NEUROPHYSIOLOGICAL CORRELATES OF MOTOR SKILL LEARNING: REORGANIZATION OF MOVEMENT REPRESENTATIONS WITHIN MOTOR CORTEX

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Abstract

This thesis used a rodent model of skilled forelimb training and intracortical microstimulation to examine the relationship between learning and cortical reorganization. This thesis examines how reorganization is related to the specific changes in forelimb movements during learning. It also examines the role that task repetition plays in driving motor cortex reorganization and showed that once the skilled motor task had been acquired it was necessary to repeat the task sufficiently to produce motor cortex reorganization. This thesis also examines reorganization following skilled reach training was related to the consolidation of motor skill, finding that animals that learned the skilled reaching task after five days of training also showed cortical reorganization, which persisted for one month. These experiments show that the distribution and subsequent redistribution of movement representations within motor cortex is related to changes in motor performance that occur during motor training.

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CHAPTER ONE

GENERAL BACKGROUND

Many adult behaviours involve the expression of some acquired motor skill and the process of motor skill learning is familiar to us all. At first, skilled movements are difficult and must be continually modified. With time and continual practice, the skill becomes intrinsic and you are able to perform the behaviour without having to 'think' about it. Further, with enough practice, you are able to go for long periods of time without performing the task, and not lose the skill. Despite the importance and predominance of the ability to encode and maintain a novel motor skill, little is known about how motor skill is encoded by the motor system of the brain. Given that skill learning is characterized by significant changes in motor behaviour, some form of biological alteration within motor regions of the central nervous system must be occurring.

The brain is a highly dynamic organ that is continually changing throughout the life of an organism. The ability of the brain to change is referred to as 'plasticity'. Plasticity refers to the brain's capacity to maintain many aspects of its structure and function while at the same time changing others. Structural plasticity includes dendritic growth, axonal sprouting and the formation of new synapses (Uylings et al., 1978; Diamond et al., 1976; Darian-Smith & Gilbert, 1994; Kleim et al., 2002) and is also not limited to neurons. Glial hypertrophy (Forgie, Gibb & Kolb, 1996) and angiogenesis (Sirevaag & Greenough, 1987; Kleim, Cooper & VandenBerg, 2002) also occur. Functional brain plasticity can

be observed at a number of levels. Individual neurons can undergo long term changes in their firing rates or resting membrane potentials (Aizenman & Linden, 2000) Plasticity across populations of neurons can be observed as changes in excitatory postsynaptic potentials and population spikes (Pinsker & Kandel, 1977; Suzuki et al., 2001). Finally, changes in the global pattern of activity can be observed using imaging techniques such as functional magnetic resonance imaging or positron emission topography (Roux et al., 2001; Thiel et al., 2001) (see Table1).

Table 1: E	Examples of	of Brain	Plasticity.
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Type of plasticity	Manifestation of plasticity
Structural plasticity	Dendritic growth
Structural plasticity	Axonal sprouting
Structural plasticity	Synapse formation
Structural plasticity	Glial hypertrophy
Structural plasticity	Angiogenesis
Functional plasticity	Changes in neuronal firing rates
Functional plasticity	Changes in membrane potentials
Functional plasticity	Changes in excitatory post-synaptic
	potentials
Functional plasticity	Changes in population spikes
Functional plasticity	Changes in global patterns of brain
	activity

Plasticity occurs in response to many factors, including brain injury, drug use, or learning (Kolb & Cioe, 2000; Robinson et al., 2001; Kleim et al., 2002). Although plasticity is enhanced during development, it is not limited to development. It is believed to mediate changes in behaviour that occur in response to changing behavioural demands that occur throughout life such as those associated with learning. With this in mind, the present thesis is based on the hypothesis that motor learning is supported by plasticity within the motor system.

Arguably, the motor cortex is the primary motor structure in the brain and motor skill learning has been related to motor cortex plasticity. Although motor learning is likely encoded in the cortex in a variety of ways, this thesis focuses on changes in the topography of stimulation evoked movement representations within the motor cortex.

In this introduction, I will present a historical overview of the phenomenon of functional cortical plasticity. I will then examine some of the factors that induce plasticity. Further, I will present an overview of the history of brain stimulation, and how stimulation is used to investigate functional plasticity. Specifically, I will discuss how it is used to study the plasticity of movement representations in the motor cortex. Finally, I will discuss some of the factors that lead to motor cortex reorganization, focusing on motor learning induced plasticity.

1.1 Early History of Brain Plasticity

Johann Gaspar Spurzheim was one of the first scientists to propose that the brain was a dynamic organ. He hypothesized that if muscles increased in size with exercise, then the 'organs' of the brain would respond similarly to use. He knew that there was an increase in blood flow with exercise, and that blood flow provided 'nutrition' for the body, so he hypothesized that with increased blood flow, the brain should likewise be affected (Spurzheim, 1815).

Alexander Bain (1818-1903) was the first to incorporate plasticity into a specific neural theory of learning. Bain argued that learning occurred through modifications in connections between neurons. He believed every new experience produced the reorganization of movements and sensations, and that this reorganization involved growths at cell junctions (Bain, 1855). William James proposed a similar theory years later. He believed new habits were acquired through 'voluntary repetition' so that new neural connections could be formed (James, 1890).

In 1895 Ramón y Cajal extended the idea of Bain and James. Using Golgi's staining technique, Ramón y Cajal found that there were discrete nerve cells in the developing chick brain. Believing that neurogenesis, the growth of new neurons, did not occur in the brain, he suggested that 'cerebral exercise' led to new, more developed connections in the cortex.

Despite decades of speculation that the brain was plastic, however, there was no direct experimental evidence to support the hypothesis. The first indirect evidence came from the work of Karl Lashley. Lashley trained animals on a complex maze task and performed lesioning experiments to determine where memory was located in the brain. Lashley found that the intact brain had the ability to resume the lost function of a damaged brain area. Lashley also found that task performance was affected by the amount of cortex that was lesioned, rather than the location of the lesion.

1.2 Evidence that Differential Experience Alters the Brain

In 1947 Donald Hebb demonstrated that animals raised in a complex environment showed increased cognitive performance. The complex environment was a large cage enriched with toys and other objects for the animals to explore. Later experiments showed that animals raised in a complex environment also performed better on maze learning tasks than their control counterparts (Bingham et al., 1952; Rosenzweig, 1971; Juraska et al., 1984).

Further work revealed that rats raised in a complex environment from a young age had a greater cortical thickness than their socially or individually caged littermates (Rosenzweig et al., 1962; Diamond et al., 1967; Diamond et al., 1972). Further work showed that changes in dendritic branching and neuronal density occur in animals raised in an enriched environment, compared to animals that were not (Volkmar & Greenough, 1972; Beaulieu & Colonnier, 1989).

In addition, synapse numbers increase in animals exposed to the enriched environment (Turner & Greenough, 1985). There are also increases in acetylcholine production and RNA synthesis found in enriched animals (Bennett et al., 1964; Ferchmin & Eterovic, 1987). Although these experiments clearly showed how differential experience could alter the brain, they do not provide any information on how these changes are related to learning. Thus the specific relationship between structural plasticity and learning is not evident.

1.3 Learning-Dependent Plasticity

Further experiments have demonstrated that plasticity also occurs in response to specific learning tasks. Padeh and Soller found that there was an increase in the brain weights of mice that were trained on a double T-maze and a Y-maze compared to mice that were trained on these tasks, but were not able to learn them (Padeh & Soller, 1976). Greenough et al. (1979) found that after training animals on a Hebb-Williams maze there was an increase in dendritic spine density in layer IV of the visual cortex. They also found that this effect was specific to the occipital cortex corresponding to the trained eye (Chang & Greenough, 1982). Further, Moser et al. (1994) trained animals on the Morris Water Maze and showed that there was a significant increase in the spine density along hippocampal pyramidal neurons.

Although these experiments demonstrate learning-dependent structural plasticity, it is unclear how exactly the changes might support these behaviours.

In order to study the relationship between learning and plasticity we need an experimental model that meets three important criteria. First, a behaviour with characteristics that can be easily measured during learning is required. Second, this behaviour needs to be dependent upon a specific brain area so that we know where to look for plasticity. Third, there must be some way to derive a measure of the functional organization of this brain area that can be related to the behavioural changes during learning.

Rat skilled forelimb behaviour provided an excellent model to study the relationship between learning and plasticity. First, changes in motor performance can be readily measured. Second, forelimb movements are dependent upon forelimb motor cortex (Whishaw et al., 1991). Third, the functional organization of motor cortex can be defined through intracortical microstimulation. Finally, intracortical microstimulation has shown changes in the functional organization of motor cortex with motor learning (Nudo et al., 1996; Kleim et al., 1998). This thesis relies on intracortical microstimulation to measure functional plasticity. Therefore, some background on the technique is necessary.

1.4 History of Motor Cortex Stimulation

Some of the first evidence for the presence of a motor map within the cortex came from anecdotal observations made by John Hughlings Jackson (1835-1911). He noticed there was a pattern to the epileptic seizures his wife experienced. Seizures often started in a specific location on her body and spread throughout her body in a cascade, progressing to adjacent body parts. Hughlings Jackson also noticed that the seizure would start on one side of the body and spread to the other. This led him to conclude that cortical motor representations were in the form of a map of the body and were present in both cerebral hemispheres (Jackson, 1931).

