavioral forms of motor map plasticity. We first investigated the immediate and long-term effects of cholinergic-specific lesions of the basal forebrain on the induction of cortical motor map plasticity following a facial motor nerve transection (Fig. 1A-B). Next, in order to simultaneously study both behavioral and non-behavioral forms of plasticity, animals with a facial nerve lesion were given four weeks of skilled motor training (Fig. 1C). Thus, the requirement of cholinergic inputs was concurrently assessed for both behaviorally and non-behaviorally mediated cortical reorganization in the same animal, allowing us to test the hypothesis that cholinergic inputs are specifically required for cortical map plasticity associated with behaviorally-mediated forms of motor map plasticity.

RESULTS

A total of 65 animals were used in three separate experiments (Fig. 1): i) Initial experiments (10 rats) were carried out to define rapid changes in motor representations induced by acute facial nerve transactions and to determine whether cholinergic mechanisms were required for mediating short-term cortical plasticity in the absence of active behavior. ii) A second experiment (23 animals) examined whether cholinergic mechanisms were necessary for long-term

plasticity of motor representations following a facial nerve transection. iii) The final experiment (32 animals) examined the effects of cholinergic depletion on cortical plasticity mediated by non-behavioral (facial nerve transection) and behavioral (skilled motor training) paradigms within the same animals.

Immediate Plasticity Following Facial Nerve Lesion

In an initial experiment, we sought to characterize short-term plasticity of cortical motor representations following a facial nerve transection and to determine if cholinergic mechanisms are required for enabling cortical plasticity in a paradigm devoid of behavioral activation. Based on prior studies [18, 21], it was postulated that short-term plasticity following a facial nerve transection should involve a loss of sites evoking vibrissa movements and a subsequent increase in sites evoking neck or forelimb movements, with a simultaneous reduction in stimulation threshold for evoking neck and forelimb movements.

An important caveat in the ICMS mapping paradigm not considered in the initial reports of short term plasticity following a facial nerve transection was the possibility that an increase in the number of sites evoking neck or forelimb movements may not truly reflect plasticity but may be a result of unmasking of preexisting movement patterns. Typically, ICMS maps are derived by defining a topographic pattern of characteristic movements evoked with a minimal stimulation current. Increasing currents beyond this minimal threshold may, on occasion, evoke movements from multiple body parts. Thus, it is possible that an immediate change in motor representation following a facial nerve transection

(which prevents vibrissa movements from being evoked by any magnitude of stimulus), may only reflect the unmasking of movement patterns that would have been elicited using higher stimulation currents. While such "unmasking" can be considered a form of plasticity, it is unlikely to be one requiring any underlying neural changes and thus not an ideal paradigm to test our hypothesis. To address this potential caveat, and enable the characterization of an actual reorganization beyond simple unmasking following a facial nerve transection, we characterized all movements evoked at vibrissa-responsive sites using stimulation currents up to a maximum of 200 μA. Thus, "prelesion" measurements of neck and forelimb representations included vibrissa sites where neck and/or forelimb movements could be elicited using higher stimulation currents.

To determine whether cortical plasticity following a facial nerve lesion required a functional cholinergic system, five animals received bilateral injections of the cholinergic specific toxin, 192-IgG-Saporin (SAP) [28]. Our prior studies have demonstrated that injections of this immunotoxin directly into the nucleus basalis selectively deplectes cortical cholinergic innervation by more than 98 % while not affecting noncholinergic cell populations within the basal forebrain {Conner, 2005 #347; Conner, 2003 #103 Additional animals (n=5) recieved comparable injections of vehicle (artificial cerebrospinal fluid (ACSF)) In the present study, administration of the 192-IgG SAP resulted in a loss of AChE-positive cholinergic innervation to the sensorimotor cortex (Fig. 2). Cortical plasticity was assessed 6 weeks following SAP or vehicle injections.

To assess short term plasticity following a facial nerve transection, animals were anesthetized and detailed maps of the motor cortex were made bilaterally using intracortical micro-stimulation techniques as previously described [7], but including the additional precaution of characterizing potentially masked neck and forelimb movements as described above and in the Methods section (Fig. 3A-B). After the initial mapping was completed, the facial motor nerve was exposed and transected bilaterally. Animals were maintained under anesthesia in the stereotactic device for an additional 2 hours and then were remapped to identify rapid changes in cortical motor representations (Fig. 3C).

Under normal conditions, vibrissa movements are evoked in a region located between 1.5 and 2.5 mm lateral to bregma, and bordered laterally by forelimb, neck, and occasionally hindlimb, areas. At some sites evoking vibrissa responses, higher stimulating currents also elicited movements of other body parts, primarily elbow or neck (Fig. 3B). This "overlapping" area was included in the pre-lesion measurements of neck and forelimb size in order to differentiate unmasking of these representations with an expansion into a new cortical area. We postulated that plasticity following a facial nerve lesion would be manifested either as an increase in the total area of ICMS-evoked neck or forelimb movements compared to the pre-lesioned state, or else as a decrease in the stimulus threshold of pre-existing movements.

We found a significant increase in the area evoking neck movements following a facial nerve lesion, and no change in the area evoking forelimb movements (Fig. 3C-E). The pre-lesion area evoking distal forelimb movements

(including the area elicited from vibrissa cortex) was 4.9 ± 0.3 mm^2 in control animals, and 4.7 ± 0.2 mm^2 in SAP lesioned animals, a non-significant difference ($p = 0.8$). Following a facial nerve lesion, there were no significant changes across either group in the area evoking distal forelimb movements (Fig. 3D; AVONA $p = 0.7$). The pre-lesion area evoking neck movements was 1.0 ± 0.3 mm^2 in control animals, and 0.9 ± 0.2 mm^2 in SAP lesioned animals ($p = 0.8$). Following a facial nerve lesion, the area evoking neck movements increased to 1.6 ± 0.2 mm^2 in control animals, and 1.9 ± 0.3 mm^2 in SAP lesioned animals, in both cases a significant expansion (total ANOVA $p < 0.001$; $p < 0.01$ for both individual groups on a paired t-test; Fig 3E). There was no statistical difference in the amount of expansion between groups ($p = 0.2$ between group post-hoc comparison). This expansion of the area evoking neck movements occurred both within vibrissa area as well as at other sites across motor cortex (Fig. 3C).

The overall stimulation thresholds utilized for both neck and forelimb were compared using a one-sided paired t-test to determine whether stimulation thresholds diminish following a facial nerve lesion (Fig. 3F). The mean stimulation threshold utilized for evoking neck movements decreased by 12 ± 6 uA following a FMN lesion ($p < 0.05$). There was no difference between the SAP vs. ACSF groups ($p = 0.3$). The mean stimulation threshold utilized for evoking forelimb movements did not decrease following a facial nerve lesion (ANOVA $p = 0.8$). Because the decrease in stimulation threshold was specific to the evoked neck movements, general state-changes (such as cortical damage or varying anesthesia levels) are unlikely to be a factor resulting in this change.

Long Term Facial Nerve Plasticity

It has been previously demonstrated that both immediate and long term forms of cortical map plasticity occur following peripheral nerve lesions in the motor cortex [20, 21]. While the previous experiment clearly demonstrated that basal forebrain cholinergic mechanisms are not required for mediating short-term motor map plasticity following peripheral nerve lesions, it was still necessary to examine the role of cholinergic systems in mediating long-term plasticity following a facial nerve lesion, because it is not known whether the neural correlates underlying short and long-term plasticity are the same. To accomplish this, 11 animals received SAP injections into the nucleus basalis magnocellularis (NBM) and 12 animals received comparable ACSF injections. Two weeks after the injection procedure, animals were given bilateral facial motor nerve lesions and were returned to their cage for one month. Following this period, animals were mapped bilaterally using ICMS techniques. At the end of the mapping procedure, all animals were sacrificed for histological verification of the cholinergic lesion using previously described quantitative procedures. Quantitative histological analysis revealed that 5 of the 11 SAP lesioned animals had complete cholinergic lesions in only one hemisphere. In those animals, only maps derived from the completely lesioned hemisphere were used in further analyses. Importantly, there were no statistical differences in the area of neck or forelimb between the data from unilaterally and bilaterally SAP lesioned animals

($p = 0.4$ or above for all comparisons) so data from these two groups was pooled for further analysis.

As predicted from the previous short-term plasticity experiment, the facial nerve lesion resulted in a significant long-term increase in the area of the neck representation, with no significant differences in this expansion between the SAP and ACSF groups (overall ANOVA $p < 0.01$; Fig. 4A). The average pre-lesion area evoking neck movements (pooled data from experiment 1) was 0.96 ± 0.2 mm^2. The immediate effect of a facial nerve lesion (combining both SAP and control groups from experiment 1 because they did not differ) was an expansion of the neck area to 1.9 ± 0.2 mm^2. This reorganization was maintained at the prolonged, 6-week time point after the facial nerve lesion in both the control as well as the cholinergically depleted animals. In control (ACSF-treated) animals, the area evoking neck movement after 6 weeks of recovery was 1.7 ± 0.2 mm^2, while in SAP lesioned animals the area evoking neck movement was 1.9 ± 0.2 mm^2. Neck area significantly differed when comparing pre-lesion maps to all post-lesion maps, including both short and long-term time points (Tukey-Kramer post-hoc test, $p<0.05$). None of the lesioned maps differed significantly from one another on post-hoc testing.

As observed in the short-term experiment, there was no difference in the area of the caudal forelimb area following a more chronic facial nerve injury (Fig. 4B). The pre-lesion caudal forelimb area (averaged across SAP and ACSF groups) was 4.8 ± 0.2 mm^2, and the 2 hours following the facial nerve lesion was 4.7 ± 0.2 mm^2. Six weeks after facial nerve lesions, the size of the forelimb

region did not change significantly in either ACSF (5.1 ± 0.2 mm^2) or SAP-lesioned groups (5.2 ± 0.2 mm^2) (ANOVA p = 0.2 between groups).

Stimulus thresholds required to elicit either neck or forelimb movements did not change six weeks following the facial nerve lesion, as compared to either the pre-lesion or short-term post lesion groups (ANOVA p = 0.3 for neck; ANOVA p = 0.8 for forelimb). We postulated that, over the long-term, the largest decrease in stimulation thresholds would be seen specifically in the area previously occupied by vibrissa. To test this possibility, the neck and forelimb areas were divided into a medial portion (no further lateral than 2 mm from bregma), and a lateral portion. Based on previous maps, this division ensured that almost all of the movements elicited medially would have existed in cortical areas that previously elicited vibrissal movements. The same analysis was applied to the pre-lesion and short-term post-lesion groups of experiment 1, as a basis for comparison. There was a statistically significant difference across all groups between the medial vs. lateral stimulation thresholds ($p<0.0001$ for both neck and forelimb); however, the magnitude or ratio of this difference did not differ when comparing pre-lesion, short-term lesion and long-term lesion groups for either forelimb (ANOVA p = 0.8) or neck (ANOVA p = 0.3) regions (Fig. 4C-D).

Acetylcholine and Behaviorally Mediated Plasticity

The previous experiments, like those of Kamke [9], clearly demonstrate that cortical plasticity following a peripheral nerve lesion occurs independently of a functional cholinergic system. However, to convincingly test the hypothesis that

acetylcholine may regulate plasticity differently depending on the behavioral dependence of the plasticity, and may actually do so within the same cortical system and at the same time in a single animal, we performed an additional experiment (Fig 1C). First, 32 animals underwent either SAP (n=16) or ACSF (N=16) injections within the NBM. After two weeks (a period sufficient for the destruction of cholinergic afferents to the cortex) {Conner, 2003 #103}, all 32 animals underwent bilateral facial nerve lesions. Animals were allowed an additional week to recover in their home cages before undergoing skilled motor training using the forelimb reaching task [7]. Previous studies have demonstrated that skilled motor training results specifically in plasticity within forelimb areas of the motor cortex, but that this plasticity is blocked by cholinergic lesions [7]. Based on our previous findings including those reported in this paper, we postulated that, following a lesion of the BFCS within a subject, plasticity resulting from a facial nerve lesion would not be blocked, but that plasticity in the context of skilled motor training would be disrupted within the same subject.

As previously demonstrated, cholinergic lesions of the NBM projection to the cortex resulted in a deficit in behavioral acquisition on the skilled forelimb reach task (Fig. 5; ANOVA $p<0.0001$). The two groups differed across all days except day 1 according to post-hoc t-tests, and there was an average 51 % decrease in motor performance across the final three days of testing ($p<0.01$). The presence of a facial motor nerve lesion did not reduce acquisition of skilled reaching performance in control (ACSF-injected) animals compared to our

previous observations and did not alter the impairment in forelimb motor performance that results from a SAP lesion [7].

The facial motor nerve lesion did not change the predicted expansion of caudal forelimb area that occurs following skilled motor training in intact animals (Fig. 6A) [7]. In subjects with facial nerve lesions, skilled motor training resulted in an expansion of the area of caudal forelimb area compared to animals that underwent a facial nerve lesion with no skilled forelimb training (ANOVA $p < 0.01$; Fig. 6A). The area evoking forelimb movements following a facial nerve lesion alone (average data of both SAP and ACSF groups from experiment 2) was $5.2 \pm 0.1\ mm^2$. Following skilled motor training, the area evoking forelimb movements significantly increased by 15% to $6.0 \pm 0.2\ mm^2$ ($p<0.05$; Tukey-Kramer post-hoc tests indicates significance increase compared to all other groups, Fig. 6). However, animals that received a facial nerve lesion, skilled forelimb reach training, and a SAP lesion failed to exhibit the expected expansion in the size of the caudal forelimb area: size $4.8 \pm 0.2\ mm^2$, an amount that differed significantly from animals that underwent skilled forelimb reach training in the absence of a SAP lesion ($p<0.05$, Tukey-Kramer post-hoc). Thus, cholinergic lesions specifically block cortical motor map plasticity associated with behaviorally-dependent map plasticity.

Importantly, within the SAP-lesioned group of animals, plasticity of the neck area following the facial nerve lesion still occurred (overall ANOVA $p < 0.01$; Fig. 6E). There was no difference in the extent of neck area plasticity comparing animals with a facial nerve lesion alone, a facial nerve lesion plus

skilled forelimb training, or a facial nerve lesion plus training plus SAP lesion (Tukey-Kramer post-hoc test shows a significant difference across all groups as compared to the pre-lesion group, but no other statistical differences).

DISCUSSION

The results of ths study have clearly demonstrated that plasticity within the motor cortex following a peripheral nerve lesion occurs even in the absence of a functional cholinergic system, thereby confirming Kamke's findings of similar cholinergically-independent plasticity in auditory cortex. Together, these results generalize the observation that, following a peripheral injury that induces cortical plasticity, acetylcholine is not required. More importantly, by developing a paradigm to simultaneously study multiple forms of plasticity multiple forms of plasticity within the same animal and modality, we have demonstrated that aspects of plasticity driven specifically by behavioral experience (skilled motor training) are disrupted by a cholinergic lesion, but that aspects of plasticity elicited in a context independent of behavior (the facial nerve lesion), are unaffected by a cholinergic lesion. As both cholinergic and non-cholinergic dependent forms of plasticity occur in the same animals, within the same sensory modality and therefore with identical extent of cholinergic depletion, these findings strongly support the proposed hypothesis that the basal forebrain cholinergic system is involved selectively in behaviorally-driven forms of cortical plasticity (Fig 7).

Using this model, predictions are possible regarding the role of acetylcholine in different forms of plasticity. For example, there is evidence that aberrant plasticity following amputation of limbs is associated with phantom limb pain in some patients [29]. The results of the present study and that of Kamke would strongly argue that this plasticity is cholinergic-independent. Similarly, following central nervous system injuries there is often a dramatic reorganization of both proximal and distal brain areas that occurs both immediately and following a more prolonged time course after the injury [30]. Again, one would predict according to our model of cholinergic function that acetylcholine would play no role in this spontaneous remodeling that occurs in the absence of behavioral activation. However, those aspects of plasticity that are tied to rehabilitation-induced recovery following either central or peripheral injury would require a functional BF cholinergic system, according to this model [8]. It is possible that cholinergic mechanisms would selectively modulate cortical plasticity occurring when animals engage in behavioral tasks that require attentional mechanisms to direct cognitive focus to one or more aspects of the environment. Indeed, prior studies have demonstrated that selective cholinergic lesions primarily impair tasks in which attentional load is increased [31, 32].

It is important to note that prior studies [4, 6] have presented results suggesting that cholinergic mechanisms may be required for somatosensory map plasticity following peripheral nerve lesions, a finding potentially contradicting the premise and the results of the experiments reported here, as well as those of Kamke [9], and in opposition to our proposed hypothesis. However, those studies

were undertaken before the cholinergic specific toxin (IgG-Sap) was developed. Instead, they used lesioning techniques (electrolytic and excitotoxic lesions) that resulted in widespread and nonselective damage within the basal forebrain. These lesions were likely to damage, in addition to cholinergic neurons, GABA-ergic and peptidergic neurons providing afferent innervation to the entire cortex, glutamatergic and GABA-ergic neurons projecting to other different subcortical areas, and perhaps even destroy fibers of passage from critical ascending serotonergic, noradrenergic and dopaminergic cell populations. These GABA-ergic projections, by controlling the balance of inhibition within the cortex, may be an important factor in modulating plasticity following peripheral nerve lesions [33-35]. Further, depending on how the lesion was performed, fibers of passage from serotonergic, noradrenergic and dopaminergic projections may have been affected. It is therefore not possible to ascribe the impaired plasticity solely to the cholinergic lesion in those studies [10]. In fact, it has been previously suggested that the motor map plasticity caused by peripheral nerve lesions may be directly related to changes in the balance of inhibition within the motor cortex [33]. It may well be that the GABA-ergic projections from the basal forebrain, play an important role in modulating plasticity following peripheral lesions by directly controlling the balance of inhibition within the cortex [34].

This study therefore suggests that rapid motor map plasticity resulting from peripheral motor nerve injuries occurs through a set of neural changes distinct from cholinergic-dependent mechanisms that are engaged by prolonged behavioral engagement in a skilled motor behavior. These findings have

implications for neural treatment after nervous system injury. Our findings suggest that a focus on enhancing those aspects of cortical plasticity that are behaviorally relevant may be preferable for augmenting functional recovery following injury.

MATERIALS AND METHODS

Most of these methods have been discussed in more detail previously [7].

SAP Lesions

SAP lesions were carried out under ketamine/xylazine/acepromazine anesthesia. Intraparenchymal injections of either 192-IgG-saporin (SAP; Advanced Targeting Systems, San Diego, CA), diluted to a concentration of 0.375 mg/ml in artificial cerebrospinal fluid, or vehicle (artificial cerebrospinal fluid alone) were made using a Hamilton syringe. The following sites and volumes were injected: site #1 (0.3 µl each side), R/C = -1.4 mm, M/L = ±2.5 mm, D/V = -8.0 mm; site #2 (0.2 µl each side), R/C = -2.6 mm, M/L = ±4.0 mm, D/V = -7.0 mm.

Facial Motor Nerve Lesions

Facial motor nerve lesions were carried out similar to previously described [18, 19]. Following anesthesia induction, skin incisions were placed at a point approximately two-thirds of the distance from the ear to eye. The facial motor

nerve was dissected from fascia as it emerged from the parotid gland. Three branches of the facial nerve were transected in this experiment: the buccal, marginal mandibular and zygomatic branches, to ensure elimination of whisker movement. Further, in long-term experiments, a 2mm section of nerve was removed to ensure that no peripheral nerve regeneration occured.

Functional ICMS Mapping

Standard microelectrode stimulation techniques were used to derive maps of the motor cortex. Animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.) and received supplementary doses of the anesthesia mixture as needed. Pulled-glass stimulating electrodes (input impedance ~ .5 MOhm at 300 Hz) filled with 3 M NaCl were used. Microelectrode penetrations were made at 500 μm intervals at a depth of ~ 1800 μm (corresponding to cortical layers V–VI). Stimulation consisted of a 30 ms train of 200 μs duration monophasic cathodal pulses delivered at 333 Hz from an electrically isolated, constant-current stimulator (Axon Instruments, Union City, CA) under the control of a programmable pulse generator (AMPI, Jerusalem, Israel). Pulse trains were delivered 1.2 s apart, and evoked movements were examined with the animal maintained in a prone position and the limbs supported in a consistent manner. At each site, the current was gradually increased until a movement was detected (threshold current). If no movement was detected at 200 μA, the site was defined as “nonresponsive.” The size of the forelimb and neck

representations were determined by multiplying the number of responsive sites evoking a movement of the forelimb by 0.25 mm^2.

Skilled Motor Training

Motor training was carried out using single pellet retrieval boxes as described in detail previously [7]. Rats were tested for a total of 12 days, during which total reaches, accuracy and limb use was recorded. A “reach” was scored when the rat extended its forelimb through the slot. A “hit” was scored if the rat successfully brought the pellet back to his mouth and consumed it. The order of testing was randomized each day.

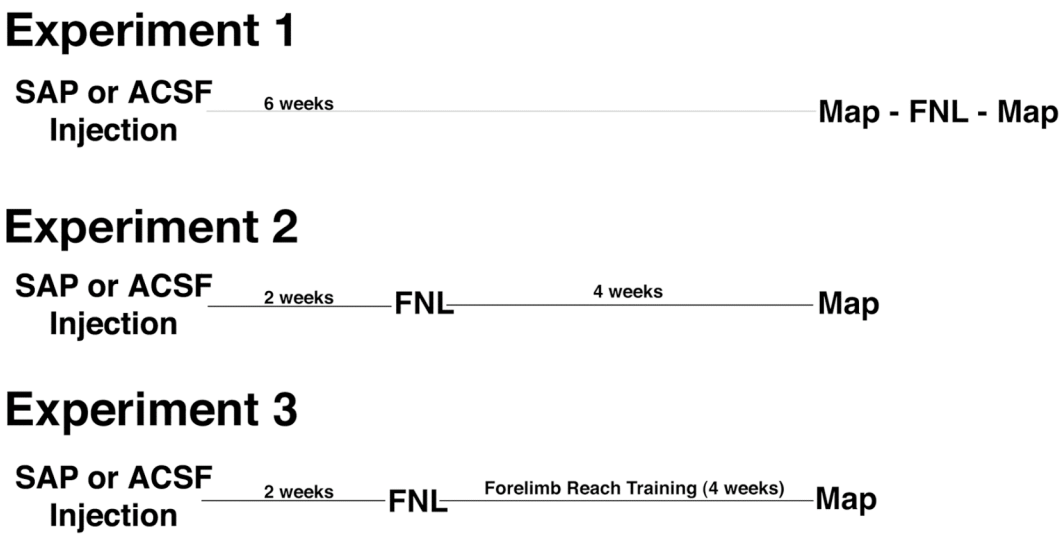

Figure 2.1: Experimental Paradigm

Three separate experiments were carried out in this study. (A) In the first paradigm, animals underwent a bilateral cholinergic lesions using the SAP immunotoxin, and were then returned to their home cage. After six weeks (to allow the same duration following SAP lesions to mapping in all experiments), the motor cortices of animals were mapped bilaterally. Then, while the animal remained anesthetized in the stereotax, the facial motor nerve (FMN) was cut bilaterally. Two hours after the FMN lesion, animals were remapped to specifically examine short-term plasticity following a facial nerve transection. (B) In the second experiment, animals underwent a SAP lesion. Two weeks after the SAP lesion, animals underwent a bilateral FMN lesion. 4 weeks after this lesion, the motor cortices of the animals were mapped. (C) In the third experiment, animals underwent a SAP lesion, followed two weeks later by a bilateral FMN lesion as in experiment 2. Animals then underwent 4 weeks of skilled motor training (4 weeks includes the total time for food deprivation, handling, behavioral shaping and 12 days of skilled motor training), followed by mapping of their motor cortices.