Gustav Fritsch (1838-1927) worked at a hospital helping wounded Russian soldiers. He was treating a soldier with a head injury, dressing a head wound, when he discovered that movement could be provoked through wound irritation. Fritsch went on to conduct further studies of this phenomenon with his colleague Eduard Hitzig (1838-1907). They demonstrated that electrical stimulation to certain areas in the frontal cortex of a lightly anaesthetized dog provoked movement. Hitzig continued this research, eventually defining the motor cortex borders of the dog and the monkey (Hitzig, 1874).

Sir David Ferrier (1843-1928) continued Fritsch and Hitzig's work and found a topographical map of movement representations within the precentral gyrus. He also studied the postcentral areas in the monkey and the dog and found that they would produce movement when stimulated.

Soon after the work done by Fritsch and Hitzig, Roberts Bartholow attempted the first direct stimulation of the human cortex through electrode placement to the scalp (Bartholow, 1874). One of Bartholow's servants had scalp

cancer that thinned regions of the skull. Bartholow stimulated her scalp and produced weak muscle contractions on the contralateral side of her body.

Sir Victor Horsley continued the work done by Fritsch, Hitzig and Ferrier. His experiments helped further the argument that the motor cortex was located in the precentral gyrus. He produced motor maps that included stimulation sites that were located in the base of sulci (Horsley & Schafer, 1888; Beevor & Horsley, 1890). In addition to these more detailed maps, Horsley found motor representations that were precentral, but outside of the primary motor cortex. These areas were later described as premotor areas. Horsley also showed that there is an ambiguous area between the precentral and postcentral gyrus that responds to both motor and sensory stimulation.

The debate continued as to whether the cortex representing movement was limited to precentral areas. In the early 1900s Sir Charles Sherrington found he could only elicit motor responses in the precentral cortex using unifocal stimulation to produce near-threshold responses (Grunbaum & Sherrington, 1901; Leyton & Sherrington, 1917). In 1905, Korbinian Brodman and Alfred Walter Campbell independently published the first papers on the cytoarchitectonics of the neocortex. Their findings showed there was a difference in cortical thickness and lamination between the precentral and the postcentral regions of the cortex. The anatomical differences in these cortical areas provided more evidence for Leyton and Sherrington's argument of a distinct functional difference between the two areas.

Wilder Penfield produced the first motor maps of humans. He described them in terms of a motor homunculus, a representational map of the body, with the size of the body parts drawn relative to the amount of motor representations (Figure 1) (Penfield & Boldrey, 1937; Penfield & Rasmussen, 1957). Although the homunculus was the first of its kind, it was based on low-resolution motor maps of epileptic patients. Interestingly, recent work has suggested that motor maps can be influenced by seizure activity, and so seizure-induced motor maps may not be representative of 'normal' motor maps. **Figure 1:** Penfield's motor homunculus. Adapted from Penfield and Rasmussen, 1950.



1.5 Recent Advances in Brain Stimulation

In the 1960s, technology had advanced so that experimenters could use a microelectrode to penetrate into the deeper layers of the cortex (Asanuma & Sakata, 1967). This intracortical microstimulation technique allowed researchers to lower a microelectrode into layer V, the output layer of the motor cortex, and elicit movements at very low current levels. The microelectrode enabled experimenters to produce motor maps while minimizing cortical damage that had been formerly produced using larger electrodes. It also allowed highresolution cortical maps to be produced (Recanzone et al., 1992a; Maldonado & Gerstein, 1996). The motor cortex is an area that has been extensively examined with intracortical microstimulation (Nudo et al., 1990; Nudo et al., 1996; Kleim et al., 1998). Detailed motor maps show a 'fractured somatotopy', a mosaic-like pattern of movement representations (Figure 2). Although the area of the rat motor cortex is fairly stable, the pattern of movement representations is different from animal to animal (Neafsey et al., 1986). The intracortical microstimulation technique has allowed researchers to confirm previous motor map details, such as the overlap of motor and sensory cortices in the rat (Sievert & Neafsey, 1986; Hummelsheim & Wiesendanger, 1985). Researchers were also able to make discoveries about the motor cortex. Using intracortical microstimulation, it was found that there is a second forelimb area in the rat motor cortex (Neafsey & Sievert, 1982). Use of the intracortical microstimulation technique also allowed researchers to examine the effects of various manipulations on the organization

of the motor maps. There is now considerable evidence that motor maps are highly dynamic and can be altered by a variety of internal and external factors. **Figure 2:** A topographical map of the motor cortex. The rat motor cortex is divided into three areas: the rostral forelimb area (RFA), the caudal forelimb area (CFA), and the hindlimb area (HLA). The different colours of the map represent different movement representations. Yellow represents neck movement representations, red represents digit movement representations, green represents wrist movement representations, light blue represents elbow movement representations and grey represents unresponsive sites. The neck representation sites and the unresponsive sites are used to determine the borders of the three areas of the rat motor cortex.



CFA

1.6 Plasticity of Movement Representations Within Motor Cortex

Since brain stimulation was first introduced, many scientists have used this technique to study how the organization of the motor cortex responds to a variety of manipulations. Some of these manipulations are artificially produced, such as cortical stimulation, limb amputation and cortical damage (see Table 1.2). Brown and Sherrington (1912) conducted one such experiment combining motor cortex stimulation and an experimental manipulation, which led to one of the earliest noted observations of cortical map plasticity. Stimulating one point in the motor cortex produced a weak muscle response, whereas stimulating the same point a short time later produced a much stronger response, and thus, the threshold of the motor representations could change. They also noticed that responses could change over time. For example, an extension response, upon further stimulation, produced a flexion response, leading Sherrington to label these sites as 'points of instability'. They also found that the size of motor maps could enlarge with stimulation. These findings represent the first evidence that motor maps are dynamic rather than static.

1.6.1 Cortical Stimulation

Since Brown and Sherrington's observations, cortical stimulation has been found to alter movement representations. Nudo and colleagues produced detailed motor maps of the rat motor cortex, and then stimulated movement representations on the border of the motor map for one to three hours. They found that repeated intracortical stimulation of the motor cortex permanently expanded the borders of the movement representations (Nudo et al., 1990). Similarly, VandenBerg and Kleim (2002) have shown that an hour following intracortical microstimulation the border of the motor cortex had expanded. Mapping the animals twenty-four hours later, the borders had returned to their original dimensions. Pathological seizure activity has also been implicated in changing the motor map (Uematsu et al., 1992). Kindling, a type of stimulation that produces seizure activity in the brain, has recently been shown to dramatically increase the area of movement representations in the motor cortex. After repeated kindling sessions involving cortical potentiation, movements are elicited outside of the normal borders of the caudal forelimb area (Teskey et al., 2002).

Focal transcranial magnetic stimulation can be used as a non-invasive method to evoke movements in a conscious human. Movements are elicited by applying a magnet to the scalp overlaying the motor cortex. It has been found that repetitive stimulation through transcranial magnetic stimulation also produces changes to the human motor cortex (Berardelli et al., 1998).

1.6.2 Limb Amputation

Forelimb removal in neonatal rats leads to reorganization of remaining muscle representations in place of the removed musculature (Donoghue & Sanes, 1988). Similar changes have also been observed in adult primates. The remaining muscle group representations expand into the area that was once devoted to the amputated musculature, and now produces movements of neighboring musculature (Wu & Kaas, 1999; Qi et al., 2000). In humans, functional magnetic resonance imaging was used to study amputees while they made imagined voluntary movements of the missing limb (Roux, Ibarrola, Lazorthes & Berry, 2001). Movement representations were activated during the virtual movement of their missing limbs. The experimenters concluded that cortical representations of missing limbs persist for several years after amputation. While amputation changes movement representations in the cortex, less dramatic manipulations, such as sensory denervation via nerve transection alone is enough to produce plasticity (Garraghty & Kaas, 1991). Rats that undergo facial nerve transection show a shift in the corresponding motor cortex from vibrissae representations to forelimb representations within a few hours of transection (Sanes et al., 1988).

1.6.3 Cortical Damage

Glees and Cole (1950) conducted some of the earliest research on motor map plasticity following motor cortex damage. They identified and lesioned thumb representations within M1 of a rhesus monkey. Two days later they remapped the motor cortex and found that there were no thumb movements elicited in the lesioned area. However, there were thumb representations in areas that had previously been dedicated to the hand. Although some spontaneous reorganization occurs following damage to the motor cortex (Nudo & Milliken, 1996), the amount and type of reorganization that occurs after cortical damage is dependent on post-lesion experience. Nudo et al. (1996) trained monkeys to retrieve pellets from small wells, using skilled digit movements. After the animals were sufficiently trained, topographical maps of the motor cortex were derived, using intracortical microstimulation. After the animals were mapped they were given a stroke in the hand area in order to affect task performance. The animals were then retrained for three to four weeks on the pellet retrieval task, until their task performance was equal to pre-lesion performance. When the animals were mapped after rehabilitation, it was found that there was a significant increase in hand representations, compared to animals that had not received rehabilitation. In a related study Nudo and Friel (1998) demonstrated that after giving an animal a stroke, restraint of the unaffected limb must be accompanied by forced use of the affected limb, or the cortical representations are not retained or recovered.