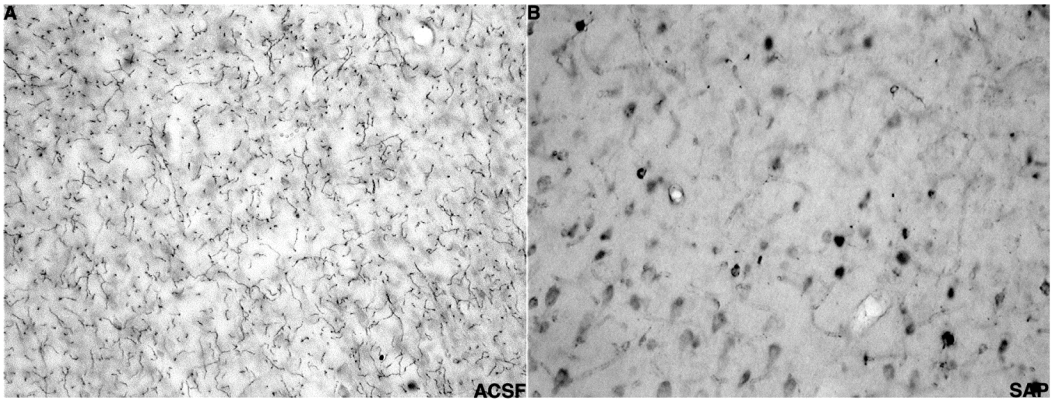

Figure 2.2: Cholinergic Density Within Motor Cortex Following ACSF and SAP Injections.

Figure 2: Cholinergic Density Within Motor Cortex Following ACSF and SAP Injections. Figure (A) demonstrates the normal distribution of acetylcholinesterase-positive cholinergic fibers within layer 5 of the motor cortex . Figure (B) demonstrates that, following an injection of the P192-IgG-Saporin conjugated immunotoxin within the nucleus basalis, the cholinergic innervation within the motor cortex is almost entirely depleted.

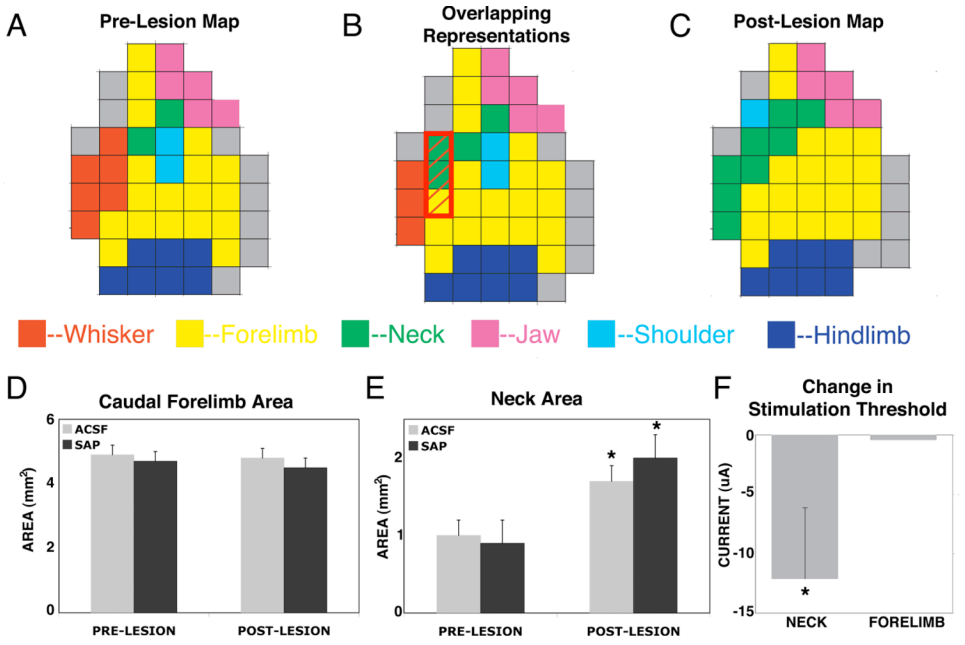

Figure 2.3: Short Term Plasticity Following a FMN Lesion Does Not Require a Functional Basal Forebrain Cholinergic System.

Figures (A-C) represent sample maps derived from a SAP-lesioned animal. Figure (A) is an example of normal somatotopic map derived from within motor cortex using Intra-Cortical Micro-Stimulation (ICMS) techniques, wherein the movement that is evoked with the lowest stimulation threshold is recorded over the entire cortical area. (B) Using a slight modification ofthat the standard ICMS protocol, in which at all whisker sites, the stimulation threshold was increased until another movement could also be evoked (or the maximum of 200 μA was reached), subtle differences in map topography were noted that could potentially account for artifacts in reported plasticity findings (see Results sectin for details). At some whisker sites no other movement was evoked, however at others (the cross-hatches areas), underlying movements were recorded. For all further "pre-lesion" analyses, the area of neck and forelimb was calculated based on this protocol. Figure (C) demonstrates that, following a FMN lesion, the area of neck expands a great deal. However, the area of forelimb does not appear to expand, despite having some spatial overlap with the whisker area. (D)No increase was observed in the caudal forelimb area in either SAP or ACSF groups following a FMN lesion. (E) The FMN lesion resulted in a statistically significant increase in the area of the neck representation (overall ANOVA $p < .001$; individual paired t-tests $p < 0.01$ for both ACSF and SAP groups). There was no statistical difference in the total pre-lesion or post-lesion areas of either neck or forelimb between SAP and ACSF groups ($p > 0.2$).between SAP and ACSF groups.

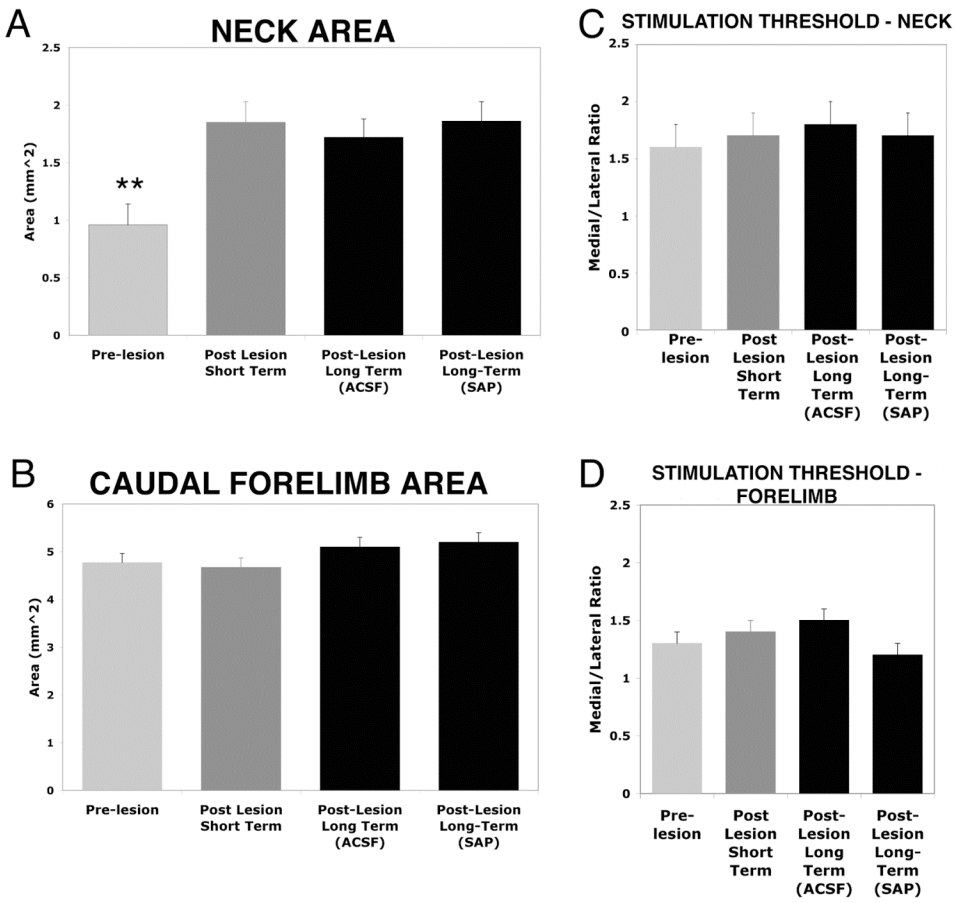

Figure 2.4: Long Term Plasticity Following a FMN Lesion Does Not Require a Functional Basal Forebrain Cholinergic System.

Figure (A) demonstrates that there is a statistically significant increase in the area of neck following a FMN lesion in both the short-term (data pooled from experiment 1), as well as in the long-term (ANOVA $p < 0.01$). Moreover, there were no statistical differences between either the SAP or ACSF groups ($p = 0.8$), as well as between either of those groups and the short-term plasticity seen two hours following the lesion (Tukey-Kramer post-hoc test). Further, stimulation thresholds (graphed as the ratio of the medial to lateral in order to assess changes specifically in areas previously within whisker, normalized to the non-whisker thresholds) within the neck area were not statistically different from each other across any group ($p = 0.3$). (B) No change in the caudal forelimb area was seen following a FMN lesion. Further, there is no change in the medial/lateral stimulation threshold ratio.

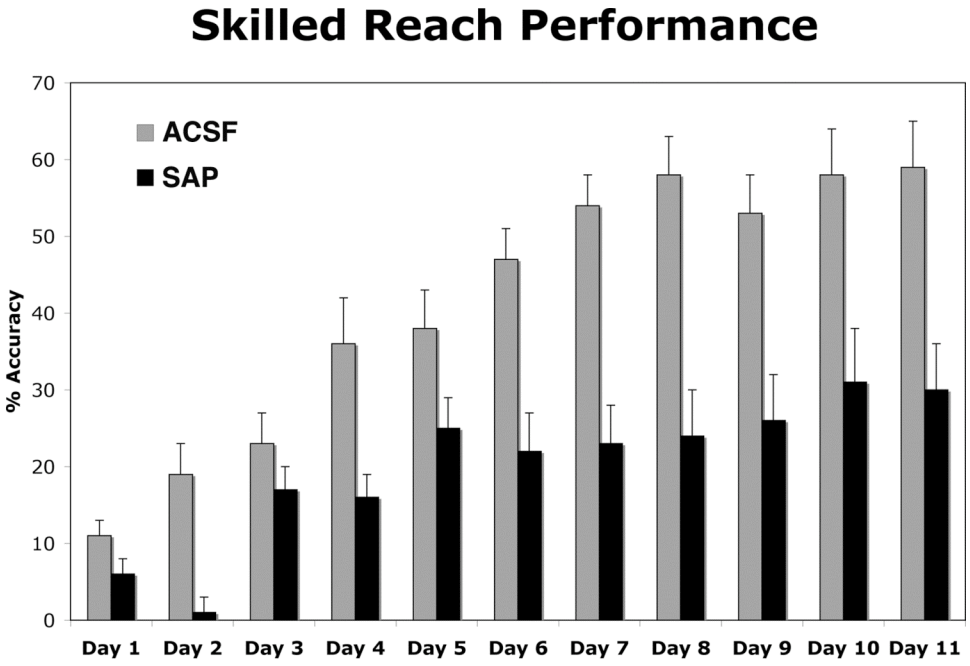

Figure 2.5: SAP Lesions Impair Acquisition of a Skilled Motor Task. Following cholinergic lesions of the nucleus basalis, acquisition of the skilled forelimb reach task is significantly impaired. Using a repeated measures ANOVA, impairments were seen from day 2 onwards (overall ANOVA $p < 0.0001$), and there was a 51 % decrease in final performance as averaged across the last three days of testing ($p < 0.01$).

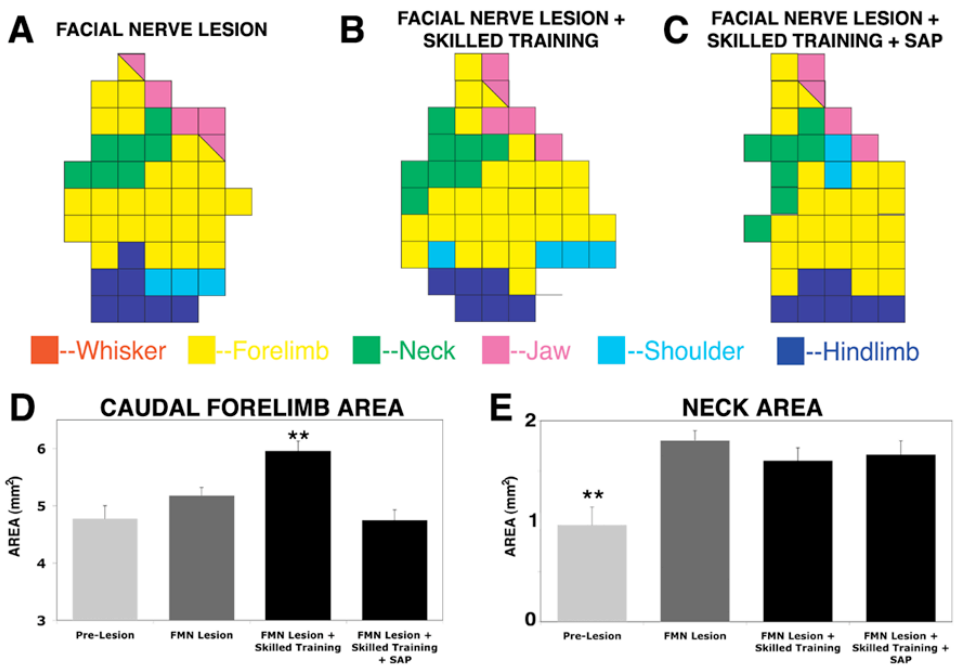

Figure 2.6: Cholinergic Lesions Abolish Plasticity Associated With Skilled Motor Learning, but Allow Plasticity Associated With a FMN Lesion.

(A) Skilled motor training, within the context of a FMN lesion, results in an expansion of the caudal forelimb representation (overall ANOVA $p < 0.0001$). The pre-lesion caudal forelimb area was 4.8 ± 0.2 mm2. Following a long-term FMN lesion (collapsed across both SAP and ACSF groups because there was no difference between them), the caudal forelimb area was 5.2 ± 0.2 mm2 (non-significant according to Tukey-Kramer post-hoc test) Skilled motor training resulted in a significant expansion of the caudal forelimb area to 6.0 ± 0.2 mm2 (significantly different than all other groups according to Tukey-Kramer post-hoc test). Skilled motor training in the absence of a functional basal forebrain cholinergic system completely blocked this plasticity (CFA = 4.8 ± 0.2 mm2). (B) In the animals where the cholinergic lesions prevent plasticity associated with skilled motor training, there is no change in the aspect of plasticity associated with the facial nerve transection (the expansion of the neck representation) (overall ANOVA $p < 0.01$). The pre-lesion area of neck is 1 ± 0.2 mm2, a long-term facial nerve lesion (averaged across SAP and ACSF animals) is 1.8 ± 0.1 mm2. Training with a FMN lesion results in a total neck area of 1.6 ± 0.2 mm2, and skilled motor training with a FMN lesion and a SAP lesion results in a total neck area of 1.7 ± 0.2 mm2. Tukey-Kramer post-hoc tests reveal statistically significant differences between the pre-lesioned group and all other groups only.

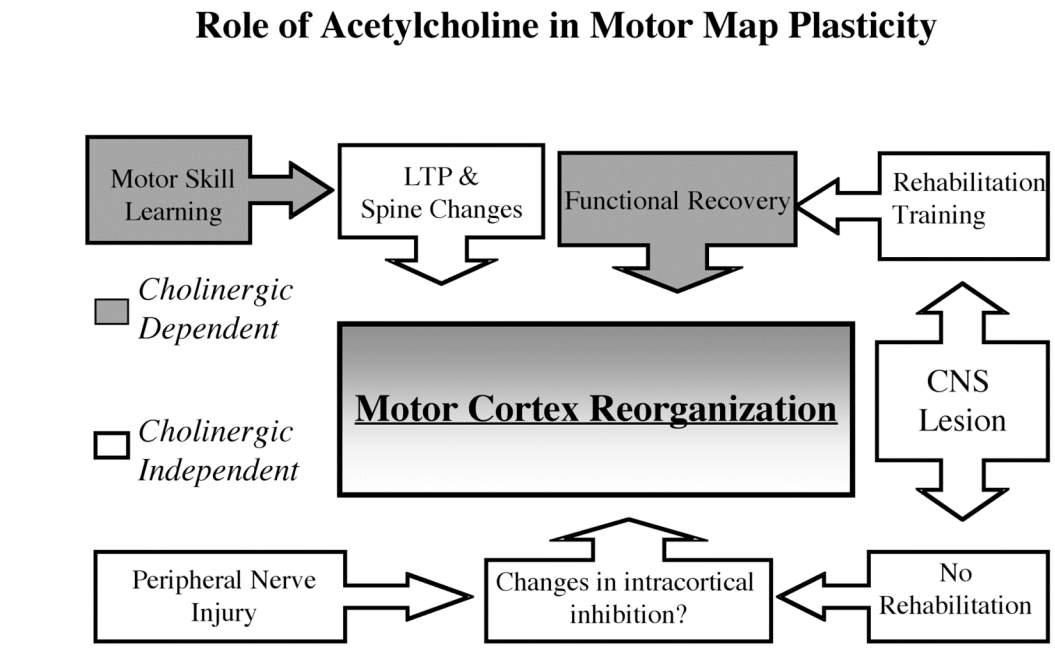

Figure 2.7: The Role of Acetylcholine in Motor Map Plasticity. Based on past findings and the results of the present study, we put forward a model wherein acetylcholine selectively modulates the behaviorally-mediated neural changes that result in cortical map plasticity. This model proposes that cholinergic mechanisms are not required for motor map lasticity that comes about by manipulations that are devoid of behavioral experience.

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Chapter 3:
Acetylcholine is Necessary for the Developmental Maturation of Cortical Motor Systems

ABSTRACT

The basal forebrain cholinergic system is essential for map plasticity in the motor cortex of adults. The present study examined whether the cholinergic system is also required for postnatal maturation and function of cortical motor systems. We find that a selective lesion of cholinergic neurons within sensorimotor cortex in 24-day old rats prevents the normal post-natal maturation of the cortical motor map. A cholinergic lesion delivered after animals are fully matured does not result in any map impairments, demonstrating this effect is specific to a developmental time period. Further, this impairment in maturation of the cortical motor map is associated with significant deficits in skilled forelimb use. This study establishes a novel and critical role for cholinergic modulation in the post-natal development of cortical plasticity and function.

INTRODUCTION

Several studies have demonstrated the importance of the basal forebrain cholinergic system in regulating adult cortical map plasticity [1-4]. However, few studies have examined its role in affecting postnatal developmental plasticity and the maturation of cortical maps.

Previous studies on the effects of neonatal cholinergic lesions have demonstrated only mild effects on cortical anatomy and behavior. Immunotoxic lesions, using the specific agent 192-IgG SAP (SAP), in newborn rat pups (P1), result in a decrease in cortical thickness by 10 %, and a reduction in apical branching and spine density of cortical neurons [5, 6]. However, SAP injections delivered only six days later, at neonatal day P7, demonstrate none of these gross anatomical changes [7, 8]. Rats with neonatal cholinergic lesions (at P1 and later) perform normally on water maze tests, but do show slight impairments on open-field and passive avoidance tests [7, 9, 10]. Thus, evidence suggests that neonatal cholinergic lesions, especially when given at P7 or later, have few overall effects on cortical development and only mild behavioral deficits.

Neonatal cholinergic lesions do result in impairments in neural plasticity. SAP lesions delivered at day P4 block the induction of TBS induced LTP in visual cortical slices [11]. Neonatal electrolytic lesions of the basal forebrain also stunt somatosensory plasticity of mouse barrel fields following whisker plucking [12]. Acetylcholine, along with other neuromodulatory systems, also play a role in modulating ocular dominance plasticity, a form of developmental plasticity that

occurs in visual cortex [13-18]. However, studies have shown that ablation of either acetylcholine or norepinephrine alone is not enough to completely block this plasticity – instead, both neurotransmitter systems must be abolished to block plasticity [19].

Studies summarized above indicate that while neonatal cholinergic lesions in isolation do not result in gross abnormalities in development or behavior, they may impair certain aspects of neural plasticity. However, recent findings raise the possibility that cholinergic systems in isolation may exert a particularly critical role in modulating motor cortex developmental plasticity. Conner et al reported that cholinergic systems are critical for plasticity of adult motor cortex in the context of acquistion of novel behavioral tasks and to support functional recovery following rehabilitation training after injury in adult rats [2, 20]. These studies suggest that acetylcholine is important during behaviorally-mediated forms of cortical plasticity. There is much evidence to suggest that the cortical motor system requires behavioral activity for normal development to occur [21, 22]. Blocking motor activity at a specific developmental time point, either peripherally by injecting botulinim toxin, or centrally using GABAergic inactivation of sensorimotor cortex, results in aberrant maturation of cortical motor maps, aberrant development of the corticospinal tract and very specific motor defecits as adults [21-25].