Rehabilitation-induced cortical plasticity following cerebral ischemia has also been observed in rats (Goertzen et al., 2001) and humans (Liepert et al., 2000). Goertzen et al. (2001) trained rats on a skilled reaching task and then used intracortical microstimulation to derive topographical maps of the motor cortex. The animals then received a stroke. Following recovery, the animals were divided into three groups. The skilled rehabilitation condition animals received training on the original skilled reaching task. In this task the animal learns to use its wrist and digits in a skillful manner, which forces the animal to increase forelimb use. In the unskilled rehabilitation condition, animals were trained on a task that involved increased forelimb use with no skillful digit and wrist movements. The non-skilled rehabilitation animals did not use their forelimbs. Animals in the non-skilled rehabilitation condition did not recover any of the lost elbow, shoulder, wrist or digit representations, while the unskilled rehabilitation condition animals regained some of the lost elbow and shoulder representations. The skilled rehabilitation animals regained both elbow and shoulder as well as wrist and digit representations. This study provides evidence that the type of rehabilitation is directly involved in functional recovery seen in the motor cortex.

1.6.4 Motor Learning

Although the motor cortex can alter its organization in response to artificial or pathological manipulation, natural differential motor experience can also produce motor cortex reorganization. Skilled reach training has been widely used to study plasticity of the motor representations in rodents and primates. Nudo et al. (1996) trained monkeys to perform a task that involved learning novel digit movements. The animals had increased digit representations and decreased elbow representations. Thus, areas in the motor cortex that once produced elbow movements subsequently produced wrist movements.

Although motor learning tasks are useful to study functional cortical plasticity, the increased motor activity of the task is a confounding variable. To

compensate for the increase in motor activity Kleim et al. (1998) trained rats in an unskilled reaching condition. These animals were taught to press a bar to receive a food pellet, which increased the amount of forelimb activity that the animals experienced. The motor maps of the unskilled reaching condition animals were then compared to maps of skilled reaching condition animals. They found that animals in the skilled reaching condition had a significant increase in the number and size of distal forelimb representations, whereas the unskilled reaching condition animals did not (Figure 3). Plautz, Milliken and Nudo (2000) provide further evidence that skilled motor learning is required to produce changes to the motor map. Monkeys were trained to retrieve a pellet from a large well. This simple motor task involved a small amount of finger movements to successfully retrieve the pellet. The purpose behind this simple task was to see if the motor cortex reorganizes with the repetition of a non-challenging task. They found that there were no changes in the movement representations of the animals. Therefore, learning a skilled movement drives functional plasticity in the motor cortex.

Figure 3: Topographical motor maps from animals trained on the skilled reaching condition and the unskilled reaching condition. In the caudal forelimb area (CFA), green and red represents distal (wrist and digit) movement representations, and light blue represents proximal (elbow and shoulder) movement representations.



UNSKILLED REACHING CONDITION



Finally, Kleim et al. (2002) found that rats that had access to running wheels had motor maps that were similar to rats in an inactive condition, Remple et al. (2001) found that rats that had been trained extensively on a forelimb strengthening task did not significantly differ from reaching controls. Both results suggest that motor learning is necessary to produce functional reorganization in the motor cortex.

The time course of cortical reorganization in skilled reach trained rats has also been studied. A recent experiment found that although rats typically reach the asymptote of behavioural performance after seven days of training, the motor maps show reorganization only after ten days of training (Kleim et al., 2001) (Figure 4). This study also showed that after ten days of training, these plastic changes persist in the absence of training for as long as 220 days.

Figure 4: Time course of plasticity in motor cortex. Motor skill acquisition precedes synaptic changes in the motor cortex, which precedes motor map reorganization.





There have been similar findings in humans. Karni et al. (1995) found that training subjects on a digit sequence-learning task over a period of a few weeks produced changes in motor cortex activation, which persisted for several months. These changes in activation may reflect motor map reorganization. Classen et al. (1998) found, using standard transcranial magnetic stimulation techniques that the motor cortex has the ability to adapt rapidly to new skills. Transcranial magnetic stimulation is a procedure that involves placing a magnet on top of the skull, so that the brain can be stimulated externally. Classen and colleagues used transcranial magnetic stimulation to elicit a unidirectional thumb movement. After a brief training session in which the subjects produced thumb movements in the opposite direction of the original stimulated response, transcranial magnetic stimulation now evoked the trained thumb direction. These movements reverted back to their original direction after a brief time This experiment demonstrated that functional changes could be period. produced through a short training session, and that these changes were transient.

1.7 Summary of General Introduction

Motor learning is essential to mammalian survival. Motor learning provides the ability to respond to a continually changing environment. Motor behaviours are supported in many areas of the brain. The motor cortex is one of the primary motor areas, and has been shown to possess the capacity to change. This plasticity likely supports motor learning. This thesis will examine the relationship between functional cortical plasticity and motor skill learning.

1.8 Thesis Objectives

Although there is substantial evidence for changes in movement representations in motor cortex with motor learning, the relationship between map plasticity and motor skill training is still unclear. With the exception of one prior experiment (Kleim et al., 2001), all previous experiments have examined motor maps only after extensive training.

My thesis will involve training rats on a skilled reaching task, and then deriving maps of motor cortex using intracortical microstimulation. The experiments will address the following three questions:

- Do changes in motor map organization reflect specific changes in motor behaviour that occur during learning? The first experiment will examine exactly how forelimb movements change during learning and then relate these findings to the functional reorganization in motor cortex.
- 2. What role does practice/repetition play in driving motor map reorganization? We have previously found that motor maps change after the skill has already been acquired. The second experiment examines two possible hypotheses that explain this result. The first hypothesis is that the cortex may require a sufficient amount of time to

reorganize. The second hypothesis is that the skill must be practiced after the initial acquisition in order to produce cortical reorganization.

3. Does motor map reorganization represent consolidation of motor skill? If performance levels on the skilled reaching task are associated with cortical reorganization, and if this cortical reorganization persists in the absence of practice, it is plausible that motor cortex reorganization represents the permanent neural encoding of the skill.

CHAPTER TWO

DEVELOPMENT OF NOVEL SKILLED FORELIMB MOVEMENTS AND REDISTRIBUTION OF FORELIMB MOVEMENT REPRESENTATIONS WITHIN RAT MOTOR CORTEX

Theresa M. Hogg

2.1 Abstract

Adult rats were trained for thirteen days on a skilled reaching task. Frame by frame video analysis was performed on reaching movements each day to assess changes in forelimb movement sequences and accuracy. Intracortical microstimulation was then used to define the topography of movement representations within contralateral forelimb motor cortex. Results showed that increased accuracy was accompanied by the acquisition of skilled wrist and digit movements. The development of the skilled movement sequences was associated with an expansion of wrist and digit movement representations in forelimb motor cortex. The results show that changes in movement representations within rat motor cortex that are driven by learning a novel motor skill can be directly related to specific changes in movement sequences that mediate improved motor performance.

2.2 Introduction

The topography of movement representations within adult motor cortex is highly dynamic and can be altered in response to a variety of manipulations (Jacobs & Donoghue, 1991; Huntley, 1997). The capacity for functional reorganization is thought to support the development of novel motor skills. Within rat motor cortex, skilled forelimb training has been shown to cause an expansion of wrist and digit movement representations into regions of elbow and shoulder representations. This reorganization does not occur in response to extensive repetition of unskilled movements (Kleim et al., 2002) or increased forelimb strength (Remple et al., 2001) suggesting that the plasticity is dependent upon learning a novel skill. Despite the robust nature of the reorganization, however, there has been no demonstration that the expansion of wrist and digit representations reflects specific changes in distal forelimb movement sequences during training. All previous experiments have measured reaching accuracy during training (Kleim et al., 1998; Kleim et al., 2002) without examining exactly how forelimb movement sequences change. If the expansion of distal (wrist and digit) movement representations is related to the performance of skilled reaching behaviour, then changes in distal movement sequences should be observed over the course of training and be related to improved performance. In the present experiment we examined changes in both distal and proximal forelimb movement sequences across several days of skilled reach training. These changes in movement strategy were then related to changes in success on the
task and the topography of movement representations within the forelimb motor cortex.

2.3 Materials and Methods

2.3.1 Subjects: Animals were housed in standard animal housing cages and were kept on a steady 12:12 light/dark cycle throughout the experiment. Prior to training they were placed on a restricted diet (approximately 15 g of food/day for 2-3 days) until they reached approximately 90% of their original body weight.

2.3.2 *Reach training:* All animals were trained for 2-3 days on a pre-training task in order to familiarize them with the food pellets and assess paw preference. The pretraining task used was the Whishaw Tray Reaching Task (see Whishaw, 2000). The animals were trained to reach out of the front of plexi-glas cage towards a tray of food pellets located at the front of the cage. After they successfully retrieved ten food pellets in one training session, they were randomly assigned to either a Skilled Reaching Condition (SRC; n=8) or a Non-Reaching Condition (NRC; n=8). SRC animals were trained on a single pellet reaching task for thirteen consecutive days (see Figure 5). The task required the animals to reach through an opening in the front of a Plexi-Glas box to retrieve a food pellet (Bioserv, 45 mg) located on a platform outside the front of the cage (see Dunnett et al., 1987). Each training session lasted for ten minutes and was videotaped for further analysis. NRC animals were only trained on the pretraining task to assess their paw preference.