Based on this evidence, we hypothesized that lesions of the basal forebrain cholinergic system would interfere with the behaviorally-dependent maturation of cortical motor maps. To test this hypothesis, cortical motor map plasticity and

behavioral function were assessed in adult animals that had received lesions specifically depleting cholinergic inputs to the motor cortex while they were juveniles. Results of these studies demonstrate that the basal forebrain cholinergic system exerts a critical role in the developmental plasticity and functionality of the motor cortex.

MATERIALS AND METHODS

Juvenile SAP Lesions

Post-natal day 24, Fisher-344 rats underwent specific depletive lesions of the cholinergic system by injections of the 198 IgG Saporin toxin (SAP, from Advanced Targeting Systems). These juvenile rats were anesthetized using a mixture of ketamine, xylazine, and acepromazine, and placed in a rat stereotactic apparatus. SAP was diluted to a concentration of 0.375 mg/mL, and a total of 0.5 uL was pressure injected into the motor cortex using a 33 gauge Hamilton syringe at each of the following coordinate sites relative to bregma: M/L: ± 3.5 mm; A/P: 0; 1.5 mm; D/V: 1.3 mm. After the final injection, rats were placed back into their cages and left to mature for eight weeks. Control animals (n = 6) received comparable injections of vehicle (ACSF).

Functional ICMS Mapping

Standard microelectrode stimulation techniques were used to derive maps of the motor cortex. Animals were anesthetized with ketamine hydrochloride (70

mg/kg i.p.) and xylazine (5 mg/kg i.p.) and received supplementary doses of the anesthesia mixture as needed. Pulled-glass stimulating electrodes (input impedance ~ 0.5 MOhm at 300 Hz) filled with 3 M NaCl were used. Microelectrode penetrations were made at 500 μm intervals at a depth of ~ 1800 μm (corresponding to cortical layers V–VI). Stimulation consisted of a 30 ms train of 200 μs duration monophasic cathodal pulses delivered at 333 Hz from an electrically isolated, constant-current stimulator (Isoflex Stimulus Isolator, AMPI, Jerusalem, Israel) under the control of a programmable pulse generator (Master-8, AMPI, Jerusalem, Israel). Pulse trains were delivered 1.2 s apart, and evoked movements were examined with the animal maintained in a prone position and the limbs supported in a consistent manner. At each site, the current was gradually increased until a movement was detected (threshold current). If no movement was detected at a maximum stimulus intensity of 200 μA, the site was defined as "nonresponsive." The size of motor representation was determined by multiplying the number of responsive sites evoking a movement of the forelimb by 0.25 mm^2. Because the hindlimb motor area extended beyond the area normally mapped, the total area representing this body part was not quantified.

Skilled Motor Training

Motor training was carried out using single pellet retrieval boxes as described in detail previously [2]. This task requires animals to use the forepaw to reach through a small slit in a plexiglass chamber, grasp, and retrieve a small food pellet positioned on a platform near the chamber. Rats were tested for a total of

12 days, during which total reaches, accuracy and limb use was recorded. A "reach" was scored when the rat extended its forelimb through the slot. A "hit" was scored if the rat successfully brought the pellet back to his mouth and consumed it. The order of testing was randomized each day.

RESULTS

An initial study was carried out to characterize the normal post-natal maturation of the motor system in rats. For this purpose 16 rats between the ages of p25 and p50 wereelectrophysiologically mapped using techniques of intracortical microstimulation (ICMS. Qualitatively, it appeared that motor maps underwent significant change in overall size across time, with more mature animals having larger overall maps and an increase in distal forelimb sites (Figure 2A-C). To quantify these effects, animals were broken into three distinct age groups as follows: P < 30 days (n=3), P = 31-40 days (n=7) and P = 41-50 days (n=3). The quantitative analysis confirmed the qualitative observations by demonstrating that the total area of the motor map (sum of the area evoking whisker, neck, forelimb, shoulder and jaw motor responses, excluding hindlimb as explained in methods) increased from 4.7 ± 0.8 mm^2 in the youngest age group to 7.9 ± 0.9 mm^2 in the P41-50 group (ANOVA $p< 0.001$; Fig. 2A). The size of the caudal forelimb area also enlarged over time, from 0.8 mm^2 in the youngest animals to 2.3 mm^2 in the P41-50 group (ANOVA $p<0.001$; Fig 2B). The expansion of the CFA over time resulted almost equally from expansion of both

the elbow and wrist regions of the cortical map. The wrist area expanded from 0.2 ± 0.3 mm^2 to 1.9 ± 0.4 mm^2 (ANOVA < 0.05; Fig. 2C). The elbow area increased from 0.6 ± 0.2 mm^2 to 1.7 ± 0.2 mm^2 (ANOVA < 0.01; 2D). In contrast to the enlargement seen with most representations, the area of the shoulder representation demonstrated a non-significant trend towards decreasing over time (Fig 2E; $p = 0.08$). This trend resulted in a significant inverse relationship between the area representing shoulder and the caudal forelimb area ($R^2 = 0.4$; $p < 0.05$), suggesting that the shoulder region may be converted into caudal forelimb area as animals mature (Fig. 2A-C). Other motor areas, including neck (ANOVA $p < 0.05$; Fig. 2F) and whisker (ANOVA $p < 0.05$; not shown), also exhibited significant expansion over time.

To investigate the role of basal forebrain cholinergic mechanisms on the functional maturation of cortical motor maps, juvenile rats received lesions selectively depleting cholinergic innervation to the motor cortex. Cholinergic-specific immunotoxic lesions were performed on 24 day old rats, thereby minimizing non-specific effects of a neonatal cholinergic lesion on gross cortical morphology, [5, 7, 26, 27]. In a further attempt to minimize the non-specific effects of a lesion of the entire basal forebrain, the immunotoxin was injected directly into the motor cortex. Based upon prior studies [28] and our own unpublished observations, the injection of SAP into the motor cortex selectively eliminates afferent cholinergic innervation within the sensorimotor cortex, but does not damage cholinergic innervation of other cortical regions, thus allowing

for a direct interpretation of cholinergic influences on motor cortical development.

SAP injections at P24 targeted to the motor cortex resulted in long-lasting loss of innervation of sensorimotor cortex measured in adulthood (Fig. 3A-B). Further, this cholinergic deficit was specific to the sensorimotor cortex; adjacent cortical areas demonstrated normal patterns and density of cholinergic innervation (Fig. 3C). One animal was excluded from analysis because of incomplete lesion of cholinergic fibers.

Five animals lesioned with SAP at age P24 and six vehicle-injected animals underwent subsequent electrophysiological mapping of their motor cortex at P80, a time when maps are fully matured. Notably, specific motor cholinergic lesions placed at P24, during cortical motor map development, significantly impaired subsequent maturation of the motor map (Fig. 4). The overall size of the motor map was reduced by 34 % in animals with cholinergic lesions limited to the motor cortex relative to vehicle injected controls (10.5 ± 0.3 mm^2 in control subjects and 6.9 ± 0.3 mm^2 in animals with juvenile cholinergic lesions (ANOVA $p < 0.0001$; Fig 5A)). The caudal forelimb area, which has been demonstrated to play a crucial role in for skilled manipulation of the wrist and digits, diminished in size by 34 %, from 4.1 ± 0.1 mm^2 in control animals to 2.7 ± 0.1 mm^2 in cholinergic lesioned animals (ANOVA $p < 0.0001$; Fig. 5B). There was no significant difference in the size of the elbow representation when comparing subjects with SAP lesions against vehicle-injected ones (ANOVA $p = 0.3$; Fig. 5D). Thus, the decrease in size of the caudal forelimb area was caused primarily

by a reduction in the area of the wrist representation from 2.5 ± 0.2 mm^2 in controls to 1.3 ± 0.2 mm^2 in SAP lesioned animals (ANOVA < 0.01; Fig. 5C). Concomitant with the decrease in CFA, the size of the shoulder area was significantly larger in animals that underwent juvenile cholinergic lesions (ANOVA $P < 0.05$; Fig. 5E). This is consistent with previous observations that the shoulder region normally matures into caudal forelimb area during post-natal development. Other motor representations, such as neck (ANOVA $p < 0.01$; Fig. 5F were also smaller in SAP lesioned animals.

To control for the effects of cholinergic lesions alone on motor map topography, SAP lesions were also performed on adult rats (ordered from Harlan Sprague at 225 – 250 grams) with fully matured cortical motor maps. Following the injection of either SAP or vehicle (ACSF) into the Nucleus Basalis Magnocellularis (NBM), these adult animals were returned to their cages for 6 weeks before undergoing electrophysiological mapping. Cholinergic lesions administered in adult animals, at a time when cortical motor representations had fully matured, resulted in no differences in either total map size or in the area of individual movement representation (Fig. 6). Thus, cholinergic lesions appear to specifically impair post-natal development of cortical motor map plasticity. After maturation occurs, cholinergic lesions have no effect on the size of motor maps.

Since prior studies have demonstrated the critical role played by the CFA in mediating skilled motor learning, it was postulated that impairments in motor map development (specifically within the CFA) may have a long-lasting impact on subsequent motor learning in adulthood. To assess the behavioral relevance of

this impaired motor map maturation, animals with juvenile SAP lesions were tested in a skilled reaching task as adults. Juvenile cholinergic lesions result in impaired behavioral performance of the skilled reach task relative to control (vehicle-injected) animals (Fig. 7) [2].

DISCUSSION

This study demonstrates that cholinergic systems are essential for the development of cortical motor map plasticity during a developmental "critical period." Cholinergic lesions limited to the motor cortex, administered during a critical window in the development of cortical motor maps, disrupted the normal maturation of cortical motor representation. Cholinergic lesions applied after these mature representations were established had no effect on motor map topography. Perturbation of cortical map development resulted in long-lasting behavioral consequences, reflected by the impaired ability of rats to acquire a skilled forelimb reaching motor behavior.

Previous studies detailing the development of cortical motor development have primarily been restricted to cats. The M1 motor representation in cats is completely absent prior to postnatal day 45, and undergoes initial development at postnatal day 60 [29]. From postnatal day 60 - 90, the motor map gradually develops, growing in both size and complexity. Before day 71, stimulation within the forelimb motor cortex produces primarily shoulder or elbow movements. After this time point, first wrist and later digit sites begin to emerge. Further, after

day 71, sites with multi-joint movements appear with greater frequency [29]. This developmental time frame and sequence of events parallels our findings in the rodent: motor maps exhibit increasing size and complexity over time, with a proximal-to-distal establishment of forelimb representation over time.

This study provides the first evidence that a neuromodulatory system influences a critical period of developmental plasticity in the cortical motor system with long-lasting consequences on behavior. Solitary disruption of cholinergic inputs, and indeed elimination only of those cholinergic axons specifically projecting to motor cortex, perturbed cortical plasticity during a critical period of post-natal system maturation. To our knowledge, this is the first demonstration of an essential and independent role for the cholinergic system in modulating critical period plasticity. Further, these findings are derived from an unperturbed model of cortical plasticity, highlighting the importance of this finding in the context of normal nervous system maturation. Previous experiments investigating the role of neurotransmitters in developmental plasticity used paradigms of developmental perturbation (e.g., monocular deprivation) [14, 15, 19, 30], whereas we examined normal cortical motor plasticity without additional experimental manipulation. Further, previous experiments suggested that lesions of the basal forebrain cholinergic system alone were not sufficient to prevent ocular dominance column plasticity [19]. In contrast, we find that solitary and indeed highly focal elimination of cholinergic cortical inputs prevents developmental plasticity of motor cortex representations.

Mechanisms whereby cholinergic elimination perturbs cortical map plasticity merit further investigation. It has been reported that motor activity during a critical period of development is essential for normal development of cortical motor maps and corticospinal tract projections to their targets [31, 32]. Blocking intracortical motor activity using the GABA agonist, muscimol, between 5-8 weeks of age resulted in an impairment of motor map development and a reduction in corticospinal tract branching, density and innervation of the cervical spinal cord [31, 32]. Further, the presence of motor maps has been associated with myelination and increased synaptic efficacy of the corticospinal tract [33, 34]. It is postulated that damage to the basal forebrain cholinergic system during development may disrupt attentional mechanisms that enable activity derived from behavioral experience to modify cortical circuits as needed in order to result normal patterns of motor map development. Cholinergic blockade may thus perturb development by impairing excitatory mechanisms in the motor cortex that are a necessary component of activity-dependent cortical map development. For example, post-natal lesions of the nuclus basalis result in a loss of cortical muscarinic receptors and impairments in visual cortex long term potentiation (LTP) [11]. Post-natal cholinergic lesions also reduce dendritic branching and spine complexity in cortical neurons [5]. Cholinergic systems are also sensitive to nerve growth factor (NGF), and both NGF and its receptor trkA have been implicated in critical period plasticity [35, 36]. Specifically, expression of NGF increases and expression of TrkA peaks in the visual cortex at the height of visual cortical plasticity [35, 36], and NGF infusions prevent the consequences

of monocular deprivation [37]. Thus, several mechanisms link functions of the cholinergic system with cortical development.

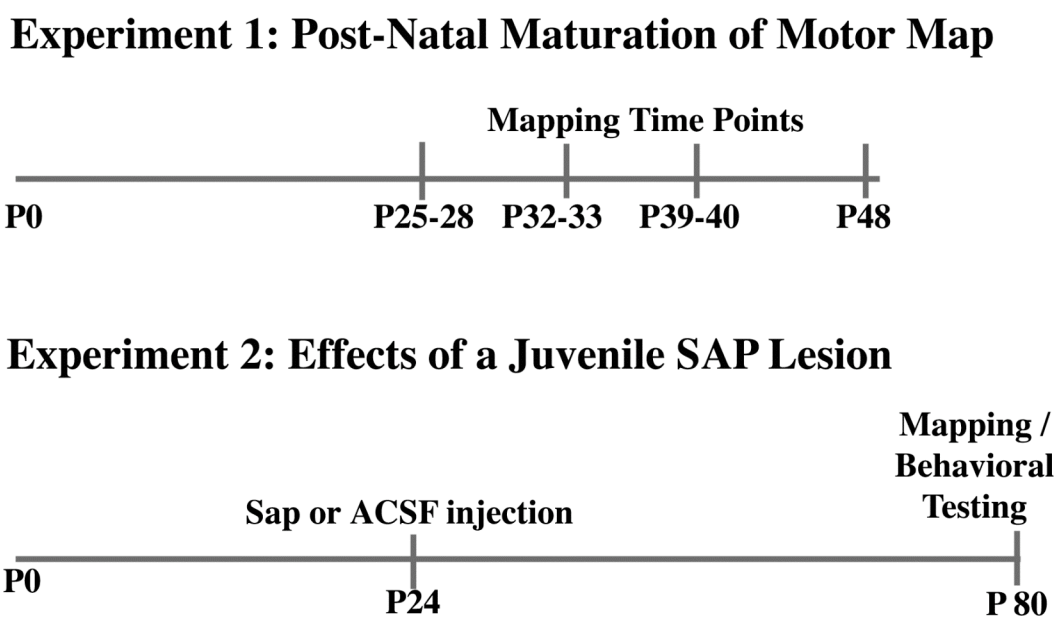

Figure 3.1: Experimental Methodology.
Two experiments were carried out in this study. In the first, the normal maturation of the motor cortex was studied by electrophysiologically mapping animals at different postnatal time points. In the second experiment, animals underwent cholinergic depletion at P24, and then underwent eletrophysiological mapping and skilled motor testing as adults.

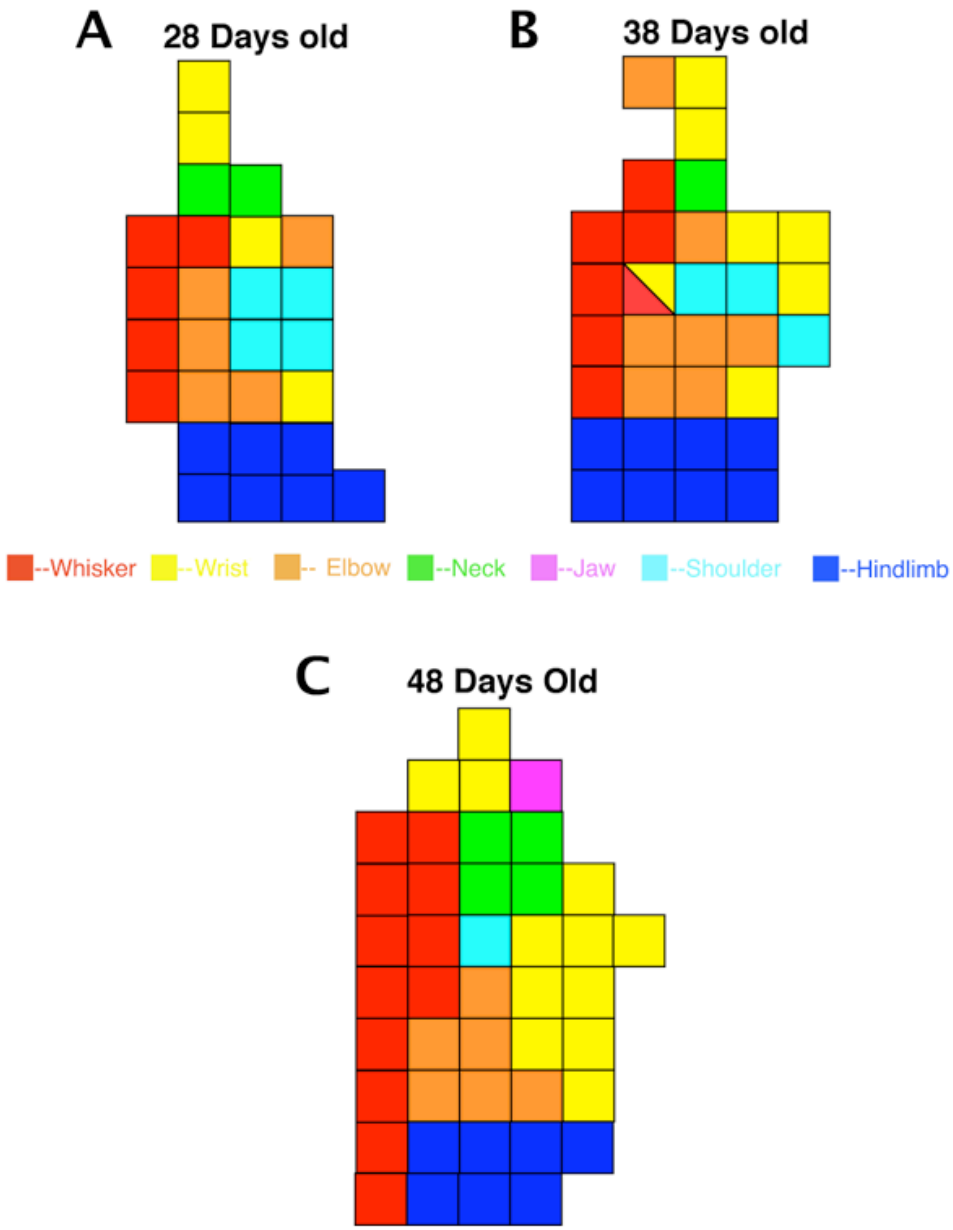

Figure 3.2: Motor Maps at Various Developmental Time Points.
Male fisher rats were mapped at developmental time points ranging from postnatal day 24 to 50. Sample maps are shown at (A) postnatal day 28, (B) postnatal day 38, and (C) postnatal day 48. Progressive changes in the size of different motor representations can be observed over time.

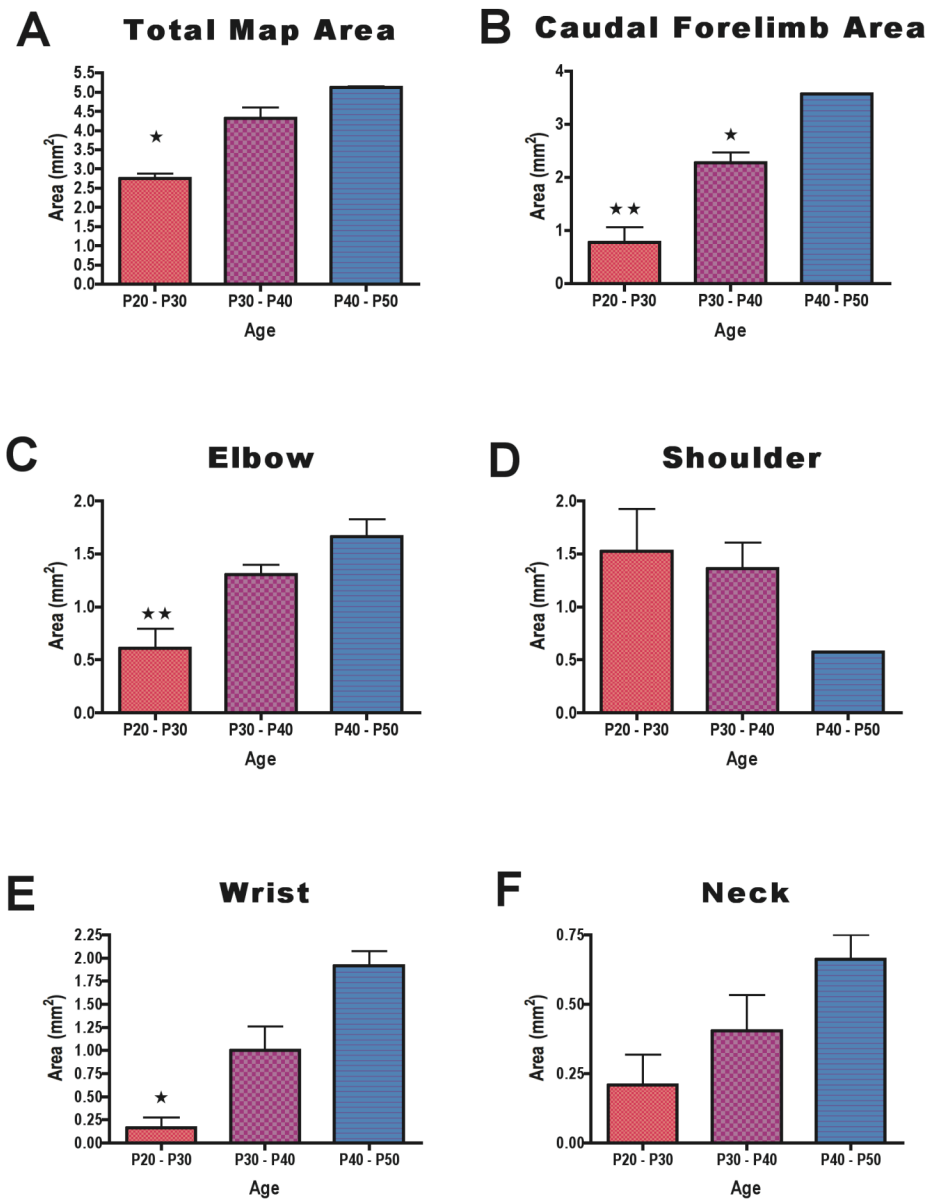

Figure 3.3: Development of the Cortical Motor System.