2.3.3 Movement Analysis: The first ten successful reaches from each training session were analyzed using a movement rating system based on the ten element system developed by Whishaw et al. (1993). A reach qualified as successful if the animal retrieved a pellet from the platform on the front of the reaching box and placed the pellet in its mouth in the first attempt. The nine components of the reach were: (1) Digits Close, the palm is supinated and the digits are semi-flexed in anticipation of the reach. (2) *Aim*, the elbow is brought towards the midline of the body and the palm is brought to midline. (3) Advance, the elbow is kept in midline and the limb is advanced towards the target. (4) Digits Open, the wrist is not fully pronated and the digits open. (5) Pronation, the elbow is turned outward as the palm is placed over the food pellet in an arpeggio. (6) Grasp, the arm is kept still as the digits close over the pellet. The palm is then lifted from the shelf. (7) *Supination I*, the elbow is rotated inward and the palm is turned at a 90° angle as it leaves the slot. (8) Supination II, the palm is turned straight up in anticipation of the animal eating the pellet. (9) *Release*, the digits are opened and the animal puts the food in its mouth (see Figure 5).

These movements were rated on a 0 to 2 point system, in which 0 represented the complete performance of a movement, 1 represented an intermediate performance of the movement, and 2 represented the absence or

poor performance of a movement. The nine reach components were then categorized as either a predominantly proximal (elbow and shoulder) movement, or a predominantly distal (wrist and digit) movement. The aim and advance components were classified as proximal movements, and the digits close, digits open, pronation, grasp, supination i, supination ii and release components were classified as distal components. Figure 5: Elements of movement analysis (taken from Whishaw, 2000). The lift component of the elements was not used in this experiment because it did not fit into the distal or proximal movement category. Further, the advance and digits open components are represented in a single heading on the chart, and the release component of the scale is not present on the chart.



Lift

Pronation



Lift - Dig. close



Grasp



Supination 1



Supination 2

2.3.4 *Reaching Accuracy:* Three, two minute samples of the videotaped training sessions were analyzed for reaching accuracy from each day of training for each SRC animal. The number of successful reaches was divided by the number of total reaches attempted. This produced the accuracy percentage within the training session.

2.3.5 Intracortical Microstimulation: The day following the final training session, a map of the motor cortex was produced for each animal. A map of the motor cortex contralateral to the trained paw was derived using standard intracortical microstimulation (ICMS) techniques.

The animals were first anesthetized with ketamine hydrochloride (70 mg/kg ip) and xylazine (5 mg/kg im). The animals received supplementary injections of ketamine (20 mg/ kg ip) and acepromazine (0.02 mg/ kg ip) as needed.

The skull directly over the motor cortex, contralateral to the trained paw, was removed. The dura mater was removed, and the skull was covered with warm silicon oil. A digital image of the brain was taken for use as a guide for microelectrode penetrations. A 375 µm grid was superimposed over the digital image of the cortex. Using a hydraulic microdrive, a glass microelectrode was lowered to a depth of approximately 1500 µm, which corresponds to cortical layer V. A 40-ms train of thirteen thousand, two hundred-µs monophasic cathodal pulses delivered at 350 Hz from an electrically isolated, constant current

stimulator was used to stimulate the cortex. The animal was in a prone position, with its trained paw held slightly extended in front of the rat in a constant position. At each penetration site, the current was gradually increased until a movement was evoked (threshold current). The current was increased at most to 60 μ A. If no movement was evoked at 60 μ A, the site was labeled non-responsive.

The caudal forelimb area (CFA) of the motor cortex was the area that was mapped, because it is the only area of the rat motor cortex that has been shown to undergo reorganization in response to the skilled reaching task (Kleim et al., 1998). Forelimb movements were categorized as either distal representations (digit and wrist movements) or proximal representations (elbow and shoulder movements). The head, neck, vibrissae, and non-responsive sites were used to determine the border of the CFA.

After the motor maps were derived, a computer program (Canvas 3.5.4) was used to determine the area of each type of representation.

2.4 Results

2.4.1 Reaching Accuracy: A repeated measures ANOVA with DAY as a within subject factor revealed a significant effect of DAY on reaching accuracy in the SRC animals (F(6, 72) = 13.481; p<0.001). The mean reaching accuracy significantly increased with training (Figure 6).



Figure 6: Reaching accuracy on the skilled reaching task across thirteen days.

2.4.2 *Movement Analysis:* A repeated measures ANOVA with DAY as a within subject factor revealed a significant of DAY on the percentage of successful reaches containing specific movement elements. The results are summarized in Table 2.

Table 2:	Elements	of movement	analysis
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Movement	Туре	Significantly Changed
Digits Close	Distal	No (F(12,78) = 1.4)
Aim	Proximal	No (F(12,78) = .74
Advance	Proximal	No (F(12,78) = .80
Digits Open	Distal	Yes (F(12,78) = 5.0
Pronation	Distal	Yes (F(12,78) = 6.5
Grasp	Distal	Yes (F(12,78) = 6.2
Supination I	Distal	Yes (F(12, 78) = 12.0
Supination II	Distal	Yes F(12,78) = 8.5
Release	Distal	Yes (F(12, 78) = 2.6

2.4.3 Topography of Movement Representations: A Student's t test (p<0.05) showed the SRC animals to have a significantly greater percentage of the CFA occupied by distal forelimb representations than untrained animals (t(12) = 3.32; p<0.01). Conversely, untrained animals had a significantly greater proportion of CFA occupied by proximal representations than SRC animals (t(12) = 3.32; p<0.01).

2.5 Discussion

Several experiments have recently shown that skilled forelimb training leads to an expansion of distal movement representations in rat forelimb motor cortex (Kleim et al, 1998; Kleim et al, 2002). Although the trained, stereotyped reach of the rat has been demonstrated to be predominantly proximally-driven (Whishaw et al., 1990), it is in fact the distal components of the reach that change and develop as the animal is learning the task. The present experiment demonstrates that this functional reorganization is accompanied by the development of skilled distal movement sequences. Although the individual components of the movement sequence may be present prior to motor learning, it is the grouping of these movements into a skilled movement sequence that drives motor learning. The existing motor map supports existing movement sequences. Prior to training, the animals are able to produce a wide variety of movements, and thus the animals are learning to produce novel movement sequences in a skillful manner, which in turn requires cortical reorganization. The development of skilled distal movements also occurs at the same time that reaching accuracy improves indicating that these novel sequences are related to improved performance. The results are consistent with similar experiments in primates where an increase in movement representations resembled those used during training on a skilled digit task was found (Nudo, 1996).

These data show that novel motor sequence learning drives specific physiological changes within motor cortex that reflect the acquired skill. However, a recent experiment has shown that the functional reorganization and improved accuracy do not occur at the same time. Despite an increase in reaching accuracy after seven days of training, no significant changes in motor cortex organization were observed until ten days of training (Kleim et al., 2000). Thus the acquisition of skilled movements alone is not sufficient to induce functional reorganization. Further work has shown that acquired skilled movements must be sufficiently repeated before the cortex undergoes reorganization (Hogg et al., 2001). Similar findings in humans have shown that one training session transiently alters the activation pattern of the motor cortex (Karni et al., 1995), while more extensive training is required to alter the pattern of activation permanently (Classen et al., 1998). Collectively, these experiments show that changes in motor behaviour are reflected as functional reorganization in the motor cortex and that this reorganization requires the acquisition and repetition of novel skilled movement sequences.

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CHAPTER THREE

REORGANIZATION OF FORELIMB MOVEMENT REPRESENTATIONS WITHIN RAT MOTOR CORTEX IS DEPENDENT UPON SUFFICIENT REPETITION OF NOVEL SKILLED FORELIMB MOVEMENTS

Theresa M. Hogg

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3.1 Abstract

Motor learning is associated with a redistribution of movement representations within the motor cortex. However, the relationship between cortical reorganization and learning is still unclear. Recent work found that rats trained on a motor learning task acquired the skill well before significant changes in the organization of movement representations occurred (Kleim et al., 2002). The present experiment examined the role movement repetition plays in driving functional reorganization. Intracortical microstimulation (ICMS) was used to derive topographical maps of the caudal forelimb area (CFA) of the motor cortex from rats on three different training schedules. Adult male rats were trained on a skilled (SRC) or an unskilled reaching condition (URC) for 3 or 13 days. Another group of animals was trained for 3 days on either the URC or the SRC and left without training for 10 days before the cortex was mapped. Animals in the SRC that were trained for 13 days had a significant increase in the percentage of distal (wrist and digit) representations in the CFA in comparison to URC No significant differences were found between the URC or SRC animals. animals that were trained for 3 days, or trained for 3 days and then left for 10 days. These results suggest that motor cortex reorganization requires acquisition of motor skill and sufficient performance of the skilled movements.