Animals were mapped at various 4 general time points. For simplicity, the middle two groups (P32-33 and P38-39) were collapsed for statistical and graphical comparisons. (A) There is a significant increase in total motor map size over time. (B-D): The total area of motor cortex caudal forelimb area also increases over time, including wrist and elbow regions. (E) Area of proximal forelimb (shoulder) does not increase over time. (F) Other non-forelimb areas, such as neck, also expand over time.

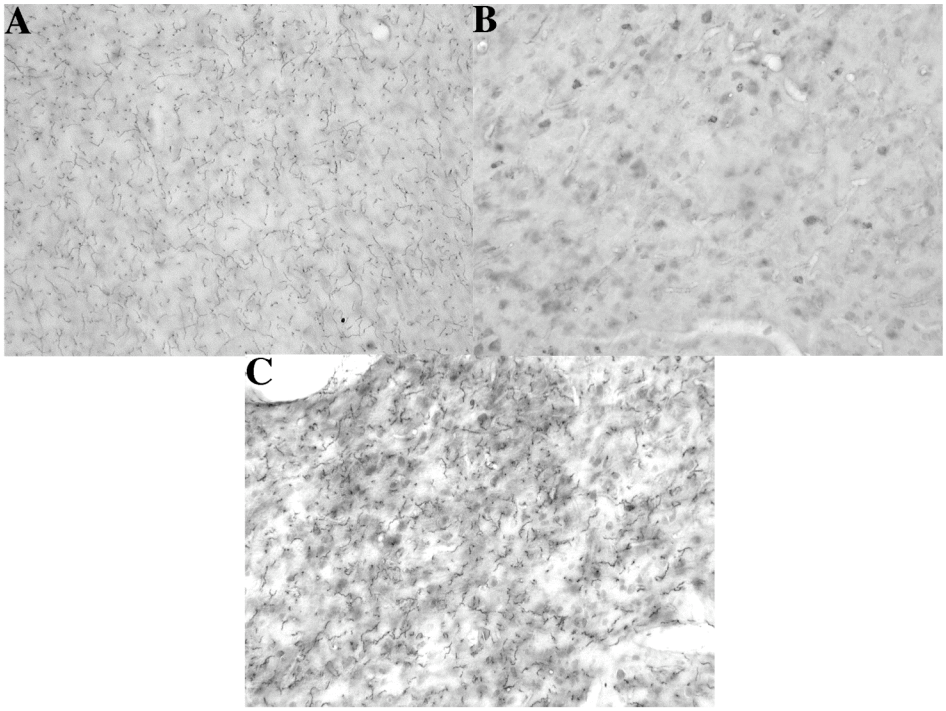

Figure 3.4: Juvenile Cholinergic Lesions Result in Long-Lasting Cholinergic Deficits.

SAP injections at P24 targeted to the motor cortex resulted in long-lasting loss of innervation of sensorimotor cortex measured in adulthood (Fig. A-B). Further, this cholinergic deficit was specific to the sensorimotor cortex; parietal cortex of the animal shown in Fig. 4B demonstrated normal patterns and density of cholinergic innervation (Fig. C).

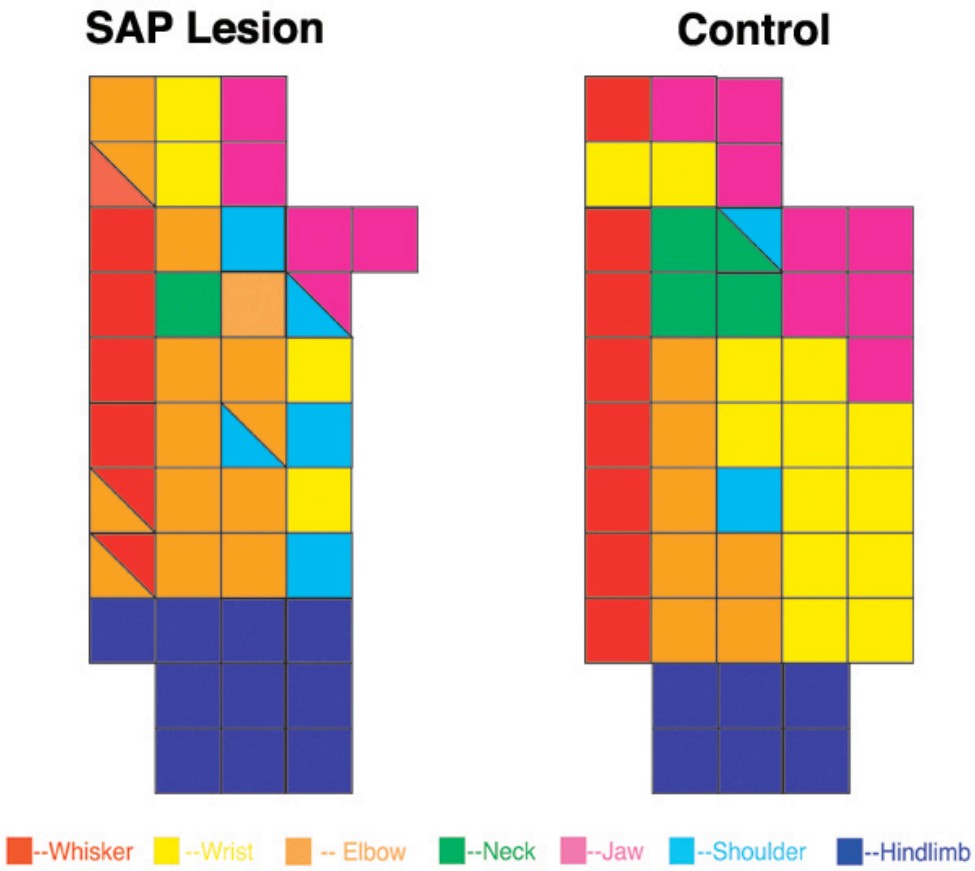

Figure 3.5: Juvenile Cholinergic Lesions Result in Impaired Map Development

Sample maps show that juvenile cholinergic lesions impair the normal development of cortical motor maps.

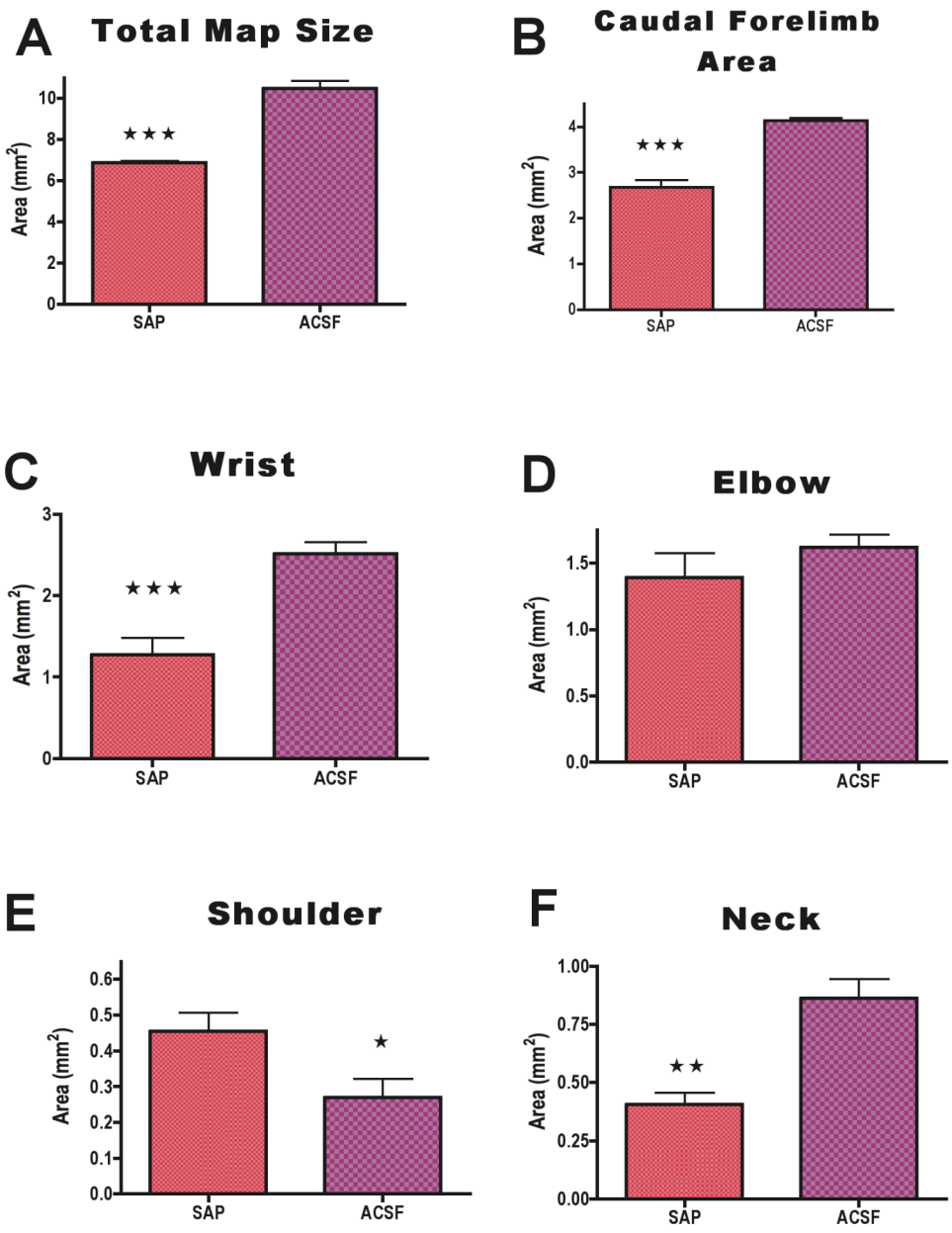

Figure 3.6: Cholinergic Lesions During the Cortical Motor Critical Period Impair Map Plasticity.

(A) Focal cortical cholinergic depleting lesions result in a reduction in the size of the total motor map in adulthood, and (B) in the size of caudal forelimb area and (C) neck area. (D) Most of the reduction of the caudal forelimb is a result of a reduction in wrist area but not (E) elbow. (F) Developmental cholinergic lesions also result in a larger representation of proximal forelimb area, the only motor area to increase as a result of cholinergic lesions. Notably, proximal forelimb mediates non-skilled movement of the upper extremity.

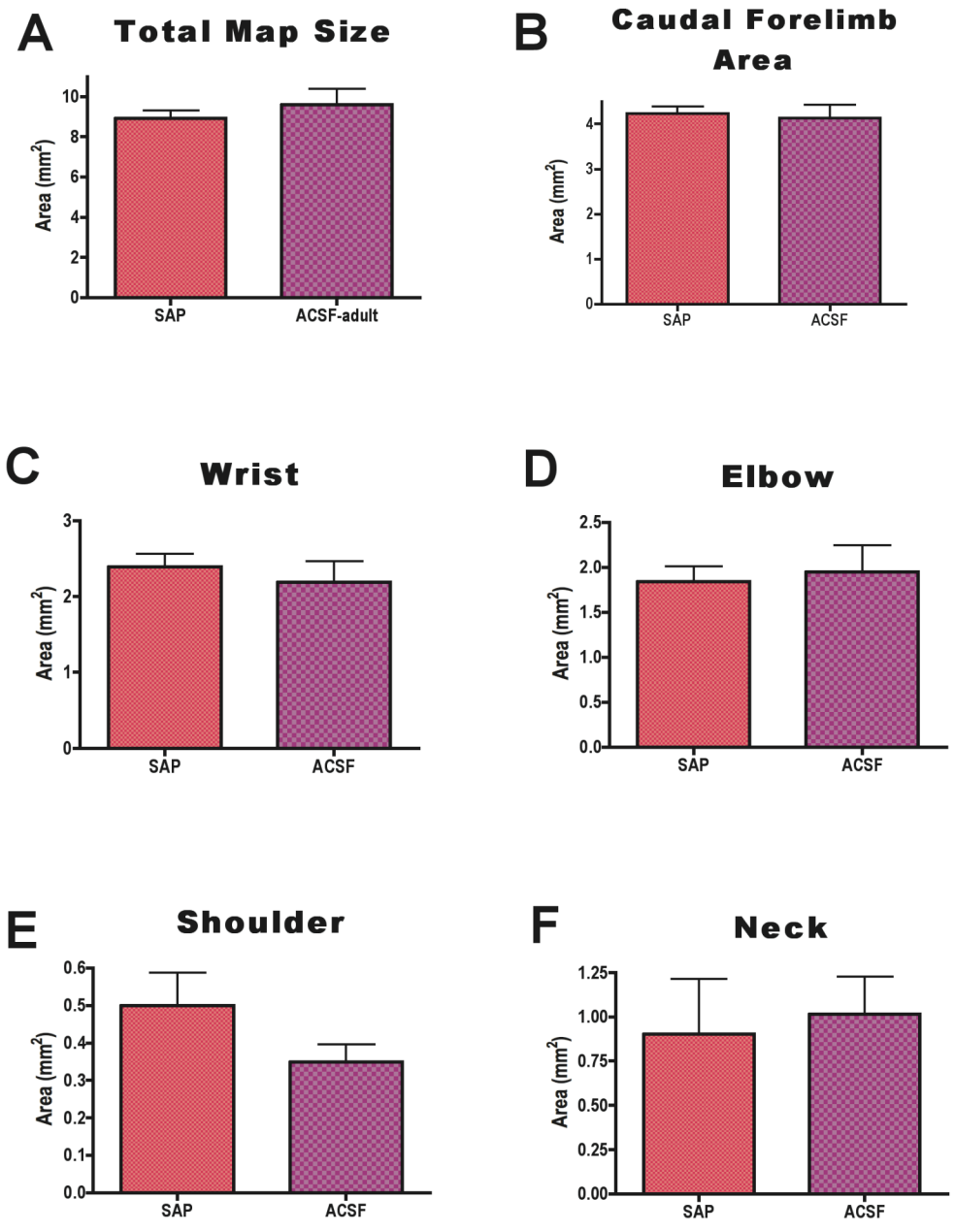

Figure 3.7: Cholinergic Lesions Delivered in Adults Do Not Alter Motor Maps

SAP lesions in adult subjects do not change any aspect of the cortical motor map as compared to vehicle injected animals.

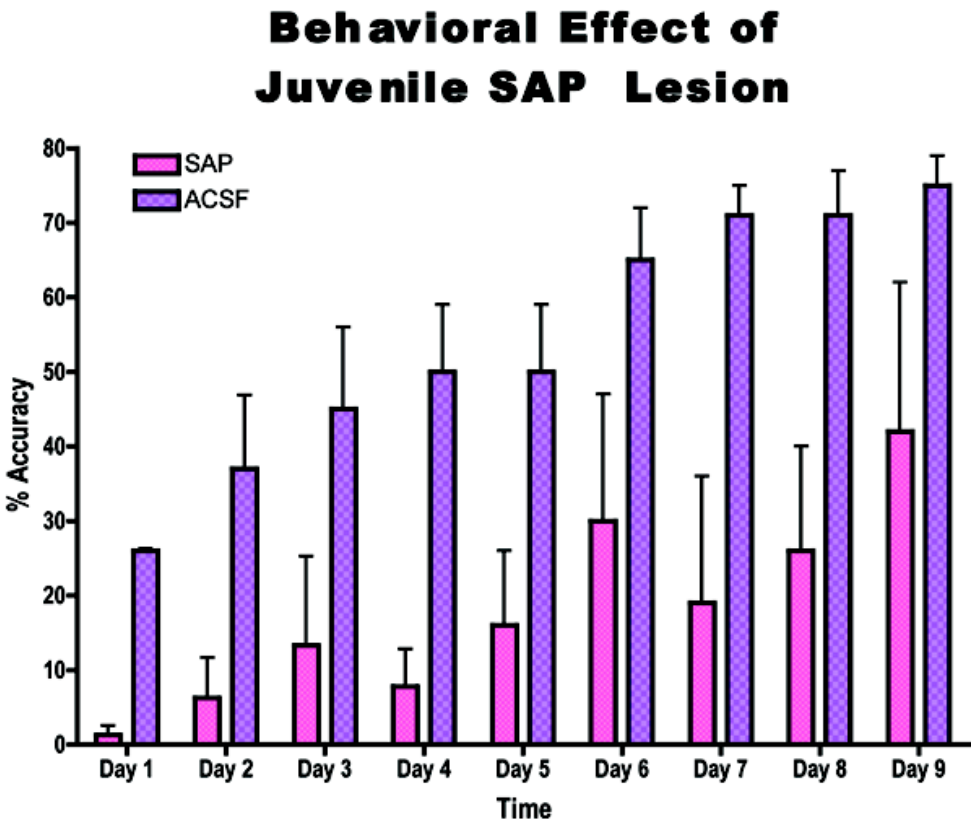

Figure 3.8: Juvenile SAP Lesions Result in Behavioral Impairments in Adults.

Acquisition of a skilled forelimb reach task is impaired following cholinergic depetion at P24 compared to vehicle injected controls (repeated measures ANOVA $p < 0.05$).

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Chapter 4:
Functional Recovery After Motor Cortex Injury Occurs in the Absence of Corticospinal Tract Plasticity

ABSTRACT

Axonal plasticity of cortical and subcortical systems has been described following both peripheral and central nervous system injuries. Only rarely has this type of plasticity been investigated within the context of motor map plasticity following injury. In this study, the motor cortex of rats was studied following a cortical lesion and five weeks of rehabilitative motor training. As in previous studies, intracortical microstimulation demonstrated functional reorganization of the rostral forelimb area (RFA) following rehabilitation training after injury compared to animals with lesions and no further rehabilitation, and compared to intact, unlesioned animals. Injecting anterograde tracers into the RFA to trace the corticospinal tract in these three groups of animals revealed no significant differences in the distribution or topology of axonal labeling within cervical spinal cord. This study suggests that axonal plasticity of the corticospinal tract is not a correlate for the functional recovery or motor map plasticity that occurs following rehabilitation training after brain injury.

INTRODUCTION

Axonal plasticity is often cited as a neuronal mechanism underlying functional recovery following nervous system injury [1, 2]. After peripheral nerve transections, sprouting of sensory axons has been described at multiple levels of synaptic relays in the lesioned projection, including subcortical and cortical levels of the nervous system [3]. Further, sprouting of intracortical axons within somatosensory cortex correlates well with the degree of somatosensory map plasticity that occurs following the peripheral nerve injury [4-6].

Intra-cortical axonal sprouting has also been described following central nervous system injuries. Following a focal cortical injury, axonal sprouting of layer V neurons was described within adjacent peri-lesional cortical areas [7]. Further, unilateral ischemic injuries of sensorimotor cortex have been shown to induce inter-hemispheric axonal sprouting of corticostriatal fibers from the unlesioned cortex [8-11]. Only one previous study has attempted to correlate axonal sprouting with motor cortex reorganization following CNS injury [12]. This study demonstrated that, many months after a cortical lesion to the hand area of M1, motor reorganization of the ventral premotor cortex (PMv) was associated with substantial sprouting of both the afferent and intracortical axonal projections from this area [12, 13]. Specifically, novel projection patterns were characterized from primary somatosensory cortex, as well as alterations in the trajectory of intracortical axons within PMv.

Increased axonal plasticity has been associated with many therapeutic interventions that enhance functional recovery. For example, D-amphetamine, a

stimulant known to increase functional recovery following brain injury, has been shown to enhance markers for neurite outgrowth and synaptic development of corticostriatal fibers [14]. Blocking the myelin Nogo receptor has been shown following brain injuries to increase axonal plasticity and to correlate with functional recovery compared to untreated controls [15-18]. Delivery of the Nogo inhibitor IN-1 following a unilateral sensorimotor cortical lesion resulted in axonal sprouting from the spared cortical hemisphere into the contrateral red nucleus, pons and striatum [15-17]. Mice with a knockout of the Nogo receptor, similarly, show increased interhemispheric axonal plasticity from the contralesional hemisphere to both red nucleus and cervical spinal cord [18].

The preceding studies demonstrate that axonal plasticity occurs following therapeutic interventions after brain injury. Another mechanism that supports functional recovery after brain injury is use or "rehabilitation" of the affected body part [19, 20]. However, systematic study on the effects of rehabilitation following brain injury in the lesioned corticospinal system have not been undertaken. This is curious for two reasons; first, rehabilitation is the most effective treatment option to induce functional recovery after brain injury [19, 21]**;** second, the corticospinal tract is one of the primary outputs from the motor cortex [22]. In previous studies, we characterized several types of cortical plasticity associated specifically with rehabilitation after brain injury in the rostral forelimb motor cortex of rats [23, 24]. In this experiment, using the same lesion and rehabilitation paradigm described in previous studies, **we tested the hypothesis that** axonal plasticity of the corticospinal system may underlie

functional recovery and motor map plasticity following rehabilitation after brain injury.

Rats underwent four weeks of skilled forelimb reach training, followed by an electrolytic lesion of the caudal forelimb area. Half of the animals underwent 5 weeks of rehabilitation training, after which all animals underwent electrophysiological mapping especially of the remaining, rostral forelimb area. Dextran amine, an anterograde tracer, was injected into the center of this area, and the corticospinal innervation pattern within C7 was characterized. We now find that plasticity of the corticospinal tract is not associated with functional recovery and motor reorganization following rehabilitation after injury.

MATERIALS AND METHODS

Behavioral Training

Motor training was carried out using single pellet retrieval boxes as previously described [25, 26]. Briefly, this task requires animals to use the forepaw to reach through a small slit in a Plexiglas chamber, grasp, and retrieve a small food pellet positioned on a platform near the chamber. During the acquisition phase of testing, 40 rats performed 60 reaches per day, five days per week, for 3 weeks. Following acquisition, rats were given a motor cortex lesion (see below). Two weeks after the lesion, animals were given three days of behavioral testing to assess the behavioral deficits following this lesion (Fig. 1A). One of the groups underwent further rehabilitation training while the other group

served as lesion-only controls. Rehabilitative training consisted of 60 trials per day, 5 days per week, for 5 weeks. Rehabilitation training resulted in a 42 % functional improvement (Fig. 1b; $p < 0.05$ repeated measures ANOVA).

Motor Cortex Lesions

The focal motor cortex lesion used in this study is a modification of lesion paradigms used by others [27-29]. Small electrolytic lesions were made bilaterally at 2 sites (Site 1: A/P = 0, M/L = ±3.5mm; Site 2: A/P = +1.5 mm, M/L = ±3.5 mm relative to Bregma), specifically targeting the distal forelimb representation in caudal forelimb motor cortex [24]. Bilateral lesions were performed to eliminate the possibility that rats would switch paw preference to the unaffected hemisphere. At each site, a 100μm, Teflon-coated, stainless steel electrode was initially lowered to a depth of 1.7mm and 1mA DC current (Grass Model DCLM5A) was passed for 20 sec. The electrode was raised 1 mm and current was applied for another 20 sec.

Functional ICMS Mapping

Standard microelectrode stimulation techniques were used to derive maps of the motor cortex. Animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.) and received supplementary doses of the anesthesia mixture as needed. Pulled-glass stimulating electrodes (input impedance ~ .5 MOhm at 300 Hz) filled with 3 M NaCl were used. Microelectrode penetrations were made at 500 μm intervals at a depth of ~ 1800

μm (corresponding to cortical layers V–VI). Stimulation consisted of a 30 ms train of 200 μs duration monophasic cathodal pulses delivered at 333 Hz from an electrically isolated, constant-current stimulator (Axon Instruments, Union City, CA) under the control of a programmable pulse generator (AMPI, Jerusalem, Israel). Pulse trains were delivered 1.2 s apart, and evoked movements were examined with the animal maintained in a prone position and the limbs supported in a consistent manner. At each site, the current was gradually increased until a movement was detected (threshold current). If no movement was detected at 200 μA, the site was defined as "nonresponsive." The size of the forelimb and neck representations were determined by multiplying the number of responsive sites evoking a movement of the forelimb by 0.25 mm^2.

Axonal Tracing

All animals underwent electrophysiological mapping to identify the rostral forelimb area. This included 20 animals that underwent a motor cortex lesion and rehabilitation training, 20 animals that underwent a motor cortex lesion alone, and 20 naïve control animals. The center of the rostral forelimb area in these animals varied from 2.25 – 2.75 mm medial to bregma, and 2 to 2.75 mm rostral to bregma. 300 nl of dextran amine coupled to alexa-488 was pressure injected into the center of this area using a pulled glass micropipette attached to a Picospritzer II (General Valve, Fairfield, NJ), at a rate of 100 nL / min.

Histology

Two weeks after the tracer injection (a time period allowing for complete anterograde transport of corticospinal neurons) animals were perfused with a 4 % PFA solution diluted in PBS. The brain and spinal cord, including vertebral column, were removed and kept overnight in a 4% PFA solution. These were then transferred into a 30 % sucrose solution for several days. Cervical level 7 of the spinal cord was identified using vertebral landmarks and by counting spinal roots. C7 was dissected cut out of the spinal cord and sectioned at 35 um thick sections using a Leica 4500 cryotome at -20^0 C.

A set of sections from each well was then immunolabeled for the tracer. Sections were washed in TBS, incubated in a 1 % triton-TBS with 5 % goat serum for one hour, and then placed in a 1:2500 dilution of rabbit polyclonal anti-αlexa-488 antibody (Jackson Laboratories). This incubation was performed at 4^0 C for 48 hours. Sections were then rinsed and incubated in a 1:200 dilution of secondary biotinylated goat anti-rabbit antibody (Molecular Probes) for 12 hours at 4^0 C. Antibodies were visualized using the ABC kit and DAB peroxidase substrates (Vectastain). Animals that with no staining of the corticospinal tract within C7 were discarded from all analyses (behavioral, motor reorganization and plasticity). 7 animals from the control group, 10 animals from the rehabilitation group, and four animals from the lesion only group were discarded for this reason, resulting in a total of 13 animals in the control group, 16 animals in the lesion group, and 10 animals in the lesion with rehabilitation group.

Image Analysis

Sections were mounted, dehydrated, coverslipped, and visualized at 200 x magnification. Captured images were automatically processed in ImageJ to remove noise and non-fiber staining. Processing involved the following steps: first, the low masking threshold was set to mean pixel density plus 2 standard deviations image (Fig. 2A-2B). Next, particles with a size greater than 11 pixels and a circularity greater than 0.3 were removed from that masked image, resulting in an image that fairly closely matched actual fiber staining with only minimal background (Fig. 2C-D). The processed images were then automatically analyzed for pixel density. In a random sampling of 21 images, unprocessed images were counted by hand to verify that the automated results derived from the processed images were comparable to data arrived at manually on unprocessed images. There was a significant correlation between density measurements arrived at automatically, and fiber counts done by hand ($p < 0.01$; $R^2 = 0.36$), suggesting the automatic images were an acceptable method of quantifying density within the spinal cord.

RESULTS

Rehabilitation training following a cortical injury resulted in an expansion of the size of the rostral forelimb area representing distal forelimb areas (wrist or digit) in the hemisphere controlling the preferred limb (Fig. 3; ANOVA $p < 0.01$). Rehabilitation resulted in a total area of 0.8 ± 0.1 mm^2, a 280 % increase in size relative to non-rehabilitated lesioned animals, and a 600 % increase compared to

control animals (Tukey post-hoc test demonstrated significant differences between the Rehab group and both other groups).

Labeling of the corticospinal tract shows comparable density and innervation patterns in control intact animals, animals with motor cortex lesions, and animals with rehabilitation training following injury (Fig. 4).-No significant differences were found in the density of axons in the grey matter across the three groups (Fig. 5A; ANOVA $p = 0.4$). Further, there was no significant difference in the center of mass of axons in either the horizontal or vertical directions (Fig. 5B-C; ANOVA $p = 0.2$ horizontal direction, $p = 0.4$ vertical direction), indicating no major difference in distribution of corticospinal axons after motor cortex lesions.

DISCUSSION

Findings of this study suggest that axonal plasticity of the corticospinal tract does not underlie the early functional recovery and map plasticity that is associated with rehabilitation after injury. Previous studies in the lesioned sensory system demonstrate extensive synaptic rearrangements of spared axons at several levels of a polysynaptic relay, evidencing plasticity of both primary projecting neurons and their transsynaptic partners in the nucleus gracilis, thalamus and cortex. In contrast, the corticospinal motor system exhibits plasticity of cortical maps after a partial lesion, but no plasticity per se in the projecting axons that constitute the mechanism for supporting functional recovery [24]. These findings suggest that differing brain regions may recruit fundamentally different

mechanisms to support recovery. Lesions to dorsal root sensory systems result in plastic responses from spared sensory axons at polysynaptic levels, whereas central motor cortex lesions result in reallocation of functional forelimb activation to rostral forelimb representations, but no change in axonal anatomy in the rostral cortex giving rise to this behavioral compensation.

There are several possible explanations for this finding. The simplest explanation is that motor reorganization that occurs within rostral forelimb area, and the existing population of corticospinal neurons projecting from this area are sufficient to assume the new behavioral function that is transferred there following an injury to the caudal forelimb area. Thus, there is no behavioral drive to elicit sprouting of these projections to mediate the observed functional recovery.

It is also possible that sprouting of the corticospinal tract occurred in a way that was not appreciated by the methods used in this study. A very subtle change in the distribution pattern, or a small increase in the overall density of axons may not have been measurable. Likewise, sprouting across the midline from the non-rehabilitated side of the cortex to the rehabilitated side would also not have been appreciated, as only the cortex contralateral to the preferred limb was traced.

Studies have demonstrated axonal plasticity in sensory, corticorubral and corticostriatal projections following unilateral brain injuries [8-11]. It is possible that injury coupled with rehabilitation training results in enhanced axonal plasticity within these other motor or sensory areas and therefore greater

functional recovery. However, ICMS-derived motor maps are believed to represent the cortical motor output to intra-spinal networks, communicated via the corticospinal tract [30, 31]. Thus, axonal plasticity of other afferent or efferent systems of the motor cortex would probably have little relevance to the motor map reorganization that occurs following rehabilitation training after injury. Thus, though it is possible that axonal plasticity of corticostriatal, corticorubral, or sensory neurons allowed for enhanced functional recovery following rehabilitation training, it is less likely that this plasticity is associated with motor map reorganization.

One final caveat to this study is that axons were traced only 5 weeks after the cortical injury. Many previous studies demonstrating axonal plasticity after injury waited many months to years after the injury to assess such plasticity [4, 12]. Thus, it is possible that corticospinal tract plasticity would have occurred if assessed many months after the injury, as oppose to only 7 weeks. However, the time course determined for studying the corticospinal tract was based on evidence from previous studies that 7 weeks of rehabilitation training is sufficient to mediate both functional recovery and motor map plasticity [24]. Thus, this study may better reflect whether axonal plasticity mediates behavioral recovery and motor cortex reorganization following injury, than chronic injury studies with longer time points and no behavioral measures [12].

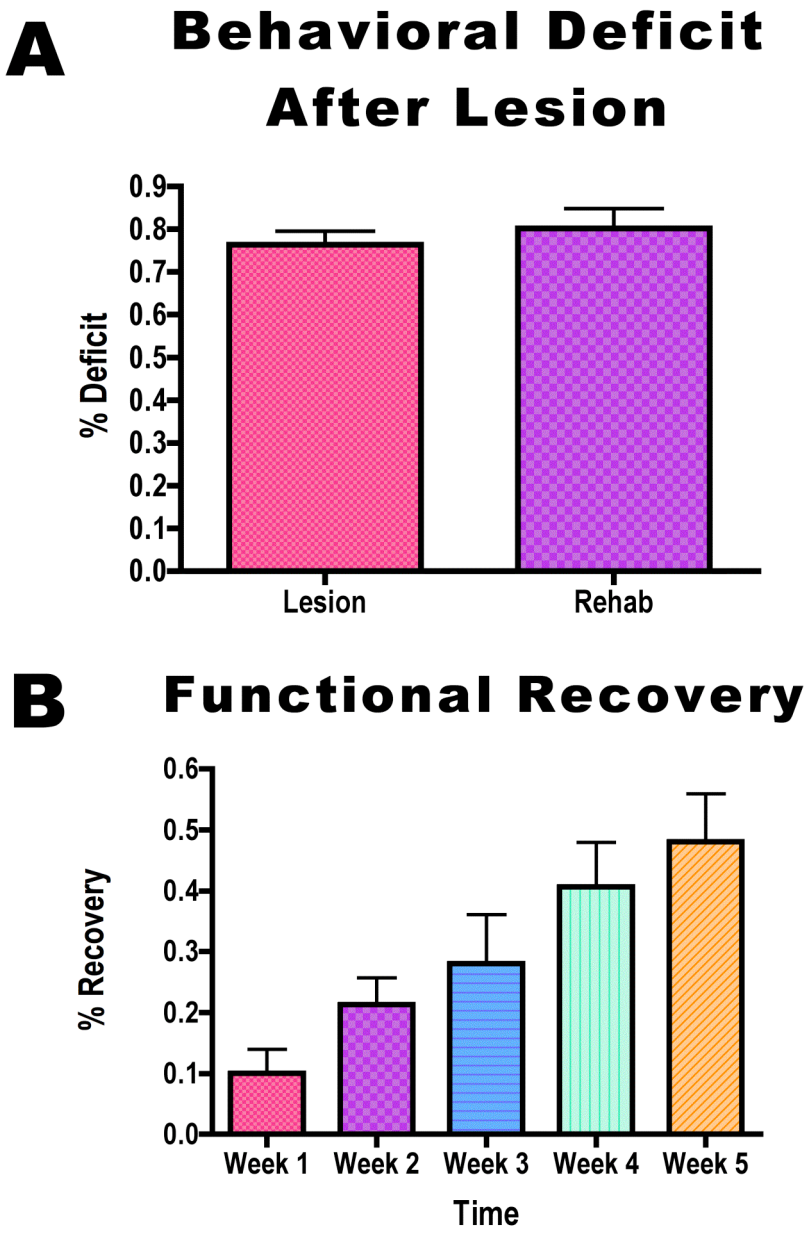

Figure 4.1 Behavioral Deficit and Functional Recovery Following Lesions of the Caudal Forelimb Area. 2 weeks after a lesion to the caudal forelimb area, animals were given three days of assessment on the skilled reach task. Animals were then split into two groups according to equivalent behavioral deficits, one group to be a lesion only control, and the other to undergo 5 weeks of rehabilitation training (A). Rehabilitation training resulted in functional recovery of 42 % compared to prelesion performance (B).

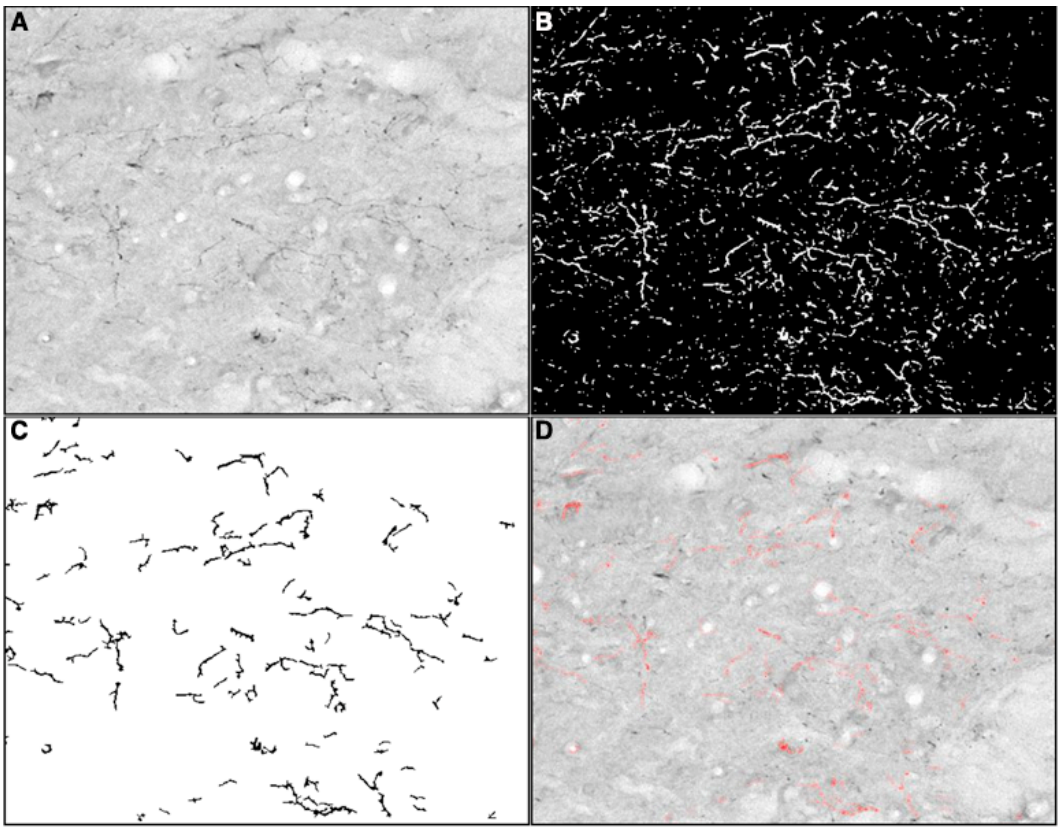

Figure 4.2: Automatic Processing of Labeled Fibers Within C7 of Spinal Cord.

Following acquisition of images at 200X (A), images were automatically processed using ImageJ. Sections were masked at greater than two standard deviations from the mean pixel density (B). Next, noise was removed by setting particle exclusion criteria at greater than 11 pixels, and 0 - 0.3 circularity (C). As a measure of how well processing algorithm worked, processed sections were overlaid onto the actual images (D).

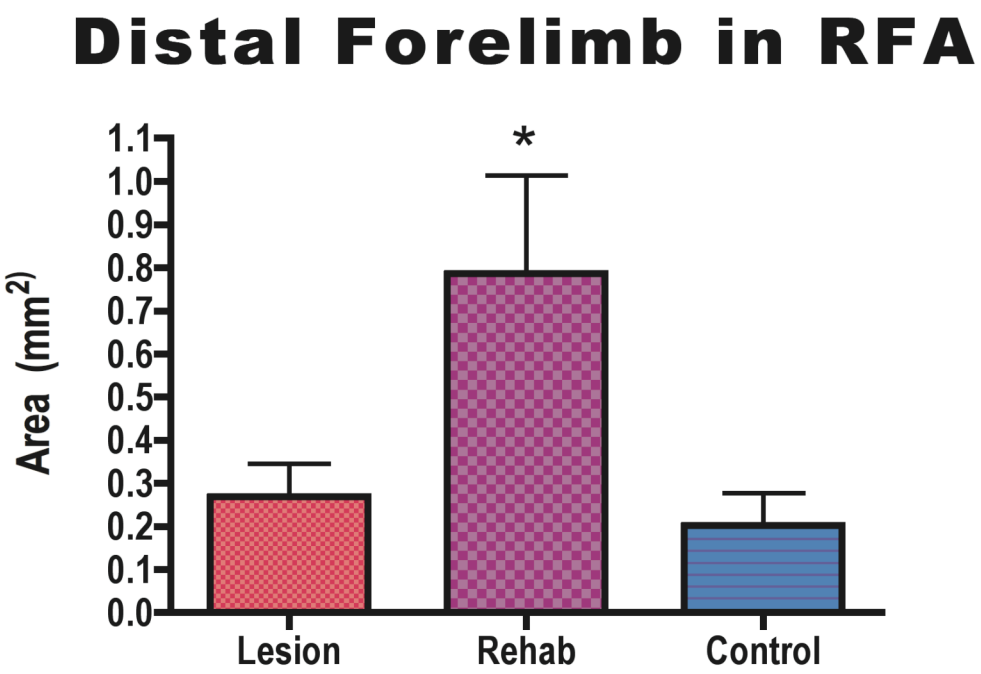

Figure 4.3: Distal Forelimb Representation Within Rostral Forelimb Area Expands Following Rehabilitation Training After Cortical Injury. Following 5 weeks of rehabilitation training, the distal forelimb representation (wrist and digits) within the cortex contralateral to the preferred limb reorganizes compared to animals with a lesion and no rehabilitation training, and with intact controls (ANOVA $p < 0.01$). Rehabilitation resulted in a total area of 0.8 ± 0.1 mm2, a 280 % increase in size relative to non-rehabilitated lesioned animals, and a 600 % increase compared to control animals (Tukey post-hoc test demonstrated significant differences between the Rehab group and both other groups).

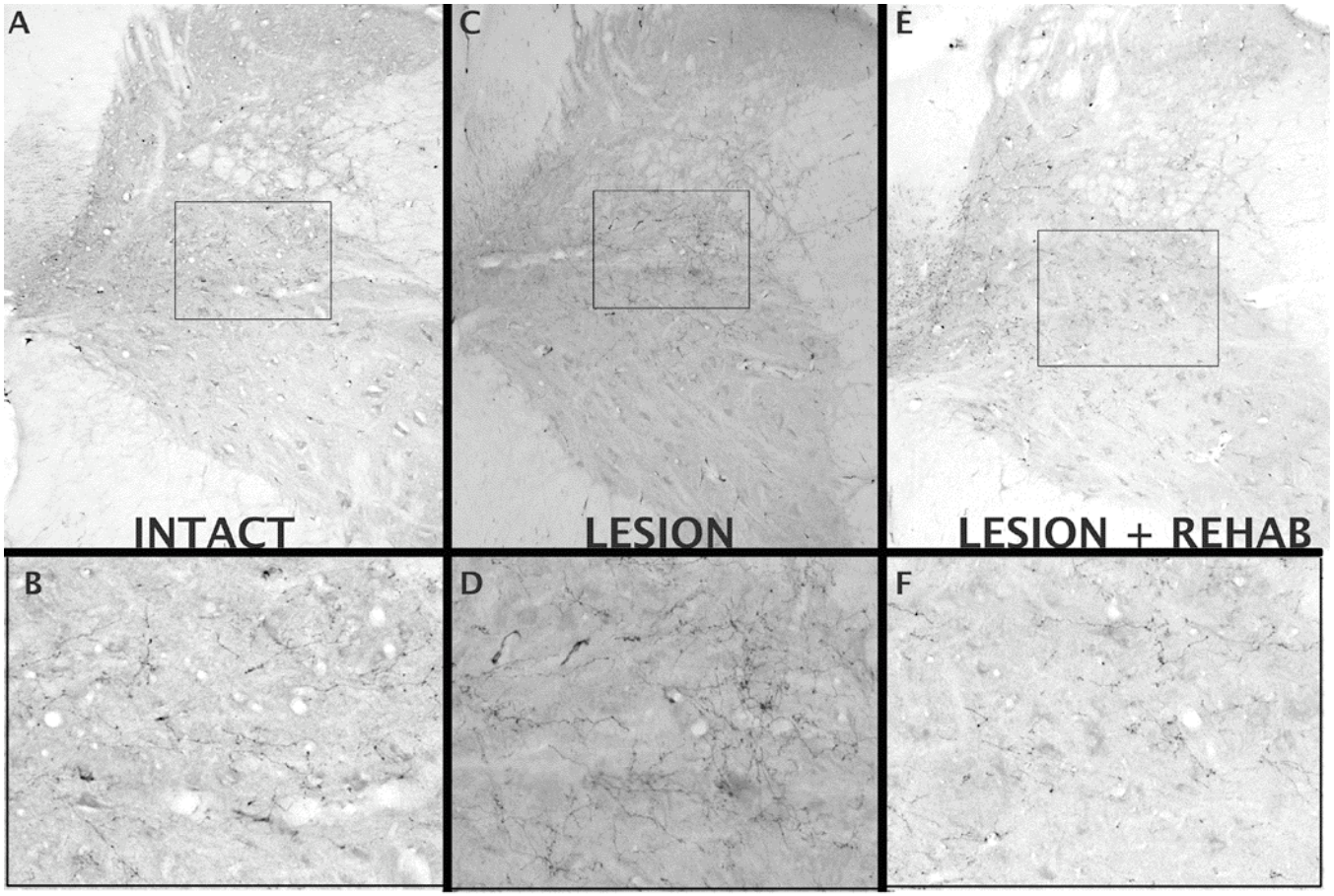

Figure 4.4: Corticospinal Axonal Profiles Within Cervical 7 in the Absence of Injury, Following Injury to the Caudal Forelimb Area, and With Rehabilitation Training Following Injury.