3.2 Introduction

It has been well documented that the organization of representations within the neocortex is not fixed but can change in response to a variety of manipulations. Cortical damage, electrical stimulation and peripheral damage have all been shown to change topographical representations in various cortical areas, including auditory cortex (Roe et al., 1990; Schwaber, Garraghty & Kaas, 1993; Maldonado & Gerstein, 1996), visual cortex (Chino et al., 1992; Godde et al., 2002), somatosensory cortex (Rasmusson, Turnbull & Leech, 1985; Garraghty & Kaas, 1991) and motor cortex (Glees & Cole, 1950; Nudo et al., 1990). Although the motor cortex is capable of changing in response to a variety of invasive manipulations, cortical reorganization is thought to mediate the development of new motor behaviours. Motor learning has been shown to induce reorganization of movement representations in humans (Cohen et al., 1993), primates (Nudo et al., 1996), and rodents (Kleim et al., 1998). Further, this reorganization is learning but not activity dependent. Kleim et al. (1998) trained animals on an unskilled reaching condition, in which they were taught to press a bar in order to receive a food pellet. This task does not require the animals to make skillful wrist and digit movements. It was found that training on the unskilled reaching condition did not lead to motor cortex reorganization. Plautz et al. (2000) trained monkeys on a pellet reaching task that required the animals to use their digits to retrieve a food pellet from a large well. The task involved repetitive movements without motor skill acquisition. When the pre-training motor maps and post-training

motor maps were compared there were no significant differences. Finally, animals exposed to running wheels for a month did not undergo cortical reorganization but did exhibit an increase in blood vessel density within motor cortex (Kleim et al., 2002). Activity alone is insufficient to drive cortical reorganization, while learning novel motor skills (i.e. novel motor sequences) drives functional plasticity in the motor cortex.

Despite the evidence that motor skill learning induces motor map reorganization, the temporal nature of this relationship is unclear. Recent work has shown that rats trained on a motor skill task showed motor map reorganization only after the skill had been acquired (Kleim et al., 2002). There are two possible interpretations of these results. The first is that the cortex simply requires time to reorganize after the initial motor learning has taken place. The second interpretation is that once the motor task has been acquired, it must be sufficiently repeated before the cortex will reorganize. The present experiment attempts to distinguish between these two possibilities and determine if motor map changes are time or practice dependent.

3.3 Materials and Methods

3.3.1 Subjects: Forty-eight adult male Long-Evans hooded rats (350-420 g) were group housed (2 animals/cage) in standard laboratory cages on a 12:12 hour light/dark cycle for the duration of the experiment. The animals were randomly distributed into a Skilled Reaching Condition (SRC; n = 24) or an

Unskilled Reaching Condition (URC; n = 24). Animals from both the unskilled and the skilled reaching conditions were then divided equally into three training schedules: a DAY 3 group (n = 16), a DAY 3 - 10 group (n = 16) and a DAY 13 group (n = 16). Animals in the DAY 3 condition received three days of training on either the SRC or the URC. Animals in the DAY 3 - 10 group were trained for three days on the SRC or the URC, followed by no training for another ten days. Animals in the DAY 13 group were trained on either the SRC or the URC for thirteen days.

3.3.2 *Reach training*: Over the course of several days, animals were placed on a restricted diet, until they reached approximately 90% of their original body weight. All the animals were given several brief pre-training sessions on the Whishaw Tray Reaching Task (see Dunnett et al., 1987). The animals were placed in test cages (10 cm x 18 cm x 10 cm) with floors constructed of 2 mm bars, 9 mm apart edge-to-edge. A tray (4 cm wide x 5 cm deep) filled with food pellets (Bioserv, 45 mg) was mounted on the front of the cage. The rats were required to reach outside the cage and retrieve pellets from the tray. The purpose of this task was to familiarize the animals with the pellets, as well as to determine the paw preference of all of the animals. All rats remained in pretraining until they had successfully retrieved 10 food pellets (approximately 1 hour/day for 2 days). Following pre-training, both groups of animals were trained in a Plexi-Glas cage (11 cm x 40 cm x 40 cm) with a 1 cm slot located at the front of the cage. SRC animals were trained for 10 minutes each day to reach through the slot and retrieve a single food pellet from a platform attached to the front of the cage (Whishaw and Pellis, 1990). Each session was videotaped and was later used to assess reaching performance. A successful reach was scored when the animal grasped the food pellet, brought it into the cage and to its mouth without dropping the pellet. The percentage of successful reaches was then calculated [(# successful retrievals/total # of reaches) x 100].

To control for the added effect of increased motor activity of the SRC, URC animals were trained on a variation of the skilled reaching task. The URC animals were also trained to reach for a food pellet outside of the slot. However, the pellet was placed out of the animal's reach. This provided the animal with the impetus to reach, without successfully retrieving the pellet. Thus, the URC animals would reach for but never obtain the food pellet and therefore not develop skilled reaching movements. To keep the animal reaching, the experimenter dropped the pellet into the cage after the animal had reached several times. Thus, the SRC and the URC animals experienced similar amounts of forelimb activity, but only the SRC animals developed motor skill.

3.3.3 Topography of Movement Representations: The day following the final training session, the motor cortex contralateral to the trained paw was mapped using standard intracortical microstimulation (ICMS) techniques. The animals were first anesthetized with ketamine hydrochloride (70 mg/ kg ip) and xylazine

(5 mg/kg im). The animals received supplementary injections of ketamine (20 mg/ kg ip) and acepromazine (0.02 mg/ kg ip) as animals needed. The skull directly over the motor cortex, contralateral to the trained paw, was removed. The dura mater was removed, and the skull was covered with warm silicon oil. A digital image of the brain was taken for use as a guide for microelectrode penetrations. A 375 μ m grid was superimposed over the digital image of the cortex. Using a hydraulic microdrive, a glass microelectrode was lowered to a depth of approximately 1500 μ m, which corresponds to cortical layer V. A 40-ms train of thirteen thousand, two hundred- μ s monophasic cathodal pulses delivered at 350 Hz from an electrically isolated, constant current stimulator was used to stimulate the cortex. The animal was in a prone position, with its trained paw held in a constant position. At each penetration site, the current was gradually increased until a movement was evoked (threshold current). The current was increased at most to 60 μ A. If no movement was evoked at 60 μ A, the site was labeled non-responsive.

The caudal forelimb area (CFA) of the motor cortex was the area that was mapped, because it is the only area of the rat motor cortex that has been shown to undergo reorganization in response to the skilled reaching task. A previous study found that the total area of the CFA did not significantly changes after skilled reach training. Rather, the area of the distal representations (sites that elicited digit and wrist movements) increases at the expense of the area of the proximal representations (sites that elicited elbow and shoulder movements) (Kleim et al., 1998). Forelimb movements were categorized as either distal representations or proximal representations. The head, neck, vibrissae, and non-responsive sites were used to determine the border of the CFA.

After the motor maps were derived, a computer program (Canvas 3.5.4) was used to determine the area of each type of representation as well as the total area of the CFA.

3.4 Results

3.4.1 Reaching Accuracy: A repeated measures ANOVA with DAY as a between subject factor revealed a significant effect of DAY on reaching accuracy for animals in the 3 (F(10,20) = 30.13; p<0.01), 3-10 (F(7, 21) = 10.65; p<0.01) and 13 conditions (F6,72) = 13.48; p<0.01). Animals in all three conditions showed significant improvements in reaching accuracy with training (Figure 7). However, animals in the 3-10 condition exhibited a significant reduction in reaching accuracy between their last day of training and the probe trial ten days later (Fisher's PLSD; p<0.05).

Figure 7: Accuracy on the skilled reaching task.



3.4.2 Topography of Movement Representations: A two way ANOVA with TIME and CONDITION as between subject factors revealed a significant TIME x CONDITION interaction for % of CFA occupied by Distal (F(2, 42) = 3.43; p<0.05) and Proximal (F(2, 42) = 3.51; p<0.05). Subsequent multiple comparisons (*Fisher's LSD; p<0.05) showed the SRC animals to have a greater proportion of distal movement representations and a smaller proportion of proximal representations within the CFA than the URC animals after thirteen days of training but not after 3 or 3-10 days of training (Figure 8). See Figure 9 for examples of topographical maps of all training schedules. **Figure 8:** Percentage of distal movement representations in caudal forelimb area following three training schedules.



Figure 9: Topographical motor maps of the caudal forelimb area. Green represents distal movements and blue represents proximal movements. A) Animal was trained for 3 days on the unskilled reaching condition (URC). B) Animal was trained for 3 days on the skilled reaching condition (SRC). C) Animal was trained for 3 days on the URC, and was mapped after 10 days of no training. D) Animal was trained for 3 days on the SRC, and was mapped after 10 days of no training. E) Animal was trained for 13 days on the URC. F) Animal was trained for 13 days on the SRC.



DAY 3 URC







DAY 3-10 SRC

Ð

DAY 3-10 URC

52

С





DAY 13 URC

DAY 13 SRC F

E

53

3.5 Discussion

The present results confirm those of previous studies showing that reorganization of movement representations within motor cortex is dependent on learning and not simply increased motor activity. Yet, activity is critical, because once the skill is acquired, it must be sufficiently repeated to induce reorganization.

These results and others have lead to the following theory. When learning a novel motor skill, initially many errors are made and occasionally a successful movement will occur. These movements are soon coordinated together to produce a movement pattern that results in consistent successful task performance. This rapid learning is associated with improved performance, but no motor map reorganization (Kleim et al., 2000). This rapid learning is followed by a period in which the animal makes minute adjustments in task performance, until the movement becomes stereotyped. The animal, however, is repeating the learned task and reinforcing the neural activity that supports it. Small improvements in the motor skill and significant changes in motor cortex reorganization distinguish this stage of learning, making it likely that once the skill is acquired, repetition is necessary to drive motor map reorganization, as this experiment has demonstrated. This theory is supported by a previous proposal by Karni et al. (1998) in which they suggest that there are two phases of motor learning. The first type is a fast learning phase, during the initial training sessions. This fast phase is characterized by fast progression in the acquisition of

a novel skilled motor task. This phase is followed by a slow learning phase. In the slow phase of learning, there is a slow, gradual improvement in behavioural performance. Several studies have shown that there is a change in motor cortex activation after a brief training session (Shadmehr & Brashers-Krug, 1997; Classen et al., 1998; Karni et al., 1998; Gandolfo et al., 2000). However, these changes in motor activity are transient, only persisting for several minutes following the training session (Classen et al., 1998; Liepert et al. 1999; Rosenkranz et al., 2000). This suggests that more training is required before these changes become resistant to decay.