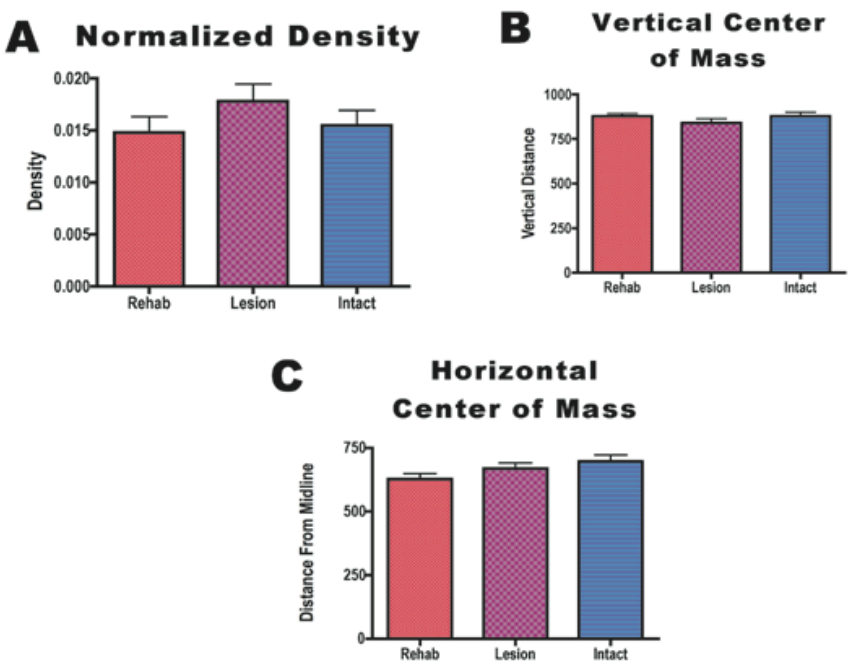

Figure 4.5: A Cortical Lesion With or Without Rehabilitation Training Results in no Change in the Density or Distribution of Fibers Within the Cervical Level 7 of the Spinal Cord.

The normalized labeling density within C7 of Intact animals is not significantly different than in animals either with an injury alone, or than in animals with injury followed by rehabilitation training (ANOVA $p = 0.4$). Further, there were no differences in the center of mass in either the vertical (Fig. 2B) (ANOVA $p = 0.4$) or horizontal (Fig. 2C) (ANOVA $p = 0.2$) directions across any group.

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Chapter 5:
A Novel Form of Motor Cortical Plasticity That Correlates with Recovery of Function After Brain Injury

ABSTRACT

To investigate functional mechanisms underlying cortical motor plasticity in the intact and injured brain, we utilized "behaviorally-relevant", long-duration intracortical microstimulation. We now report the existence of complex, multi-joint movements revealed with 500 ms duration intracortical stimulation in rat motor cortex. A consistent topographic distribution of these complex motor patterns is present across the motor cortex in naïve rats. We further document the plasticity of these complex movement patterns following focal cortical injury, with a significant expansion of specific complex movement representations in response to rehabilitative training following injury. Notably, the degree of functional recovery attained following cortical injury and rehabilitation correlates significantly with a specific feature of map reorganization, the ability to re-express movement patterns disrupted by the initial injury. This evidence suggests the existence of complex movement representations in the rat motor cortex that exhibit plasticity after injury and rehabilitation, serving as a relevant predictor of functional recovery.

INTRODUCTION

The ability of sensory and motor cortices to dynamically reorganize is an important component of normal learning and recovery after neural injury [1-7]. Cortical reorganization, or map plasticity, is believed to reflect the integration of molecular, cellular, synaptic and anatomic plasticity over large populations of neurons [7-11]. Map plasticity in the motor cortex is traditionally observed as a reorganization of cortically encoded muscle groups (such as those controlling wrist, elbow, shoulder, etc.) identified by intracortical microstimulation (ICMS) [3, 5, 12]. For example, skilled motor training of the distal forelimb in rats increases the proportion of ICMS-evoked distal forelimb movements in the motor cortex [3, 5, 12].

Plasticity of ICMS-derived cortical maps also occurs in association with functional recovery after brain injury. In both rats [4, 8, 13-16] and primates [2, 17], discrete lesions placed within the forelimb area of motor cortex result in functional deficits in skilled reaching performance. Subsequent rehabilitative training promotes functional recovery and results in the reorganization of forelimb motor representations [2, 4, 18-21]. Disrupting cortical reorganization significantly reduces recovery [4], and ablating or inactivating newly responsive forelimb sites reinstates the functional deficit after recovery has occurred [4, 15, 17, 22], suggesting that the reorganization was responsible for the functional recovery. Despite the substantial evidence demonstrating reorganization of ICMS derived cortical maps following focal brain injury, it is still not clear how this

reorganization contributes to behavioral recovery. In part, this lack of understanding can be ascribed to a limitation inherent to the technique: ICMS elicits contractions of individual muscles, but does not reveal integrative aspects of neural encoding such as coordinated actions across muscles, movement velocity or movement force [23-27].

Recently, Graziano and colleagues have reported that intracortical stimulation in primates over "behaviorally-relevant" time spans of 500 ms elicits complex, multi-joint movements coordinated through space and time [28-34]. Stimulation of specific cortical sites causes coordinated limb movements toward identical locations and postures, regardless of the initial limb position. Moreover, a topographical organization of complex movements across both motor and premotor cortex was identified. Though controversial [35, 36], it has been proposed that long-duration stimulation reveals complex aspects of motor encoding previously unattainable through short duration ICMS [28, 29, 35]. In this study we used long-duration intracortical microstimulation to identify potential neural mechanisms underlying motor learning and functional recovery after cortical injury. We now report the existence of topographical maps generating complex, multi-joint movements within the motor cortex of rats, and a significant correlation between the reorganization of disrupted complex representations and behavioral recovery after brain injury.

RESULTS

A total of 58 adult male F344 rats (225 – 250 grams starting weight) were used in this study in three separate experiments: 1) To characterize the types and topography of movements evoked by a long-duration stimulation paradigm, 11 naive animals were mapped using a long-duration stimulation paradigm [29]. 2) To assess the effects of motor learning on the distribution of these complex movement representations, 11 animals were trained to perform a skilled forelimb reaching task and were mapped using ICMS techniques; 10 additional animals served as untrained, naïve controls. 3) To examine whether plasticity of complex movement representations relates to functional recovery after brain injury, 26 rats received focal cortical lesions and rehabilitative training as described previously [4]. Briefly, rats were trained to acquire a skilled forelimb reaching task and then received bilateral focal electrolytic lesions targeting sites controlling distal (wrist) forelimb movements [4]. One group of animals (n = 15) then received 5 weeks of rehabilitative training, while a second group (n = 11) was not rehabilitated. Both groups were then mapped using the long-duration stimulation paradigm to assess potential plasticity of complex movement representations and their correlation with functional recovery.

Qualitative Features of Forelimb Movements Elicited by Long-duration Stimulation

To determine whether *complex* movement representations exist in rat motor cortex, long-duration (500 msec) ICMS was applied, as previously described [29]. At each site the stimulating current was gradually increased from 1 uA until movement was detected, and the current was then raised to magnify

that movement and facilitate characterization. Notably, raising the current did not alter the quality or sequencing of the evoked complex movement. As with short-duration stimulation [4, 5], a site was deemed non-responsive if movement could not be elicited with a maximum stimulus of 200 uA.

Long-duration stimulation elicited a variety of movement patterns, ranging from "simple movements" (muscle contractions across a single joint), to very complex movement patterns across multiple joints. Three patterns of complex movements were commonly observed, described here as *reaching*, *grasping* and *retraction*(Figs. 1-3).(Fig. 1). Along with these complex movements, long-duration stimulation also produced sequential combinations of these reaching, grasping and retraction movements (Figs. 2, 3). For example, a single 500 msec stimulation could elicit a ***sequentially coordinated*** reach, grasp, and retract movement (Fig. 2; supplementary video). Other **complex movement sequences** included combinations of reaching followed by grasping, or grasping followed by retractions. Long-duration stimulation rarely elicited dysynergic or apparently random patterns of motor activation (i.e. stimulation never resulted in a grasp coinciding with, or preceding, a reach). Rather, elicited movements nearly always occurred in the sequence of reach-grasp-retract, resembling patterns spontaneously generated by animals during purposeful behaviors. Complex movement sequences (reach-grasp, grasp-retract, reach-grasp-retract) required lower stimulation intensities than individual complex movements, (117 ± 10 uA vs. 141 ± 10 uA, respectively, $p < 0.01$), suggesting that sequential movements are not caused simply by cortical "overstimulation."

To compare the topography of movements elicited with long-duration (500 msec) and short-duration (30 msec) microstimulation, motor maps were next derived using a standard short-duration stimulation paradigm. Short duration motor maps were similar to those described previously [37, 38] and included the presence of two distinct forelimb areas (caudal and rostral forelimb area), separated by a region associated with vibrissa and neck movements (Fig. 3A). Forelimb movements evoked using short-duration ICMS typically consisted of brief twitches of the elbow or shoulder (proximal limb movements), wrist (distal limb movements), or simultaneous twitches of both muscle groups. The mean stimulation threshold measured in previous experiments for evoking forelimb (elbow) movements with short-duration ICMS was 57.7 ± 3.1 uA (4, 5).

Notably, a distinct topography of complex movements existed (Figs. 3B-C). Long-duration stimulation in the rostral-most portion of the forelimb area (classically referred to as the rostral forelimb area) most often elicited *grasping* movements; stimulation within the lateral aspect of the classically defined caudal forelimb area elicited *retraction* of both the wrist and forepaw; and stimulation in a region intermediate between the rostral forelimb area and caudal forelimb area typically elicited forward *reaching* movements of the forelimb and paw. Complex movement sequences described above were exclusively elicited by stimulation within the rostral portions of the forelimb area (Figs. 3B-C).

As noted above, long-duration stimulation also evoked movements that were simpler in nature, defined as movements across only one joint. Some of these movements, such as wrist and elbow contractions, were similar in form to

"twitches" observed after short-term stimulation. Other, single-joint movements, such as supination of the arm or extension of the elbow or hand, are never observed during short-term stimulation. These simple, single-joint movements were distributed across both caudal and rostral forelimb area (Fig. 3B), and their topography was not examined in detail in the present study.

Thus, complex movements are produced by long-duration stimulation consisting of either ***individual complex movements*** (reach, grasp or retract) or ***complex movement sequences*** (sequential combinations of reach, grasp and retract). The topographic distribution of individual complex movements (reach, grasp and retract), as well as complex movement sequences (reach-grasp, grasp-retract and reach-grasp-retract), is consistent across animals (Fig. 3C), suggesting a common neural organization underlying these movement patterns.

Complex Movement Representations Do Not Expand Following Normal Motor Learning

Having identified a general topography of complex multi-joint movements evoked by long-duration stimulation within the motor cortex of naïve rats, we next investigated whether the distribution of these complex movements changes following the acquisition of a skilled motor behavior [5]. The forelimb reaching task requires animals to use their forepaw to retrieve small food pellets from a platform next to the testing chamber [5]. Behavioral and EMG studies have suggested that success in reaching, grasping, and retrieving food pellets requires the animal to coordinate and modify complex motor synergies [39-41]. Thus, it is possible that skilled motor learning would be associated with an increase in

cortical resources devoted to the generation of complex movements, reflected by an increase in the total cortical area where complex movements could be elicited by long-duration stimulation.

Acquisition of the motor skill was measured as percent success in pellet retrieval. Animals acquired a level of skilled reaching performance comparable to previous reports (mean 70 $\pm$ 5% retrieval accuracy)[4, 5]. Skilled motor learning did not alter the distribution or qualitative nature of complex movements and sequences (reach, retract, grasp, or any combination thereof). The mean area of cortex coding for all complex movements did not differ between naïve and trained animals (2.9 ± 0.3 mm^2 vs. 2.7 ± 0.3 mm^2, respectively; p = 0.6). Further, the mean area of cortex coding for simple movements did not change between naïve and trained animals following skilled motor learning (2.9 ± 0.2 mm^2 vs. 2.6 ± 0.3 mm^2, respectively; p = 0.4). Average stimulation amplitudes used to evoke complex movements also did not differ between naïve and trained rats (138 ± 5 μA vs. 128 ± 6 μA, respectively, p = 0.2). See Supplementary Table 1 for a full description of movement topographies in these animals.

Plasticity of Complex Movement Representations Correlates With Functional Recovery Following Focal Cortical Injury and Rehabilitation

Whereas plasticity of complex movement representations was not identified in association with normal motor learning, we postulated that reorganization of complex movement representations could occur as a neural mechanism underlying functional recovery following injury. Evidence suggests that cortical plasticity occurring in response to injury and rehabilitation may differ

from that arising during normal learning [13, 18, 42-45]. Further, more extensive cortical reorganization is often required to support functional recovery after injury relative to that required for normal skilled motor learning [4, 5].

Animals were subjected to a focal injury and rehabilitation paradigm as previously described [4]. After 3 weeks of training to acquire a skilled reaching behavior, rats received bilateral electrolytic lesions of the lateral aspect of the caudal forelimb area, a region associated primarily with retraction movements (Fig. 4). To control for possible variability in lesion size, animals were reassessed for 3 days (beginning 2 weeks after the initial injury) on the reaching task to establish the magnitude of the functional deficit defined as follows:

$$\% \text{ Deficit} = \frac{(\text{pre-lesion accuracy} - \text{post-lesion accuracy})}{\text{pre-lesion accuracy}} \times 100\%$$

Animals were then divided into two groups (rehabilitated and non-rehabilitated), matched for extent of functional deficit (average deficit = 82.3 ± 3.4 % vs. 80.5 ± 4.1%, respectively; $p = 0.84$). Animals were either subjected to rehabilitation training for an additional five weeks ($n = 15$), or were treated as non-rehabilitated controls for the same duration ($n = 11$); without rehabilitation, rats do not recover forelimb function after this lesion (2, 4). Functional recovery in the rehabilitated group was then calculated as the percent improvement of each animal's initial deficit over the course of rehabilitation as follows [4]:

$$\% \text{ Recovery} = \frac{(\text{post-rehabilitation accuracy} - \text{initial post-lesion accuracy})}{\text{lesion-induced deficit}} \times 100$$

Animals that underwent rehabilitation training exhibited a 51.5 ± 5.3% recovery of function on the forelimb reach task by the fifth week of post-lesion rehabilitation, comparable to previous studies [4]. At the conclusion of rehabilitation training, long-duration ICMS was used to derive maps of complex movement representations. In all cases, the cortex contralateral to the forepaw used for grasping pellets was analyzed. In fewfour animals(n=4) that used both forepaws, each cortex was mapped and the size of the complex movement representations was averaged across hemispheres (after determining that map topographies did not differ significantly between hemispheres, and between unilaterally vs. bilaterally reaching animals; $p > 0.4$ for all comparisons).

In non-rehabilitated animals, focal lesions placed in the lateral part of caudal forelimb area (Fig. 4), centered in the region associated with retraction movements, resulted in a complete loss of stimulation-evoked retraction movements in and around the ablated region (4B-C), and an overall 66% loss in the total cortical area evoking retraction movements (ANOVA $p < 0.01$; Fisher's post-hoc $p < 0.0001$ compared to intact animals; Fig. 4). The rostral forelimb area in these non-rehabilitated subjects showed no reorganization of the complex map, indicated by a lack of change in area of cortex evoking complex movement sequences compared to intact controls (Fig. 4D).

In marked contrast, lesioned and rehabilitated rats exhibited significant plasticity of complex movement representations relative both to intact and lesioned, non-rehabilitated animals (Fig. 4). The number of cortical sites from which complex movement *sequences* could be elicited increased in rehabilitated

animals (Fig. 4D). Rehabilitation also resulted in a 90% increase in the area of cortex specifically evoking retraction movements compared to non-rehabilitated controls ($p < 0.05$ Fisher's post-hoc; Fig. 4E). Correlational analysis between the size of individual movement representations (reach, grasp or retract) and the extent of functional recovery in each rehabilitated subject indicated that functional recovery correlated significantly with the size of cortex specifically encoding ***retract*** movements (Fig. 4F; $R^2 = 0.49$; $Z = 2.93$; $p < 0.005$). Other complex movements (***reach*** or ***grasp***) did not demonstrate a statistically significant correlation with behavioral recovery ($R^2 = 0.21$, $p = 0.09$ for ***grasp***; $R^2 = 0.1$, $p = 0.26$ for ***reach***). Thus, functional recovery correlated best with plasticity specifically associated with restoration of ***retract*** movements within the cortex, the type of complex movement originally eliminated by the caudal motor cortex lesion.

Stimulation intensity thresholds to evoke complex movements did not differ between rehabilitated and non-rehabilitated groups (171 ± 10 uA rehabilitated vs. 164 ± 4 uA, respectively; $p = 0.6$).

DISCUSSION

The present study documents the existence of complex movement representations within the motor cortex of rats, extending previous reports of stimulation-evoked complex movements in primates [29, 34] to the less complex rodent system. Moreover, the present study demonstrates that complex

representations reorganize in response to injury and rehabilitation. Indeed, functional recovery following brain injury directly correlates with the ability of an animal to encode complex movement patterns (in this case, retractions) specifically abolished by the injury. These data thus provide the first demonstration of a measure of motor map plasticity that specifically correlates with recovery of skilled motor behaviors after brain injury.

It is important to consider whether the complex movement patterns elicited by long-duration stimulation reflect the activation of cortical circuitry associated with behaviorally relevant movements. Graziano and colleagues have suggested that stimulating motor cortex for "behaviorally relevant" durations activates interrelated motor networks, potentially eliciting a more realistic depiction of complex motor actions than achieved with short-duration stimulation [35]. They infer that movements elicited by long-duration stimulation are ethologically valid, and electrophysiologically and behaviorally meaningful [30-33, 46]. Others, however, have argued that the use of long-duration stimulus trains may simply lead to nonspecific current spread beyond the original stimulation site, generating seemingly complex movements by randomly activating a large number of spinal motor units [36]. Results from the present study tend to support the former interpretation, for several reasons. One would predict that random spread of current would result in the indiscriminate activation of large numbers of neurons associated with various discrete but non-purposeful movement patterns. Our findings demonstrate that long-duration stimulation paradigms result in reproducible, sequential activation of groups of muscles to achieve what are at

times remarkably complex movements in rats, including sequential reach-grasp-retract movements (see video and Fig. 2). Further, the topographic distribution of these movements is consistent across animals. Importantly, complex movement sequences always progress in an apparently purposeful order, consistent with behaviors the animal actually uses (i.e. reach always precedes grasp, and grasp always precedes retraction of the forelimb). Behaviorally "non-purposeful" sequences (for example, a grasp followed by a reach) are never seen. If multiple individual movements contributing to a complex sequence were truly generated by a random spread of current, one would expect that the order of movements would also be random based upon the pseudorandom selection of stimulation sites within motor cortex. Further, one would expect variability in the order of these sequences between different animals, but these features were not observed. The finding of a direct correlation between the plasticity of complex movement representations and the extent of behavioral recovery after lesions further supports the physiological relevance of long-duration cortical stimulation. Because cortical excitability, measured by stimulation threshold intensity, was equivalent in rehabilitated and non-rehabilitated animals, the plasticity cannot be explained by differences in random current spread between the two groups. All of these arguments strongly support the notion that long-duration stimulation reveals a physiologically relevant measure of motor function.

Plasticity of complex movement representations did not occur as a function of normal skilled motor learning in this study. It has however been reported that plasticity of "muscle synergies" occurs following motor learning

[41], and it remains possible that patterns of precise muscle activation, measured by electromyographic recording, would reveal plasticity associated with normal learning using long-duration stimulation paradigms, a possibility that can be addressed in future studies.

Notably, significant plasticity of complex motor representations was readily apparent after rehabilitative training following cortical injury. Rehabilitation training produced a significant increase in the amount of cortex evoking complex movement sequences in comparison to both non-rehabilitated lesioned animals and intact controls. This expansion resulted in a restoration of stimulus-evoked retraction movements in the cortex of rehabilitated animals. Further, the extent of functional recovery following brain injury significantly correlated with the degree of plasticity associated specifically with retraction movements. It is important to note that, while previous studies using short duration (30 msec) ICMS have reported cortical reorganization following a lesion [2, 21], no significant correlation has been reported between the magnitude of cortical remodeling and the extent of functional recovery [4]. Thus, the plasticity of complex motor sequences identified in this study appears to represent a measure of motor encoding that actually reflects behavioral performance. Future studies of detailed kinematics of forelimb movement [39] could be useful in understanding the contribution of complex motor representations to normal function and plasticity after cortical injury. These findings shed light on both mechanisms and potential limitations of cortical plasticity related to functional recovery after nervous system injury, with implications for the design of strategies

to promote recovery in humans. Complex motor actions may require specific training to optimally recover after cortical lesions: complex post-injury training could lead to better recovery than repetition of simple motor acts in rehabilitation programs. This is a testable hypothesis in the clinical realm.

MATERIALS AND METHODS

Behavioral Training and Rehabilitative Testing

Motor training was carried out using single pellet retrieval boxes as previously described [5, 47]. This task requires animals to use the forepaw to reach through a small slit in a plexiglass chamber, grasp, and retrieve a small food pellet positioned on a platform near the chamber. During the acquisition phase of testing, rats performed 60 reaches per day, five days per week, for 3 weeks. Rehabilitative training consisted of 40-50 trials per day, 5 days per week, for 5 weeks.

Motor Cortex Lesions

The focal motor cortex lesion used in this study is a modification of lesion paradigms used by others [48-50]. Small electrolytic lesions were made bilaterally at 2 sites (Site 1: A/P = 0, M/L = ±3.5mm; Site 2: A/P = +1.5 mm, M/L = ±3.5 mm relative to Bregma), specifically targeting the distal forelimb representation in caudal forelimb motor cortex[4]. Bilateral lesions were performed to eliminate the possibility that rats would switch paw preference to the

unaffected hemisphere. At each site, a 100μm, teflon-coated, stainless steel electrode was initially lowered to a depth of 1.7mm and 1mA DC current (Grass Model DCLM5A) was passed for 20 sec. The electrode was raised 1 mm and current was applied for another 20 sec.

Functional ICMS Mapping

For all mapping procedures, animals were anesthetized with ketamine hydrochloride (70 mg/kg ip) and xylazine (5 mg/kg ip) and received supplementary doses of the ketamine/xylazine mixture as needed. Pulled glass microelectrodes (input impedance ~0.5 M-Ohm at 300Hz), filled with 3M NaCl, and containing a 125 μm chloride silver wire, were used. Microelectrode penetrations were made at 500-μm intervals at a depth of ~1,800 μm (corresponding to cortical layers V-VI).

To obtain standard somatotopic maps using short-duration stimulation, a 30-ms train of 200-μs duration monophasic cathodal pulses was delivered at 333 Hz from an electrically isolated, constant current stimulator (AMPI Inc. Isoflex, Jerusalem, Israel) under the control of a programmable pulse generator (AMPI Inc.). Two pulse trains were delivered 1.2 sec apart, with additional pulse trains delivered as needed to assess body movements evoked by the stimulation. Evoked movements were examined with the animal maintained in a prone position and limbs free. At each penetration site, the stimulating current was gradually increased until a movement could be detected (threshold current). Average stimulation thresholds measured in previous experiments for evoking forelimb

(elbow) movements with short-duration stimulation was 57.7 ± 3.1 (4, 5). If no movement could be detected up to 200 uA, the site was defined as "nonresponsive".

To identify complex motor movements using long-duration stimulation, a 300 to 500 ms train of 200 us duration bipolar pulses was delivered at 200-333 Hz. Bipolar pulses are used to minimize damage that may occur during long-duration stimulation [29]. No differences were detected when changing either the stimulation time (300 vs. 500 ms) or the frequency of stimulation (200 vs. 333 Hz), similar to findings reported by Graziano using stimulation durations between 500 and 1000 msec. Evoked movements were examined with the animal supported in a fixed position in an elevated stereotaxic frame. At each site the stimulating current was gradually increased until a movement could be detected. Once a movement was detected, the current was raised to optimize that movement and ease its characterization.

Characterization of Evoked Movements

Movements were visually monitored and identified during mapping sessions or videotaped at 30 frames/sec. Videotaped movements were analyzed frame-by-frame using Quicktime and iMovie software. Complex movement sequences too difficult to visually characterize were analyzed by digitizing joint positions frame-by-frame in NIH Image software. To standardize movements from different animals and at different levels of camera magnification, movements were calibrated to each subject's arm length. "Reaches" were defined

as movement of the elbow in the horizontal direction over a distance exceeding 10% of the subject's forearm length. "Retracts" were defined as movements in the opposite direction over a distance exceeding 10% of the subject's forearm length. "Grasps" were defined as a change in angle of the digit joints by more than 30º. Other movements, including contraction, supination, pronation and extension of both the arm and wrist were grouped together as "non-complex" movements.

Statistics

Multiple group comparisons were made using analysis of variance, with a significance threshold of $p<0.05$. Post-hoc comparisons were made using Fischer's least square difference. Two-group comparisons were made using unpaired, two-tailed t-tests. Regression analysis was used to test correlations, using Statview II software.

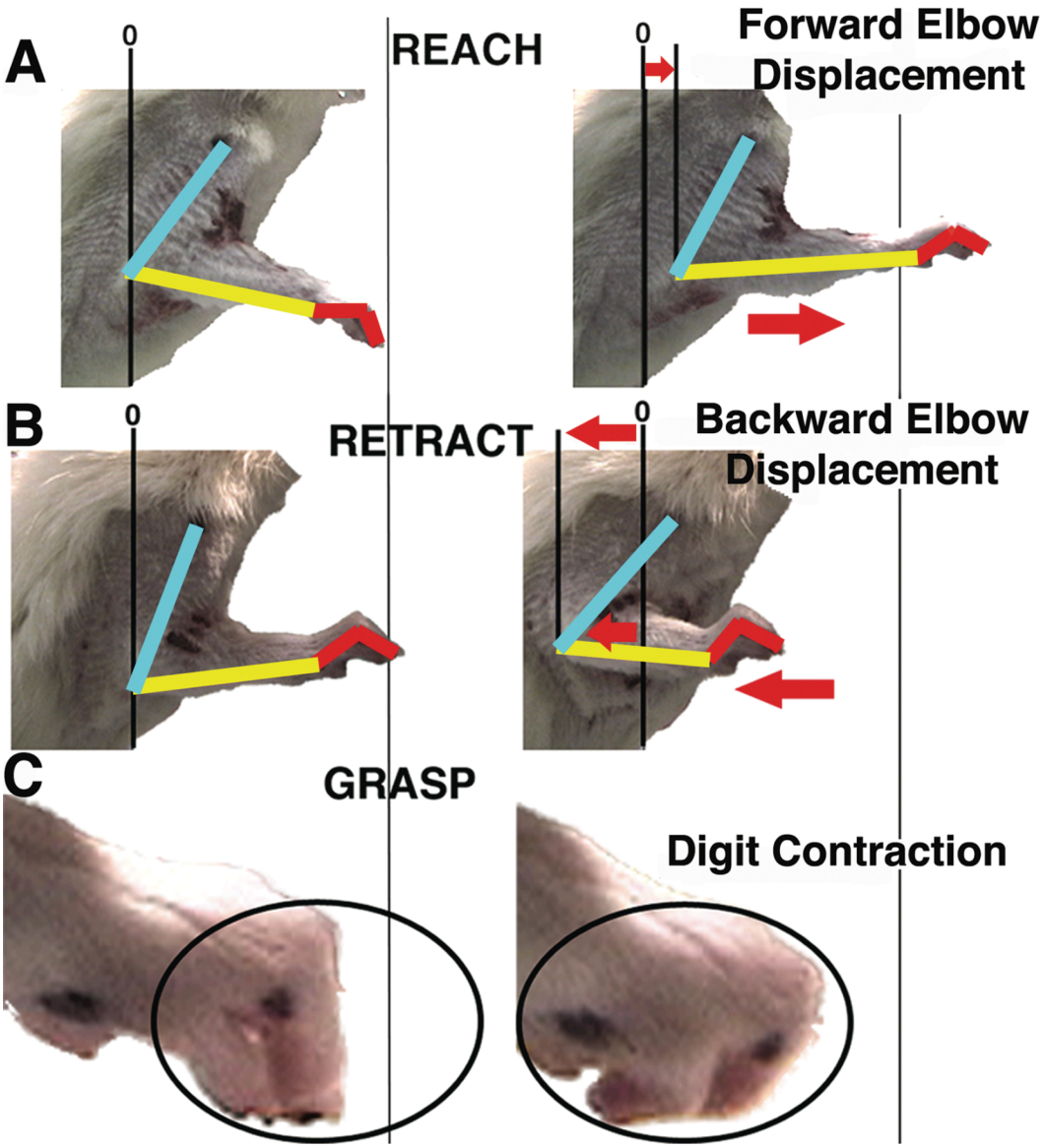

Figure 5.1: Complex Movements Elicited by Long-duration Microstimulation.

(A - C) Three types of complex movements evoked by long-duration stimulation within motor cortex. Complex movements elicited by long-duration microstimulation occur across multiple joints. **(A)** Reaching movement characterized by rostral displacement of the elbow and shoulder, without change in wrist configuration. **(B)** Retraction characterized by caudal displacement of the elbow and forepaw. **(C)** Grasping movement characterized by contraction of all digit joints simultaneously.

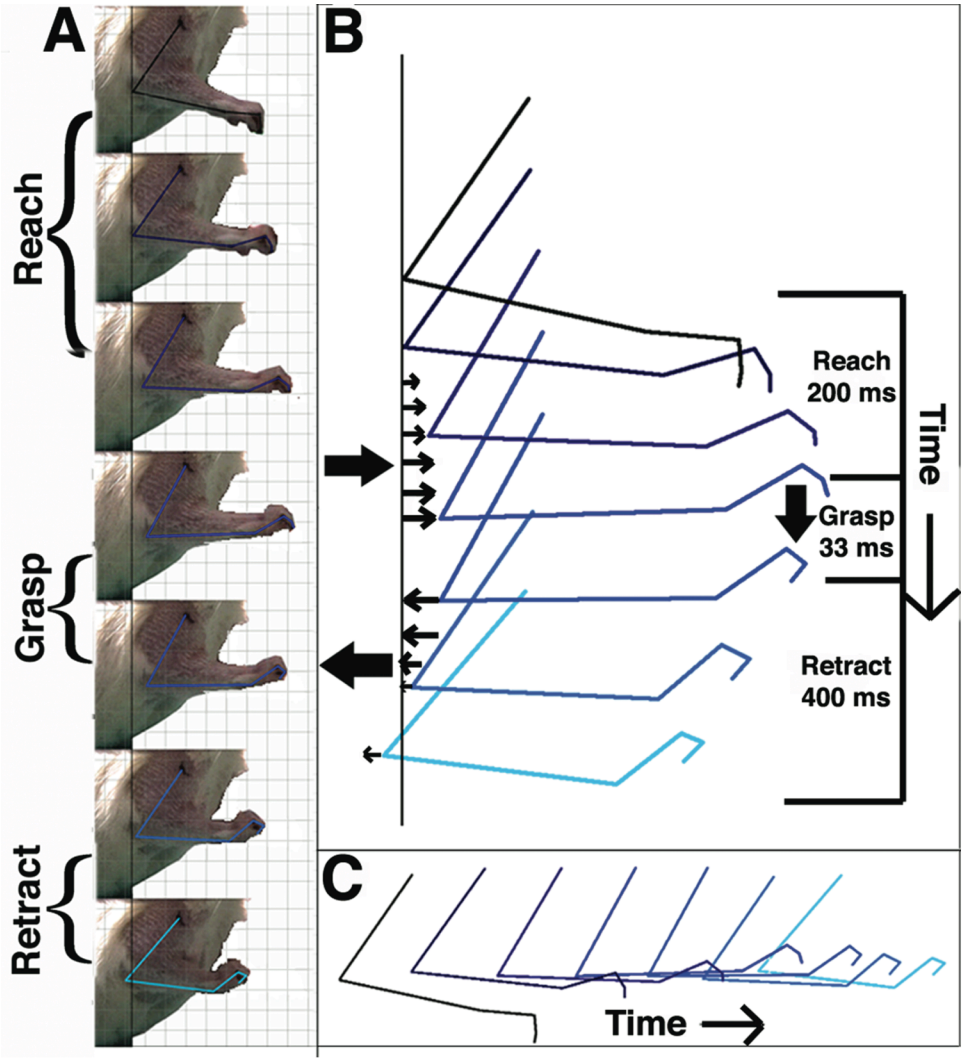

Figure 5.2: Coordinated Sequence of Complex Movements Elicited by Prolonged Simulation in Rostral Forelimb Area of Motor Cortex.

(A) Coordinated sequence of reach, grasp and retract movements elicited by a single 500 msec stimulus within the rostral portion of the motor cortex. (B) and (C) illustrate, using a digitized kinematic analysis, the temporal sequence of this complex movement. Repeated stimulation at the same site elicited the same sequence of complex movements.

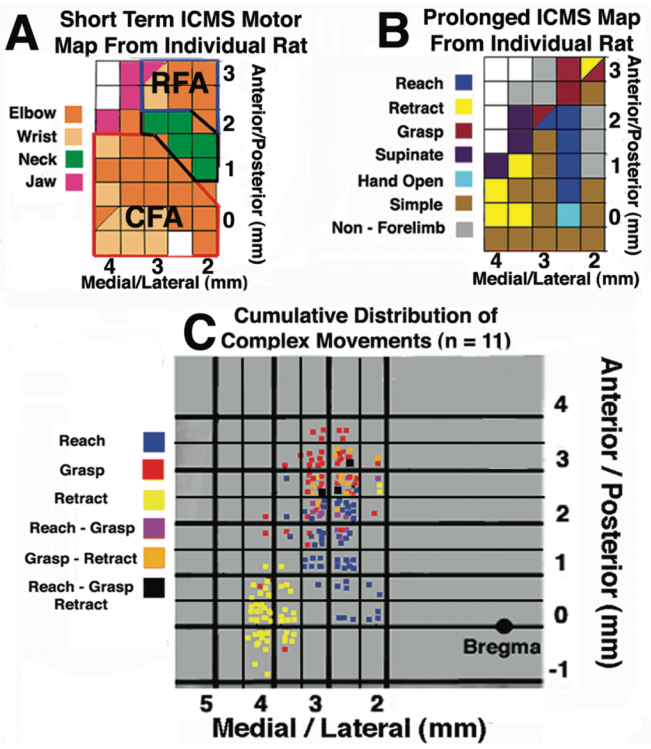

Figure 5.3: Comparison of Cortical Motor Maps Derived Using Short-duration (30 ms) and Long-duration (500 ms) Intracortical Microstimulation.

(A) Representative motor map of forelimb motor cortex derived using a short duration (30 ms) microstimulation paradigm. Two regions of classic forelimb cortex, caudal forelimb area (CFA) and rostral forelimb area (RFA), are separated by intervening neck-responsive sites. Distal forelimb movements (wrist) are generally elicited in lateral caudal forelimb areas. **(B)** Representative motor map derived using the long-duration (500 ms) ICMS paradigm, demonstrating complex movement representations. Complex movements elicited by the prolonged stimulation paradigm include reaching, grasping and retractions. Stimulation in rostral forelimb areas elicited complex sequences of movements such as grasp-retract, or reach-grasp (shown as split squares in the figures). **(C)** Cumulative distribution of complex movement patterns in 11 naïve animals. The distribution of complex movements demonstrates a clear topography across motor cortex: retractions are elicited by stimulation within lateral caudal forelimb area, reaches by stimulation spanning medial aspects of caudal and rostral forelimb areas, and grasps by stimulation within rostral motor cortex. Complex movement combinations are elicited by stimulation within rostral forelimb area (black squares).

Figure 5.4: Focal Brain Injury and Rehabilitative Training Are Associated With Significant Plasticity of Complex Movement Representations.

(A) Characteristic topography of complex movements in intact animals: retractions are located laterally, reaches are-medial, and grasps and complex movement sequences are rostral. **(B, C)** Following a lesion targeting the lateral aspect of the caudal forelimb area, forelimb movements can no longer be elicited in and around the lesion site. **(B)** Moreover, rehabilitated animals exhibit a significant expansion of complex movements (circled) within undamaged rostral forelimb area relative both to naïve control rats **(A)** and non-rehabilitated, lesioned animals **(C). (D)** Quantification of plasticity within the RFA demonstrates that rehabilitative training following a lesion results in significant expansion of *complex movement sequences* (reach-grasp, grasp-retract and reach-grasp-retract) above both naïve controls andnon- non-rehabilitated controls (ANOVA $p < 0.01$; Fisher's post-hoc between rehab vs. nonrehab $p < 0.01$; Fisher post-hoc between rehab vs. pre-lesion control $p < 0.01$). **(E)** The area encoding retraction movements is significantly reduced by 67% following the lesion in nonrehabilitated animals (ANOVA $p < 0.001$; Fisher's post-hoc $p < 0.0001$). Notably, rehabilitative training significantly increased the area of cortex encoding retraction movements ($p < 0.05$ compared to nonrehabilitated animals), partially restoring the specific loss of retraction movements imposed by the lesion. Paralleling the extent of behavioral recovery, the area encoding retraction movements in rehabilitated animals recovers to 64% of intact controls. **(F)** The area of cortex encoding stimulus-evoked retraction movements significantly correlates with the degree of functional recovery in rehabilitated animals ($R^2 = 0.46$, $p < 0.05$). No significant correlation was found between the cortical area encoding reaching or grasping movements and functional recovery (data not shown).

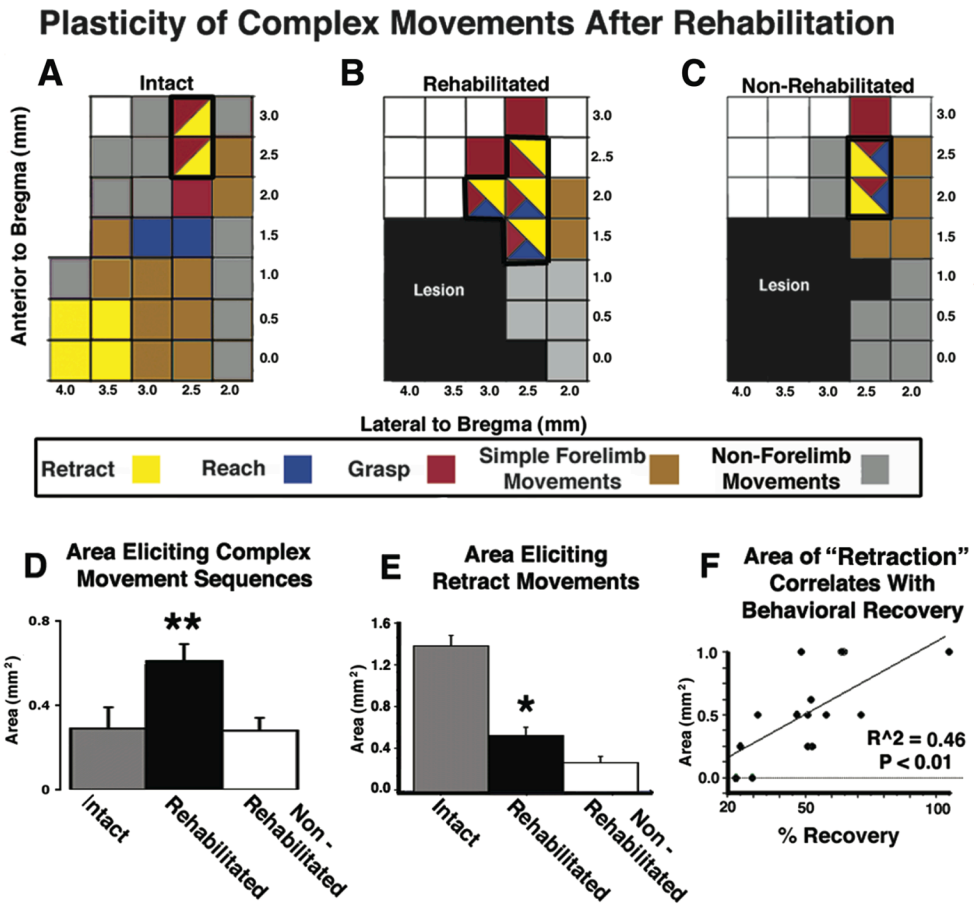
Plasticity of Complex Movements After Rehabilitation
A
Intact
B
Rehabilitated
C
Non-Rehabilitated
Anterior to Bregma (mm)
Lesion
Lateral to Bregma (mm)
Retract
Reach
Grasp
Simple Forelimb Movements
Non-Forelimb Movements
D
Area Eliciting Complex Movement Sequences
Area (mm²)
**
Intact
Rehabilitated
Non-Rehabilitated
E
Area Eliciting Retract Movements
*
F
Area of "Retraction" Correlates With Behavioral Recovery
R^2 = 0.46
P < 0.01
% Recovery

Table 1a demonstrates the mean area for each movement evoked from naïve (control) and trained animals using the long-duration stimulation protocol. Complex movements in the table above are those areas in which stimulation resulted in one movement across multiple joints. Complex movement sequences represent those areas of stimulation wherein multiple "complex movements" were evoked in a coordinated sequence. No significant differences were seen between trained and naïve animals for any movement individually, nor for the total area evoking complex movements and/or movement sequences, as described in the text.

Table 1b demonstrates the mean area for movements evoked following brain injury, either with or without further rehabilitation training. Rehabilitation results in a significant expansion in the total area evoking complex movement sequences. In addition to the complex movements and movement sequences defined above, data was also analyzed for each movement component. This analysis quantified the total area of cortex in which a particular movement could be evoked, regardless of whether it was evoked alone (as a complex movement) or as a component within a complex movement sequence. The total area evoking retract movements and grasp movements expanded significantly following rehabilitation (see text).

Table 5.1: Mean Area of Complex Movements Elicited by Long-Duration Stimulation ($mm^2 \pm SEM$)

1a: Effects of Skilled Motor Learning

	Complex Movements				*Complex Movement Sequences*				*Others*
	Reach	Grasp	Retract	**Total**	Reach-Grasp	Grasp-Retract	Reach-Grasp-Retract	**Total**	Simple Movements
Naïve Animals N = 10	0.70 ± 0.13	0.71 ± .09	1.2 ± 0.05	2.6 ± 0.22	0.13 ± 0.05	0.11 ± 0.04	0.04 ±0.03	0.28 ± 0.06	2.9 ± 0.22
Trained Animals N = 11	0.50 ± 0.17	0.65 ± 0.07	1.3 ± 0.16	2.4 ± 0.26	0.12 ± 0.04	0.12 ± 0.07	0.07 ± 0.04	0.30 ± 0.07	2.6 ± 0.22

1b: Effects of Rehabilitation Following Brain Injury

	Complex Movements			*Complex Movement Sequences*				*Summation for Each Movement Component*			*Others*
	Reach	Grasp	Retract	Reach-Grasp	Grasp-Retract	Reach-Grasp Retract	**Total**	All Retracts	All Grasps	All ***Reaches***	Simple Movements
Non-Rehab N = 11	**Lesion**	0.72 ± 0.14	*Lesion*	*Lesion*	0.16 ± 0.07	0.13 ± 0.05	0.28 ± 0.06	0.28 ± 0.06	0.92 ± 0.13	0.13 ± 0.05	0.13 ± 0.02
Rehab N = 15	*Lesion*	0.93 ± 0.10	*Lesion*	0.07 ± 0.05	0.31 ± 0.09	0.25 ± 0.09	**0.61 ± 0.08** **	**0.54 ± 0.08***	**1.6 ± 0.13** **	0.38 ± 0.1	0.11 ± 0.02

*p < 0.05; **p < 0.01

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Chapter 6:
Conclusion

GENERAL FINDINGS

Role of Acetylcholine in Motor Map Plasticity

The experiments described in this dissertation increase our understanding of the neural mechanisms underlying motor map plasticity and its behavioral implications. First, the work in this thesis helped define the role acetylcholine plays in regulating cortical map plasticity. We had hypothesized, based on previous experiments from our lab and others [1-3], that acetylcholine would be involved primarily in modulating behaviorally driven, cognitively demanding forms of cortical plasticity. We tested this hypothesis in a unique experimental paradigm in which both forms of plasticity were elicited in the same animal within the same cortical domain. Results of these studies demonstrated that lesions of the basal forebrain cholinergic system prevent cortical plasticity associated with a skilled motor learning task (behavioral task), but do not impair plasticity associated with a facial motor nerve lesion (non-behaviorally driven plasticity). Next, we studied the role acetylcholine plays in the development and maturation of the cortical motor system. Because the development of this cortical motor system is associated with the acquisition of motor skills during development, we hypothesized that acetylcholine would be necessary. As hypothesized, lesions of the basal forebrain cholinergic system in juvenile animals

resulted in impairments in the cortical motor system in adulthood. Both of these studies provide a strong foundation to support our hypothesis that the basal forebrain cholinergic system is essential for behaviorally driven forms of cortical map plasticity, but not for non-behaviorally driven forms of plasticity such as peripheral motor nerve lesions.