The present experiment utilized several short training schedules to determine the effects of time on cortical reorganization. Although it has been shown that there is a time course to motor cortex reorganization (Kleim et al., 2000), training schedule may be an important factor. Previous memory consolidation experiments have shown that massed training versus spaced training produces differences in learning. Spaced training is more conducive to forming long term memory. It has been shown that memory formation requires protein synthesis in order to occur, and that a spaced training schedule produces more protein synthesis than a massed training schedule (Tully et al., 1994). Thus, it is possible that using a massed, rather than spaced, training schedule, in which the animal produced the same number of movements of the spaced training schedule, could yield different results. Neuromodulatory systems may also play a role in cortical plasticity. Norepinephrine can act as a neuromodulator in the cortex, increasing the effects of other neurotransmitters. Bickford (1993) found that rats with a deficit in modulatory norepinephrine in the cerebellar cortex were slower to learn a skilled motor task than those animals with normal norepinephrine. Thus, it is possible that the existing levels of neurotransmitters could produce variations in cortical reorganization. It is also possible that manipulating neurotransmitter levels could produce variations in motor skill learning.

Several experiments have shown that skill learning is necessary for motor cortex reorganization, and that animals trained on an unskilled task do not show cortical reorganization (Plautz et al., 2000; Kleim et al., 1998; Kleim et al., 2002). It is unclear, however, what is neurophsyiologically different between skilled and unskilled behaviours. Behavioural relevance may play a role in learningdependent cortical reorganization. The nucleus basalis has modulatory projections throughout the cortex, and has been implicated in providing the somatosensory cortex with information about the importance of behavioural stimuli (Mesulam et al., 1983; Rye et al., 1984). Neurons in the nucleus basalis are activated when an animal is attending to an important behavioural stimulus (Richardson & DeLong, 1991). Kilgard and Merzenich (1998) have shown that extraneous activation of the nucleus basalis paired with a tone led to auditory cortex reorganization. The auditory cortex responded to the tone as if it was a significant stimulus. It is, however, unlikely that the motor cortex reorganization is dependent on behavioural salience. An experiment by Kleim and colleagues found that animals trained to reach towards a pellet held out of reach also did not show cortical reorganization (1998). Likewise, Plautz and colleagues found that animals that retrieved food pellets in a simple, repetitive motor task did not show cortical reorganization. In both of these experiments, the animals are trained to attend to the food reward. The goal of the behaviour is the salient event in both of these experiments, whether the animal's goal is to continually reach towards an unattainable food pellet so that the experimenter will provide a reward, or if the animal's goal is to directly retrieve the food pellet. In both of these studies the goal is the salient event, yet the animals do not show movement reorganization in motor cortex. Thus, it is more likely that novelty is driving cortical reorganization. The existing motor map supports existing movement sequences. Prior to training, the animals are able to produce a wide variety of movements, and thus the animals are learning to produce novel movement sequences in a skillful manner, which in turn requires cortical reorganization.

This experiment answers an important question regarding the relationship between motor learning and cortical reorganization. Time alone is not required for motor cortex reorganization. Rather, after the motor task is acquired it is the practice of the task that leads to these changes in topographical motor map changes. These findings lead to the speculation that sufficient practice of a novel motor skill sequence is necessary for its permanent neural encoding.

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CHAPTER FOUR

MOTOR MAP REORGANIZATION REPRESENTS CONSOLIDATION OF MOTOR SKILL

Theresa M. Hogg

4.1 Abstract

Motor learning is associated with reorganization of movement representations within motor cortex. However, the relationship between cortical reorganization and learning is still unclear. Recent work found that rats trained on a motor learning task showed significant learning prior to motor map reorganization (Kleim et al., 2000). Furthermore, rats trained for a longer time on the motor learning task showed cortical reorganization, and this reorganization persisted in the absence of training. If performance levels on the skilled reaching task are related to cortical reorganization, and if this cortical reorganization persists in the absence of training, it is plausible that motor cortex reorganization represents the permanent neural encoding of motor skill. To examine this possibility, we trained adult male rats on a skilled (SRC) condition for 5 days. Intracortical microstimulation (ICMS) was used to produce topographical maps of the caudal forelimb area (CFA) of the rat motor cortex prior to training, immediately following training and one month following the last day of training. Results revealed that only those animals that showed expansion of distal representations during training learned the task. Further, the expansion remained in the absence of continued training. Finally, the data suggest that the state of the motor cortex prior to training may influence motor performance. Surprisingly, rats that entered training with large distal movement representations had difficulty in learning the task.

4.2 Introduction

The functional organization of the cerebral cortex is characterized by the presence of representational maps. These include maps of the visual and auditory environment, maps of the body somatosensory system and motor maps. All of these representational maps have been shown to undergo reorganization in response to various manipulations (Rasmusson, Turnbull & Leech, 1985; Nudo et al., 1990; Chino et al., 1992; Maldonado & Gerstein, 1996). Specifically, the motor cortex has been shown to change in response to cortical damage (Glees & Cole, 1950), peripheral nerve damage (Donoghue & Sanes, 1988), repeated cortical stimulation (Nudo et al., 1990) and motor learning (Nudo et al., 1996; Kleim et al., 2002a; Kleim et al., 2002b).

The development of skilled movement has been shown to cause a redistribution of movement representations whereby trained movements expand into cortical areas previously dedicated to untrained representations (Nudo et al., 1996). Kleim and colleagues (1998) provided further evidence that motor skill learning drives motor map reorganization. Rats trained on a skilled reaching task, involving skillful wrist and digit movements, showed cortical reorganization, biasing the motor cortex towards wrist and digit representations. Motor skill learning, however, is associated with an increase in motor activity. Rats were trained on a simple bar pressing task that did not require novel skill learning to control for the increase in motor activity. Following training the

animals were mapped using ICMS, and it was found that they did not show reorganization.

Functional plasticity within the motor cortex is also directly dependent on the type of motor experience that an animal has. Animals trained on a forelimb strengthening task showed an increase in forelimb strength, but did not undergo motor cortex reorganization (Remple et al., 2001). In addition, animals that had access to running wheels for a month did not undergo cortical reorganization (Kleim et al., 2002). Therefore, learning a novel skilled task drives topographical reorganization. Similar findings have been demonstrated in human studies (Karni et al., 1995; Classen et al., 1998).

The time course of cortical reorganization in skilled reach trained rats has also been studied. In a recent experiment it was found that although rats typically reach the asymptote of behavioural performance after seven days of training, the motor maps show reorganization only after ten days of training. Further, it was found that once cortical reorganization has occurred, it persists in the absence of further motor skill training (Kleim et al., 2000). Thus, with sufficient training, the motor map reorganizes and this reorganization persists in the absence of skill practice for a long time period. There were two possible explanations for these results. The first possible interpretation is that following the initial motor skill acquisition the motor cortex requires time to reorganize. The second possibility is that the task must be repeated sufficiently in order to produce reorganization. It has been found that once the task is acquired it must be sufficiently repeated in order to produce functional plasticity in the motor cortex (Kleim at el., 2000).

Once the reorganization occurs it persists for an extended period of time (at least 200 days) without performance. Thus, it has been hypothesized that motor cortex reorganization may represent motor memory consolidation. Once motor skill learning produces cortical reorganization, this reorganization persists, and the animal still retains the ability to perform the task in the absence of training. The present experiment attempts to determine if motor cortex reorganization represents motor memory consolidation by training animals until they have started to acquire the task and then comparing motor maps elicited prior to training, immediately after training, and after a period of inactivity.

4.3 Materials and Methods

4.3.1 Subjects: Seventeen adult male Long-Evans hooded rats (350-420 g) were group housed (2 animals/cage) in standard laboratory cages on a 12:12 hour light/dark cycle for the duration of the experiment. All the animals were assigned to a Skilled Reaching Condition (SRC; n = 17) and were trained on the SRC for five days.

4.3.2 *Reach training:* Over the course of several days, animals were placed on a restricted diet, until they reached approximately 90% of their original body weight. All the animals were given several brief pre-training sessions on the

Whishaw Tray Reaching Task (see Dunnett et al., 1987). The animals were placed in test cages (10 cm x 18 cm x 10 cm) with floors constructed of 2 mm bars, 9 mm apart edge to edge. A tray (4 cm wide x 5 cm deep) filled with food pellets (Bioserv, 45 mg) was mounted on the front of the cage. The rats were required to reach outside the cage and retrieve pellets from the tray. The purpose of this task was to familiarize the animals with the pellets, as well as to determine the paw preference of all of the animals. All rats remained in pretraining until they had successfully retrieved 10 food pellets (approximately 1 hour/day for 2 days). Following pre-training, the animals were given their first surgery. After recovering from surgery, the animals were trained for five days in a Plexi-Glas cage (11 cm x 40 cm x 40 cm) with a 1 cm slot located at the front of the cage. SRC animals were trained for 10 minutes each day to reach through the slot and retrieve a single food pellet from a platform attached to the front of the cage (Whishaw and Pellis, 1990). Each session was videotaped and was later used to assess reaching performance. A successful reach was scored when the animal grasped the food pellet, brought it into the cage and to its mouth without dropping the pellet. The percentage of successful reaches was then calculated [(# successful retrievals/total # of reaches) x 100]. Prior to the third and final surgery, the animals received a probe trial on the skilled reaching task, in which they retrieved ten pellets.