Behavioral Correlates of Motor Map Plasticity

These experiments raise an interesting question: even though behavior is not necessary to drive cortical plasticity following peripheral injuries, does such plasticity in itself have behavioral or functional implications. Following a facial nerve lesion, animals have a larger cortical area representing neck. Would this translate into enhanced motor abilities? Comparing skilled forelimb reach data from animals with and without a facial nerve lesion, it was observed that lesion-induced plasticity conferred no additional benefit on a skilled-reach task. However these results are difficult to interpret as there is no reason de novo to believe an expanded representation of neck would have any impact on skilled forelimb performance anyway.

Thus, the second focus of this thesis was to develop a behaviorally relevant model of motor cortical plasticity. To do this, we used a long-term intracortical microstimulation paradigm that may reflect a cortical representation of higher-level movement parameters. We first hypothesized that, following skilled motor training of the forelimb, there would be changes in this complex motor map. However, there were no discernible differences in the complex motor

map following skilled motor training. In contrast, animals with a cortical lesion and further rehabilitation training demonstrated a significant expansion in the cortical representation of complex motor movements. The degree to which animals could shift function from caudal to rostral forelimb area correlated significantly with the behavioral recovery those animals achieved. This was especially important as previous experiments describing plasticity in the motor cortex following motor injury have still not demonstrated any correlation with functional recovery.

Axonal Plasticity of Corticospinal Tract Following Cortical Injury

After surmising possible causes of the plasticity associated with complex movements, we hypothesized, based on several pieces of evidence that this plasticity may involve axonal sprouting at the level of the spinal cord of spared corticospinal tract fibers. However, after tracing the corticospinal tract before and after injury, with or rehabilitation, we found no difference in the arborization, density or distribution of corticospinal tract fibers following cortical injury and rehabilitation, as compared to animals with just a cortical injury alone, or even naïve animals. If axonal plasticity does occur as a result of rehabilitation after injury, it is probably occurring within different sensory or motor systems, or following a much longer duration after injury. However, sprouting in an ectopic location from motor cortex is less likely to be a neural correlate underlying motor cortex reorganization.

DEVELOPING A MODEL OF MOTOR MAP PLASTICITY

In these experiments, we have studied many different paradigms to elicit motor cortical plasticity at many different levels. We have found some key differences as well as certain similarities. Using this knowledge, we can begin to construct a complete model of the motor cortex by integrating what is known about the neural correlates underlying function, plasticity and behavior.

Neural Correlates Underlying Motor Maps

Retrograde labeling of the corticospinal tract shows that neurons within the motor cortex controlling a specifc body part exist in a loose topographical organization, and with a great deal of overlap with neurons controlling other body parts. Thus, the precise organization of the motor cortex as demonstrated in motor maps is only partially constructed by anatomical constraints. The other main determinant of this organized topography is the intracortical connectivity that exists between these neuronal populations [4]. During postnatal development of the motor maps, incomplete development of horizontal connections results in smaller maps and many areas where no movements can be evoked via intracortical microstimulation (Fig. 1A) [5]. Following normal development, these lateral excitatory and inhibitory networks develop, resulting in electrophysiological segregation of partially overlapping motor networks across motor cortex (Fig. 1B). These networks are created in two opposing manners. First, neurons within the motor cortex are heavily interconnected through

horizontal excitatory projections originating both in layers 2/3 and layers 5/6. As previously mentioned, activation of this intracortical network is essential to evoke motor responses following intracortical microstimulation. The motor cortex also contains a large horizontally projecting inhibitory network from the same layers 2/3 and 5/6. This inhibitory network is instrumental in refining and defining motor maps. Blocking this inhibition results in coactivation of movements and thus a loss of the normal cortical organization [4] (Fig. 1C).

According to this model, motor map plasticity can be induced in several ways: by a reorganization of anatomical projection patterns via intracortical sprouting; or through strengthening / weakening of intracortical networks within the motor cortex. In the following section, we will discuss evidence for how, for peripheral injuries vs. central injuries vs. skilled motor learning, very different neural correlates are likely to be involved in producing cortical map plasticity. We will explore the evidence for neural correlates underlying these different plasticity inducing paradigms below.

Plasticity Following Peripheral Nerve Injury

1. Role of Inhibition

There is much evidence to suggest that intracortical disinhibition results in plasticity following peripheral lesions [6, 7]. Following a facial nerve lesion, plasticity occurs specifically within areas that already contain lateral intracortical connections, suggesting some tonic suppression of these conections normally [8].

When bicuculline methiodide is injected intracortically to block this tonic inhibition, these suppressed motor representations are revealed [4]. Facial nerve lesions directly result in disinhibition of primary motor cortices of both hemispheres, possibly secondarily to changes in somatosensory input [9, 10]. Minutes following a peripheral lesion in anesthetized animals, intracortical GABA release decreases as measured by microdialysis [11]. Pharamacological manipulations to prevent the decrease of GABA (using benzodiazepines) can prevent plasticity within sensorimotor cortex of humans following peripheral nerve block [11, 12].

Thus, numerous pieces of evidence show that peripheral lesions result in a disinhibition within the motor cortex, and this disinhibition is necessary for cortical map plasticity. However, it is far less clear what drives this disinhibition. A facial nerve lesion, typical of any lower motor nerve injury, would produce a decrease in deep-tendon reflexes and a general decrease in concurrent proprioceptive activity from the affected muscle groups. In normal animals, there is a great deal of tonic proprioceptive activity that feeds very specifically onto motor neurons that control that body part [13, 14]. Thus, a peripheral lesion should result in a decrease of tonic activity to specific neural circuits within the motor cortex, which may well lead to a concomitant decrease of lateral intracortical inhibition from these networks (Fig. 2A-D) [15, 16].

2. Axonal Sprouting

The long-term effects of a peripheral nerve lesion or amputations are very different than the short-term effects. Axonal sprouting has been described at every level of the sensory nervous pathway, including within the spinal cord, brain stem, thalamus and cortex, following long-term peripheral amputations in primates [17, 18]. However, intracortical sprouting in particular was deemed responsible for the sensory cortex reorganization that occurred following these lesions [19]. Long-standing forelimb and hindlimb amputations of motor cortex demonstrate both invasion of adjacent cortical regions as well as an expanded cortical representation of the stump area as determined by ICMS [20, 21]. It has been suggested that the enlargement of the cortical representation of the stump area is, in part, mediated by novel connections formed by the injured peripheral motor axons [22].

3. Clinical Consequences

Researchers have postulated that plasticity following peripheral nerve lesions may have harmful clinical consequences [23]. Many human patients who undergo peripheral limb amputations will continue to have sensations as if their amputated limb is still attached to their body. These sensations include non-painful sensations - including both sensory and motor - as well as painful sensations, often referred to as “phantom limb pain” [24, 25]. Several studies suggest that phantom limb pain is associated with aberrant plasticity within deafferented areas of both sensory and motor cortex [23, 26, 27]. Amputee patients experiencing phantom limb pain demonstrate increased motor and

somatosensory cortical plasticity relative to amputee patients without such pain [28]. More importantly, the degree of map reorganization in both sensory and motor cortices correlates with the extent to which patients experience phantom limb pains [29-31]. On the other hand, non-painful phantom limb sensations were not correlated with plasticity in either sensory or motor cortices [29, 32]. These data suggest that aberrant plasticity is associated specifically with phantom limb pain.

However, there is also data suggesting that insufficient plasticity may be associated with perceived phantom limb sensations. For example, patients experiencing phantom limb pain have an area within motor cortex associated with perceptual phantom limb movements that remains decades following the peripheral amputation [33, 34]. Stimulation of this area using TMS resulted in EMG activity within the stump even while subjects experience the perception of their phantom limb moving [35]. Surgically implanting an electrode to block neural activity in the area associated with phantom limb movements in the motor cortex was effective at reducing phantom limb pain [36, 37]. These studies suggest that the pain may arise from a lack of plasticity within the cortex that fails to remap its perceptual representation with the actual new afferent projections from the stump.

These studies suggest the following: aberrant plasticity may be associated with phantom limb pain, but not with the overall sensation of having a phantom limb; insufficient plasticity, resulting in ectopic activity within the area of motor cortex formerly representing the amputated body part, also results in phantom

limb pain; and finally "phantom" motor activation of the phantom limb exists and seems to be related to the experience of phantom limb pain. Because of the impossibility of asking rats if they "feel", such studies in humans that correlate perceptual phenomenon with plasticity are essential to understanding the correlates of such plasticity. Thus, understanding how to prevent such aberrant plasticity may be instrumental in preventing this pain.

Plasticity Following Skilled Motor Learning

1. Role of Motor Cortex

At its broadest, the motor cortex appears to be important for the planning and execution of motor behaviors. Single unit activity has been correlated with low-level aspects of movement such as joint angle and muscle force, as well as higher-level aspects such as movement direction and velocity, to the highest-level aspect of movement such as end-location. It is most likely that single units within the motor cortex are involved in both lower-level and higher-level aspects of movement [38, 39].

If the motor cortex is primarily responsible for controlling skilled movements, it stands to reason that plasticity in the motor cortex is essential for an animal to learn new skilled movements. While there are many types of motor learning, skilled motor learning as we discuss here implies stringing together a series movements into a novel, coordinated sequence an animal has not performed many times before (such as might happen when one learns how to golf, for

example). Since the motor cortex is able to code for both low and high-level parameters, it could potentially modify either one in order to create this novel sequence of behaviors. In this dissertation, we demonstrated that simple motor maps, reflecting low-level control parameters, change following motor learning. However complex motor maps, reflecting higher-level parameters of control, do not change following skilled motor learning. This evidence suggests that, during the learning of novel skilled behaviors, motor aspects related to low-level parameters (control of individual muscle groups) are changed, while motor control related to higher-level parameters (the complex movements evoked with long-term microstimulation) are not changed.

2. Hypothesized Neural Correlates

Upon prolonged and repeated activation of neurons involved in the novel motor behavior the neural network that produces the skilled motor behavior eventually becomes stronger [40-42]. This strengthening allows for a more precise and reliable output of the specific sequence of motor actions required in the execution of the skilled motor behavior, and thus "acquisition" of the skilled motor behavior. The acquisition of these new motor behaviors is attentionally demanding, and thus depends on the basal forebrain cholinergic system. For this reason, lesions of the basal forebrain cholinergic system prevent the plasticity and much of the motor learning.

Strengthening the horizontal connectivity between the network of neurons controlling forelimb movements eventually leads to map plasticity (Fig 3). By

specifically strengthening the connectivity between a population of neurons representing a certain body part, that network is more likely to be activated upon intracortical microstimulation, resulting in an "expanded" representation and thus map plasticity.

Plasticity Following Rehabilitation After Cortical Injury

As described above, evidence suggests that peripheral nerve injuries lead to cortical disinhibition and thus cortical map plasticity. This occurs rapidly (within hours) and spontaneously, even while the animal is anesthetized. In contrast, evidence suggests that skilled motor training results in cortical map plasticity via increased lateral connectivity between specific networks of neurons. Plasticity associated with rehabilitation following cortical injury combines neural injury with behavioral training, so one can speculate that, perhaps, multiple types of plasticity may be involved.

In fact, however, it may be more complicated. Following cortical lesion paradigms, many studies have suggested an increase in inhibition near perilesional areas rather than a decrease [43]. Thus, cortical injuries appear to the have opposite effect as compared to peripheral injuries. Further, in the paradigm used in this paper, plasticity occurs ectopically to the injury site, though still within motor cortex. This ectopic reorganization of the rostral forelimb area suggests that the functional recovery occurs by a transfer of function from the now-lesioned CFA to the intact RFA. It is unclear how this functional transfer occurs. However, this dissertation demonstrated plasticity of both simple and

complex ICMS-evoked motor maps, suggesting this functional transfer occurs via a modification of both low-level and high-level parameters of encoding within the motor cortex.

As others have shown axonal plasticity from cortical to subcortical areas occurs following central lesions and rehabilitation training, it is possible that the underlying neural mechanisms associated with recovery after neural injury may involve such rewiring in additional to any other electrophysiological changes that occur within the motor cortex. It is even possible that focused rehabilitation either enhances this axonal plasticity that occurs following a lesion, or else focuses the plasticity to make it functionally relevant. However, evidence from this dissertation suggests such plasticity does not occur to the primary output of the motor cortex, the corticospinal tract.

FUTURE DIRECTIONS

The previous model suggests a number of future experiments that would be interesting to carry out. First, the neural mechanism underlying cholinergic modulation of cortical plasticity is not yet kown. A detailed understanding that process is essential to both furthering our knowledge of the system, as well as predicting in what contexts cholinergic drugs may be used to enhance recovery in clinical settings.

It would also be useful to further understand the role acetylcholine plays in the development of the cortical motor system. The research presented in this dissertation simply suggested that acetylcholine was necessary for the

development of this system. The behavioral implications of this development have not been elucidated, nor the neural mechanisms underlying it. Possible experiments include the following:

- temporarily blocking cholinergic activity during development to see if there is a criticial period of cholinergic activity needed.
- Measuring the electroyphysiological intracortical connectivity within the motor cortex following the cholinergic lesion
- Studying the anatomy of neurons within the motor cortex to see if the impaired development of the motor map was a result of intracortical spine loss, dendritic and/or axonal changes.

There is also much opportunity for further research on the functional recovery after cortical injury. Several studies, including those reported in this dissertation, have indicated the RFA as the cortical area underlying functional recovery after injury to the CFA. It would be interesting to investigate whether it is possible to enhance plasticity within this area, and see if such a manipulation results in enhanced functional recovery. Possibly ways to do this include injecting lentiviral constructs with NGF, BDNF or other molecules associated with neural plasticity. Further, even though our studies indicate that plasticity of the corticospinal tract does not occur following cortical injury, it is possible that eliciting plasticity of this system may result in additional functional recovery. Thus, growth factors delivered at the level of the spinal cord may result in enhanced plasticity of corticospinal tract fibers, and thus enhanced recovery.

Finally, much recent work has promoted the use of cortical stimulation as a method to enhance functional recovery following brain injury [44]. Based on the work in this dissertation, it appears possible that stimulating the motor cortex in a meaningful way may enhance functional recovery. By using a long-term stimulation paradigm to evoke both high-level and low-level parameters may increase the effectiveness of cortical stimulation than a stimulation protocol that only evokes low-level paramaters. This may be tested by implanting intracortical electrodes within the rat motor cortex, and assessing the effectiveness of such long-duration intracortical microstimulation on their functional recovery following brain injury.

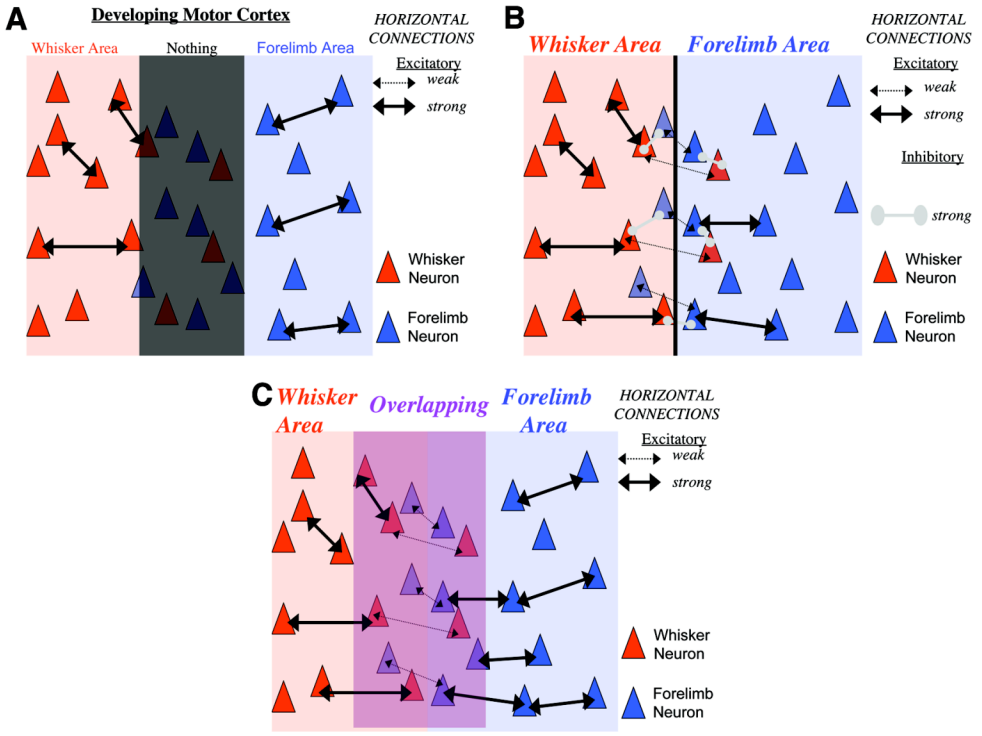

Figure 6.1: Model for the Development of Motor Maps.

(A) demonstrates a conceptual model for early development, when only few,and weaker, intracortical connections are present. Because many of these connections are not strong yet, stimulation within much of the cortex does not evoke movements. **(B)** With normal development, both excitatory and inhibitory conections develop, creating large maps with general boundaries. **(C)** Blocking inhibition eradicates the normal boundaries within these maps, creating large overlapping areas.

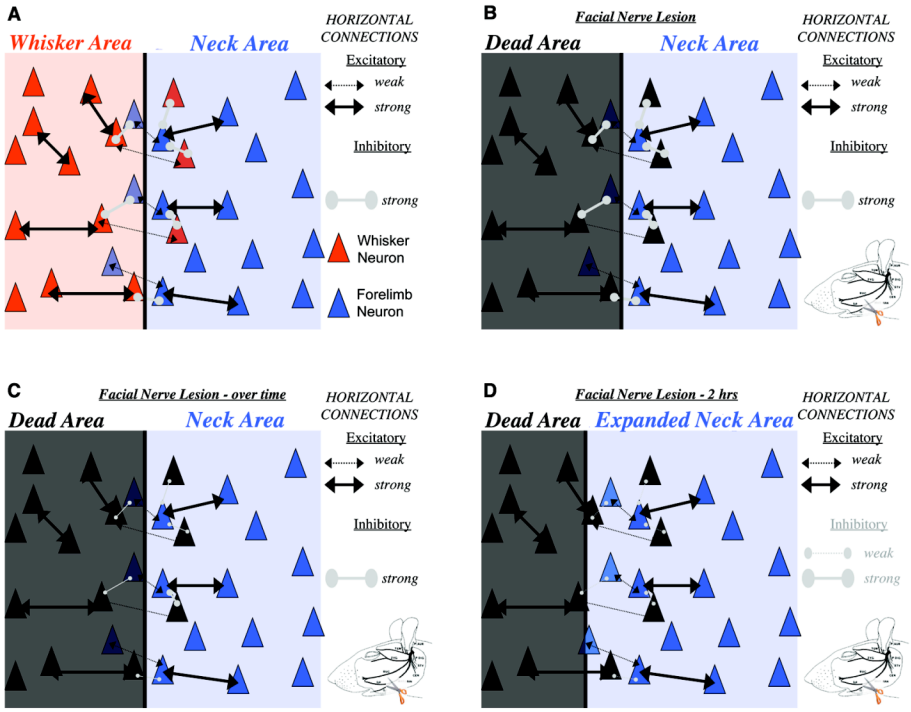

Figure 6.2: Model for the Effects of a Peripheral Nerve Lesion. Normal motor cortex creates the appearance of organized motor areas through intracortical inhibition **(A)**. Following a facial nerve lesion **(B)**, the motor area that previously controlled whisker will no longer have any functional outputs. More importantly, it will receive a significant decrease in proprioceptive activity following this motor nerve lesion. It has been postulated that this decrease in activity will specifically result in disinhibition of these motor areas **(C)**, and eventually to an unmasking and expansion of motor areas into this now lesioned motor area **(D)**.

The Effects of Skilled Motor Training

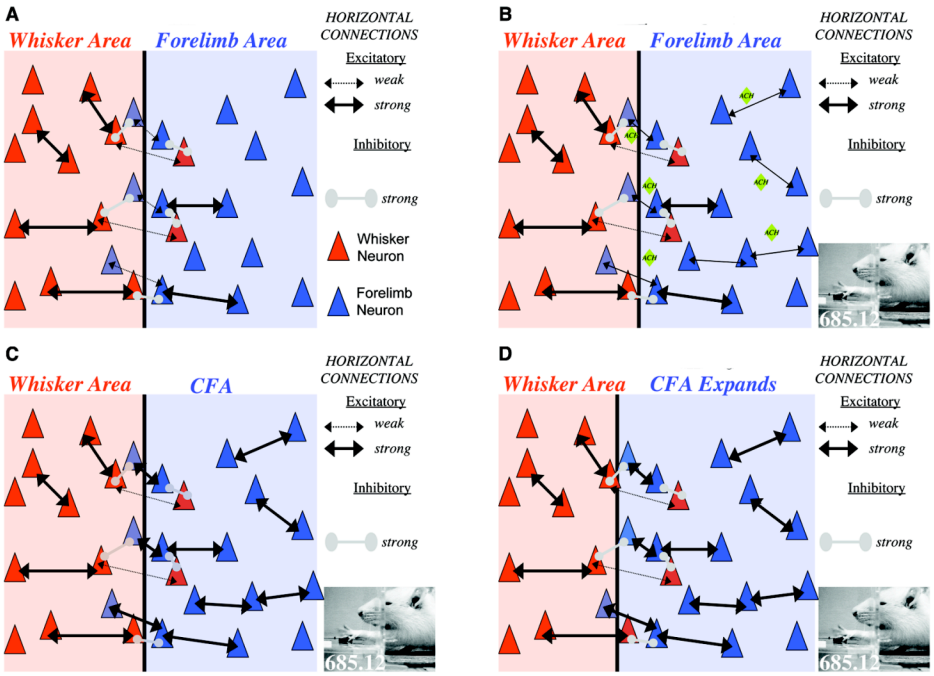

Figure 6.3: Model for the Effects of Skilled Motor Training

(A) Normal motor cortex has many overlapping areas of cortex that are tonically inhibited. Prolonged skilled motor training **(B-C)** result in LTP and increased spine numbers, specifically strengthening the intracortical connections between forelimb neurons. This may result in a stronger network of excitability specifically for these neurons, and thus a shift in the map to favor the stronger networks **(D)**.

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