4.3.3 Electrophysiological mapping: The animals received three surgeries. The first surgery was performed after pre-training but prior to training when paw preference had been established. The second surgery was performed on the day following the fifth and final training session. The third surgery was performed approximately thirty days after the second surgery. The animals received no training between the second and third surgeries. The surgeries consisted of producing a topographical map of the motor cortex contralateral to the trained paw using standard intracortical microstimulation (ICMS) techniques. The animals were first anesthetized with ketamine hydrochloride (70 mg/ kg ip) and xylazine (5 mg/kg im). The animals received isoflurane gas (.15%-.25%) and supplementary injections of ketamine (20 mg/ kg ip) and xylazine (1 mg/ kg ip) as needed.

The skull directly over the motor cortex, contralateral to the trained paw, was removed. The dura mater was retracted, and the skull was covered with warm silicon oil. A digital image of the brain was taken for use as a guide for microelectrode penetrations. A 375 µm grid was superimposed over the digital image of the cortex. Using a hydraulic microdrive, a glass microelectrode was lowered to a depth of approximately 1500 µm, which corresponds to cortical layer V. A 40-ms train of thirteen thousand, two hundred-µs monophasic cathodal pulses delivered at 350 Hz from an electrically isolated, constant current stimulator was used to stimulate the cortex. The animal was held in a prone position, with its trained paw held in a constant position. At each penetration

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site, the current was gradually increased until a movement was evoked (threshold current). The current was increased at most to 60 μ A. If no movement was evoked at 60 μ A, the site was labeled non-responsive.

The caudal forelimb area (CFA) of the motor cortex was the area that was mapped, because it is the only area of the rat motor cortex that has been shown to undergo reorganization in response to the skilled reaching task (Kleim et al., 1998). Forelimb movements were categorized as either distal representations (digit and wrist movements) or proximal representations (elbow and shoulder movements). The head, neck, vibrissae, and non-responsive sites were used to determine the border of the CFA. After the motor maps were derived, the area of each type of representation was determined.

4.4 Results

4.4.1 Group Assignment: Prior to analysis, animals were assigned to Treatment condition on the basis of the difference between Map1 and Map2. If an animal exhibited a 10% or more shift in the percentage of the CFA occupied by distal representations, they were placed in the Reorganized condition (n=5). All other animals were considered Unreorganized (n=12). There was a discrepancy between the number of animals in the Reorganized and Unreorganized groups because only five of the seventeen animals in this experiment showed motor map reorganization based on the experimental criteria. If the training time had been

increased, it would be expected that more animals would have undergone cortical reorganization.

4.4.2 Reaching Accuracy: A within subject Analysis of Variance (ANOVA) with DAY as a within subject factor and CONDITION as a between subject factor revealed a significant DAY \times CONDITION interaction on reaching accuracy (F(5,75) = 2.38; p<0.05). Subsequent multiple comparisons showed that Reorganized animals had a significantly higher reaching accuracy than Unreorganized animals on all but the first day of training (*Fisher's PLSD; p<0.05) (Figure 10). The Unreorganized animals, however, also showed no significant increase in reaching accuracy from the first to the last day of training (Figure 11A) while the Reorganized animals had a significantly higher reaching accuracy on the last day of training (Figure 11B). Furthermore, there was no significant difference in reaching accuracy between the last day of training and the probe trial in either the Reorganized or Unreorganized animals (Figure 11C and Figure 11D).

Figure 10: Reaching accuracy on the skilled reaching task. There was a one month period with no skilled reach training between Day 5 and the Probe Trial. Reorganized animals had a significantly higher reaching accuracy than Unreorganized animals on all but the first day of training. Unreorganized animals showed no significant increase in reaching accuracy from the first to the last day of training while the Reorganized animals had a significantly higher reaching accuracy on the last day of training. There was no significant difference in reaching accuracy between the last day of training and the probe trial in either the Reorganized or Unreorganized animals



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Figure 11: A comparison between days of reaching accuracy on the skilled reaching task. A) Animals with Unreorganized maps did not show a significant difference in reaching accuracy between the first and last days of training. B) Animals that showed Reorganization did show a significant difference between the first and last days of training. C) Unreorganized animals did not have a significant difference in reaching accuracy between the last day of training and the probe trial. D) Reorganized animals did not have a significant difference in reaching accuracy between the last day of training and the probe trial.







4.4.3 Topography of Movement Representations: A within subject Analysis of Variance (ANOVA) with DAY as a within subject factor and CONDITION as a between subject factor revealed a significant DAY x CONDITION interaction of the percentage of the CFA occupied by distal movement representations (F(5,75) = 5.53; p<0.05) (Figure 12). Subsequent multiple comparisons showed that the Unreorganized animals had a significantly greater percentage of the CFA occupied by distal movement representations (*Fisher's PLSD; p<0.05) (Figure 13A). Furthermore, Reorganized animals showed a significant increase in the percentage of distal representations between the pretraining and posttraining maps (Figure 13B) while the Unreorganized animals showed no such difference. Finally, no significant difference between Map2 and Map3 was found for either the Unreorganized or Reorganized animals (Figure 13C and Figure 13D). See Figure 14 for examples of topographical maps from each condition.

Figure 12: Percentage of distal movements seen in caudal forelimb area in Map 1 (pretraining), Map 2 (posttraining) and Map 3 (following probe trial). Reorganized animals showed a significant difference between the pretraining and the posttraining maps.



Figure 13: Motor map comparisons. A) Percentage of distal movements representations prior to training in Reorganized and Unreorganized animals. B) Percentage of distal movement representations in Reorganized animals from Map 1 and Map 2. There was a significant difference between the two maps. C) Percentage of distal movement representations in Unreorganized animals in Map 2 and Map 3. D) Percentage of distal movement representations in Reorganized animals in Map 2 and Map 3.



В.

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D.

Figure 14: Example of topographical caudal forelimb motor maps. Green represents distal movements and blue represents proximal movements. The first three maps are from an Unreorganized animal, and the last three maps are from a reorganized animal. A) Map 1. B) Map 2. C) Map 3. A) Map 1. B) Map 2. C) Map 3.



A. Unreorganized animal, Map 1

B. Unreorganized animal, Map 2



C. Unreorganized animal, Map 3



D. Reorganized animal, Map 1



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E. Reorganized animal, Map 2



F. Reorganized animal, Map 3



4.5 Discussion

This experiment examined whether motor cortex reorganization represents the consolidation of motor learning. Consolidation of a skilled motor task could be represented as a permanent change in cortical reorganization. If cortical reorganization represents consolidation of motor learning, then once reorganization has occurred it would become resistant to decay, and the animal's behavioural performance on the task would persist in the absence of training. The present experiment found that some of the animals that were trained for five days on a skilled reaching task showed cortical reorganization. Once cortical reorganization had occurred, it persisted in the absence of training for one month. Further, animals that showed cortical reorganization learned the task, while the animals that did not show reorganization did not learn the task. These findings provide evidence that cortical reorganization represents the permanent neural encoding of a skilled motor behaviour.

The results from the present experiment also suggest that the state of the motor cortex prior to motor learning is an important factor in how quickly an animal is able to learn a skilled motor task. Animals that had a large amount of distal representations in the caudal forelimb area of motor cortex prior to training did not learn the skilled reaching task as quickly or as well as animals that had more elbow representations. Kleim and colleagues (1998) showed that training animals on a skilled reaching task leads to an increase in wrist representations. This implies that if the motor cortex is unable to undergo

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reorganization then the animals are unable to learn the task as quickly as animals that are. It was also found that animals exhibiting distal movement reorganization were able to learn the task faster than animals that had a small or no amount of distal movement reorganization. This implies that task learning may depend in part on the predisposition of the motor cortex to reorganize.

It has been shown, however, that cortical reorganization and motor skill learning do not occur at the same time. Kleim and colleagues found that animals that were trained on a skilled reaching task successfully acquired the task prior to cortical reorganization. Thus, there must be some cellular mechanisms that support the initial acquisition of a skilled motor behaviour. Rioult-Pedotti and colleagues found that the intracortical horizontal connections of layer II/III of the motor cortex were strengthened following training on a skilled reaching task. It was also found that long-term potentiation (LTP) could not be induced as readily in the trained hemisphere of the motor cortex compared to the contralateral hemisphere. This suggests that LTP is involved in motor learning (Rioult-Pedotti et al., 1998). Further, it has been found that there is an increase in the number of synapses per neuron in the caudal forelimb area of motor cortex, the same area where functional reorganization is observed (Kleim et al., 2002). Thus, these cellular mechanisms may be involved in the motor learning process prior to motor cortex reorganization.

Several experiments have shown that there are changes in motor cortex activation following a brief motor skill training session (Shadmehr & BrashersKrug, 1997; Classen et al., 1998; Karni et al., 1998; Gandolfo et al., 2000). These changes in motor activity patterns are transient, only persisting for several minutes following the training session (Classen et al., 1998; Liepert et al. 1999; Rosenkranz et al., 2000). This suggests that brief training sessions can lead to immediate changes in motor cortex function, but that more training is required before these changes become resistant to decay. Further, Kleim and colleagues found that with extensive training on the skilled reaching task, the skill persists in the absence of training (Kleim et al., 2000).

The current experiment and as well results from experiments mentioned in this discussion have contributed to a theory of motor memory consolidation. Once an animal has learned a novel motor task and repeated it sufficiently, the motor cortex recognizes that the ability to perform the task is important, and so the cortex reorganizes to support this new behaviour. Once the motor cortex has 'consolidated' this new information through cortical reorganization, the new representational map becomes resistant to change or decay.

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CHAPTER FIVE

GENERAL DISCUSSION

Motor learning is an essential aspect of mammalian behaviour. Despite the prevalence of skilled motor behaviour, the neurobiological mechanisms underlying motor learning are unclear. This thesis examined how one form of plasticity (motor map reorganization) within one motor area (motor cortex) was related to motor learning. The first experiment examined how forelimb movements change during learning and how these changes relate to the functional reorganization in forelimb motor cortex associated with skilled reach training. It was found that the distal components of the stereotyped reach develop as the animal learns the task. This is consistent with the increase in distal representations in forelimb motor cortex observed after such training. The second experiment discovered that repetition of the skill movements is required to produce cortical reorganization. The third experiment found that once the motor cortex reorganizes, it does not revert to the original organization in the absence of task performance. Thus, it is likely that cortical reorganization represents the consolidation of motor skill. It was also found that the state of movement representations within motor cortex prior to motor learning dictates how fast an animal is able to learn a motor task and helps provide evidence of how the motor cortex adapts to support the novel skill.

5.2 Specificity of Motor Experience (Activity versus Learning)

Previous experiments have found that strength training and aerobic activity do not produce motor cortex reorganization, while skilled reach training does (Kleim et al., 1998; Remple et al., 2001; Kleim et al., 2002). Thus, it has been shown that it is the motor skill component of skilled reach training that produces cortical reorganization rather than the increase in motor activity associated with the task. Although there is evidence that the type of motor learning drives specific functional changes in motor cortex, a recent experiment found that functional reorganization of motor cortex and increased reaching accuracy do not occur at the same time. It was found that there is a significant increase in reaching accuracy by seven days of training, yet significant functional reorganization was not observed until ten days of training (Kleim et al., 2001). Therefore, learning a novel motor skill is insufficient to induce motor cortex reorganization. The second experiment of this thesis found that once the skill is acquired it must be repeated sufficiently before cortical reorganization will occur. Karni and colleagues found that a brief training session is enough to transiently change cortical patterns of motor activity (1995). Classen and colleagues found that extensive training sessions produced long-term changes in cortical patterns of activation (1998). Taken together, the findings from the first two experiments suggest that the learned movement patterns dictate the movement representation reorganization that occurs, and that once this novel skill is acquired it must be sufficiently practiced before the motor cortex will reorganize.

5.3 What Is It About Skill Learning That Drives Reorganization?

The results from this thesis show that although activity alone is insufficient to drive cortical reorganization, sufficient skilled activity is required. It is unclear, however, what is different between skilled and unskilled motor behaviours. Some insight may be gained from studies of cortical reorganization and perceptual skill learning. Behavioural relevance appears to plays a critical role in somatosensory cortex reorganization. The simple presentation of sensory stimuli alone is insufficient to drive changes in sensory representations. However, when an animal must learn to use that sensory information the representation of that stimulus in sensory cortex expands. Recanzone et al. (1992b) found that owl monkeys trained on a tactile frequency discrimination task on one hand showed sensory cortex reorganization compared to the sensory cortex representing an 'untrained' hand or a passively stimulated hand. The salience of a sensory stimulus appears to be selected via activation of the nucleus basalis (Mesulam et al., 1983; Rye et al., 1984). Richardson and DeLong (1991) found that neurons in the nucleus basalis are activated when an animal is attending to an important behavioural stimulus. Kilgard and Merzenich (1998) have shown that auditory cortex reorganizes in the presence of extraneous nucleus basalis activation paired with a tone. The auditory cortex responded to the tone as if it was a significant stimulus.

Can an analagous system be used to explain learning-dependent reorganization within motor cortex? This is unlikely. Plautz and colleagues have shown that animals trained on an unskilled task, in which they are attending to and retrieving a food reward, do not show cortical reorganization (2000). Further, animals trained on an unskilled reaching task, in which they reach outside of the cage towards a food pellet, but never learn to grasp and retrieve the pellet, also do not undergo reorganization (Kleim et al., 1998). Yet, in both experiments, the animals are still attending to the food pellet. Thus, it is more likely that learning novel movement patterns, and not behavioural saliency, drives cortical reorganization. The individual muscle contractions produced during skilled reach training are not novel. We suggest that it is the generation and repetition of novel patterns of muscle contractions, that are selected from the animal's existing movement repertoire, that drive reorganization. Thus, it is the novelty of movement sequences combined with repetition that produces motor cortex reorganization.

5.4 A Theory of Motor Learning in the Motor Cortex

The results from these experiments and others have led to the following theory of motor learning. This theory is based on results from Karni and colleagues (1998), in which they demonstrate two phases of motor learning. The fast phase occurs first, during which the animal makes rapid improvements in performance. The slow phase of motor learning occurs after and is characterized by repetition and fine-tuning of the movements acquired during the fast phase with more subtle improvements in performance. With respect to the present results, the initial fast phase of motor learning, in which the animal makes rapid progress on the skilled reaching task, occurs before the motor cortex reorganizes (Kleim et al., 2000). Motor map reorganization occurs after the task has been successfully acquired. Therefore, there must be a different neural substrate, other than cortical reorganization, that supports fast phase learning. Kleim et al. (2002) found that there is an increase in synapse numbers in layer V of the rat motor cortex after five days of training. Likewise, Rioult-Pedotti and colleagues found that following training on a skilled reaching task the horizontal cortical connections of layer II/III of the rat motor cortex were strengthened. This is interesting given that intracortical microstimulation, the technique used in this experiment to examine cortical reorganization, produces movements via activation of horizontal afferents. Thus, it has been proposed that a possible mechanism behind fast phase learning may involve synaptic plasticity that is not manifested as changes in motor maps.

Following the fast phase of learning is a slow phase, in which the animal makes minute adjustments to its reaching behaviour, until the movements become stereotyped. The animal is repeating the learned behaviour, but is not making significant improvements in task performance. It has been found that cortical reorganization occurs at this time (Kleim et al., 2000). The second experiment in this thesis suggests that once the novel motor behaviour is acquired it must be repeated sufficiently before it becomes permanently encoded in the cortex by a redistribution of movement representations.

The third experiment of this thesis examines whether motor cortex reorganization represents the consolidation of motor learning. If cortical reorganization represents consolidation of motor learning, then once reorganization has occurred it would become resistant to decay, and the animal's behavioural performance on the task would persist in the absence of training (i.e. it would become 'permanent'). The third experiment found that this was the case. Some animals that were trained for five days learned the skilled reaching task. These animals also showed cortical reorganization, which persisted in the absence of motor skill training for one month. Conversely, animals that did not learn the task in this short time period did not undergo cortical reorganization. This experiment answers an important question about the relationship between motor learning and cortical reorganization. These findings provide evidence that cortical reorganization represents the permanent neural encoding of a skilled motor behaviour.

This experiment also found that animals with naïve motor maps that consisted of predominantly distal movement representations did not learn the task as well as other animals. When these animals were mapped following five days of training, it was found that their maps did not show a significant increase in distal movement representations. These findings persisted in the absence of skill training. Thus, it can be hypothesized that the state of the brain prior to motor learning is also an important factor on how well an animal learns the task. If an animal is trained on the skilled reaching task but does not have the capacity to reorganize for distal movement representations, then the animal will not be able to successfully learn the task.

The results from the third experiment as well as results from previous experiments support the consolidation theory of motor learning, in which a novel motor skill is encoded in the motor cortex through movement representation reorganization. There are several experiments that have shown that there are changes in motor cortex activation following a brief motor skill training session (Shadmehr & Brashers-Krug, 1997; Classen et al., 1998; Karni et al., 1998; Gandolfo et al., 2000). However, these changes in motor activity patterns are transient, only persisting for several minutes following the training session (Classen et al., 1998; Liepert et al. 1999; Rosenkranz et al., 2000). This suggests that brief training sessions can lead to immediate changes in motor cortex function, but that more training is required before these changes become resistant to decay. Further, Kleim and colleagues found that with extensive training on the skilled reaching task, the skill persists in the absence of training (Kleim et al., 2000).

5.5 Conclusions

Many aspects of human behaviour involve motor learning. The present thesis has attempted to examine the relationship between motor learning and motor cortex reorganization. Using a different experimental design, behavioural training paradigms and a single neurophysiological technique, this thesis has examined how motor map reorganization is related to learning a novel motor behaviour. The first experiment investigated the developing components of the rat's stereotyped reach, and how these components relate to cortical reorganization. The second experiment discovered that once a novel motor skill has been acquired, it must be repeated before the cortex reorganizes to support it. We have also examined whether cortical reorganization represents consolidation of motor skill, discovering that once a motor skill is encoded in the motor cortex through cortical reorganization, it is permanently represented in the cortex. Thus, this thesis has helped provide evidence for a direct relationship between motor learning and functional plasticity within motor cortex.

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