TRAINING-INDUCED POTENTIATION IN THE NEOCORTEX AND ITS INTERACTION WITH STIMULATION-INDUCED LONG-TERM POTENTIATION AND LONG-TERM DEPRESSION

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A Thesis
Submitted to the School of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree
Doctor of Philosophy

McMaster University

NEOCORTICAL PLASTICITY AND MOTOR SKILL LEARNING

DOCTOR OF PHILOSOPHY (2001) McMaster University
(Psychology) Hamilton, Ontario

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NUMBER OF PAGES: viii, 126

Abstract

Long-term potentiation (LTP) is a long-lasting increase in synaptic efficacy following high-frequency electrical stimulation. Long-term depression (LTD) is a low-frequency stimulation induced reduction in synaptic efficacy. LTP-inducing stimulation is delivered pre-synaptically through an electrode which has been lowered into a neural pathway in an animal's brain. The stimulation parameters used to induce LTP produce alterations which have many of the same properties as memory. As such, LTP is a popular laboratory model of learning and memory. However, the relationship between LTP and memory remains unclear. The fundamental problem associated with linking the two phenomena is that the experimental paradigms used to study them are fundamentally different. Memory is typically measured behaviorally; LTP is typically assessed by measuring the size of evoked potentials -- an indication of synaptic efficacy. New methods allow the relationship to be more closely examined. The best of these paradigms involves training animals on a task which requires them to encode information, then monitoring for changes which resemble LTP in the regions of the brain associated with the acquired skill. Collectively this approach is known as behavioral LTP. These paradigms have had limited success, because it is difficult to isolate a memory trace in the brain and rule out confounding variables which may be responsible for any recorded changes. In this thesis a behavioral paradigm is employed which requires animals to reach with one limb to retrieve a food reward. This reaching task paradigm is more effective in dealing with these problems than those used in the past. Because the task is unilateral, the animals can serve as their own controls, eliminating some confounds of previous experiments (such as stress level). The cortical region, which encodes the information necessary to acquire the skill, is relatively circumscribed compared to other tasks and structures. In the current experiments, animals unilaterally trained on the reaching task had larger evoked potentials in the trained hemisphere relative to the untrained hemisphere. Furthermore, subsequent LTP induction was reduced in the trained hemisphere compared to the trained hemisphere. These

results are consistent with the hypothesis that LTP and memory have the same underlying mechanism (the training-induced potentiation is believed to "use up" some of the modifiability of the affected synapses). Also consistent with this hypothesis is the finding that either long-term depression LTD- or LTP-inducing stimulation delivered following the acquisition of the task disrupted memory storage. Collectively these data support both the conclusion that memories are stored as synaptic changes and that the reaching task paradigm is a useful tool for investigating the relationship between LTP and memory.

Acknowledgments

First, I would like to thank my family for the tremendous support they provided as I worked toward the completion of this thesis. My parents, Bob and Bobbie provided me with undying support and encouragement and every time I was getting a little discouraged, they found a way to right my ship and keep me on course. I want them to know that this thesis was written largely for them and their contribution was essential. Here in Hamilton, John and Gayle gave me a family away from home for which I will be forever grateful.

Of course I would also like to thank my supervisor Ron Racine. I have Ron to thank for my advancement as a scientist. Ron's door was always open and he was always willing to listen and think about my research, as well as help me think about it. The insightful comments of my other committee members Denys deCatanzaro and Shepard Siegel made this a better thesis.

In the lab, many people helped with me with the collection of my data and deserve credit for the work they completed. They are Zhanxin Ji, without whose technical expertise I could not have completed chapter 2, Shay Standish, Tiffany Boyd, Vera Mpandare, Kasha Karaban, Lyndsey Kay, Erin Pichora, and Amy Henderson. I seem to have been enormously fortunate to have always been surrounded by hard working and team-oriented collaborators. I owe each of you an enormous thanks not only for the contribution you all made to this body of work, but also for always making my time spent in the lab better.

During my time here, I made many friends with whom I will maintain a friendship long after we have all left Mac. I will not forget all the great and crazy times I shared with and the loyal friendships I had with Marco Baptista and Amy Young. During my last year at Mac, Amy and Marco made what would have been a bad year a good one that I will never forget. Kevin Duffy was one of the first people I met at Mac and is someone with whom I shared all the highs and lows of being a grad student. Kevin provided constant encouragement and friendship. Joe Kim and I shared countless hours devising new projects and devising ways to fund our graduate careers. I shared many laughs with Louis Schmidt. Chris Horn and I devoted more brain power to the memorization of useless sports facts than we should have, but I enjoyed every minute of it. And Tiffany Boyd, I will never forget all that we shared and the incredible depth of our relationship. All aspects of my life here were richer because of what we shared.

Finally I would like to thank Colleen O'Neill. Colleen was with me from the beginning and was there when the stress of grad school was overwhelming and helped me through the bad times. Colleen also made a point of sharing in all of my successes and making sure I never forgot to enjoy all the accomplishments I had during the past four years. Her enthusiasm always made the good times much better.

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Chapter 1

General Introduction

The properties of memory acquisition and storage are known to differ as a function of a variety of characteristics of the information being encoded. Examples of these characteristics include the sensory modality, the environmental context, the cognitive processing required to encode the information, the degree to which the information is emotionally meaningful, and numerous other characteristics of the information to be stored and the neural systems engaged in the storage process. Memory can be separated into different categories based on the type of information stored, and the different categories obey fundamentally different rules. For instance, many aspects of the acquisition and retrieval of a motor skill differ from the acquisition and retrieval of verbal information. The conditions under which information is most easily and accurately stored and retrieved and the common types of encoding and retrieval errors have been well delineated. This knowledge has found applications in the classroom, courtroom, and other settings. These properties are typically measured behaviorally; internal events are sometimes inferred, but rarely measured directly. Thus, despite the success outlining memory's properties, the neural substrate of memory has long eluded both researchers and theoreticians.

Progress in memory research has been impeded by uncertainties about even the most basic properties of the physical manifestation of a memory trace. This difficulty is

reflected in the work of Karl Lashley who attempted to identify the engram (his word for the physical change in the brain that stores the information and serves as the memory trace). Lashley made numerous lesions to specific regions of the cortices of rats in an attempt to interfere with existing memories, but showed that even relatively large lesions often had little effect on memories. He concluded that memory traces were widely distributed throughout the cortex but provided no insight into the nature of this physical manifestation.

The most influential theory to guide the search for memory mechanisms was proposed by Donald Hebb in 1949. Hebb agreed with Lashley that memories are stored as distributed and redundant neural traces, but he also proposed a physical mechanism of their instantiation in the brain. He postulated that when neurons experience episodes of concurrent activity, the connection between them becomes strengthened. Such strengthened connections allow the creation of new neural circuits which serve as the neural representations of the events that stimulated the original activity. Brown, Kairiss, and Keenan (1990) outlined the three fundamental features of Hebbian synaptic modification. First there must be an exact temporal occurrence of pre- and post-synaptic activity. Second, there must be a spatial component, such that modifications are restricted to synapses where correlated activity occurs. Third, there must be a sufficient level of interaction. Both the pre- and post-synaptic cells must attain a certain threshold of activity before modification will occur.

Addressing Hebb's postulate experimentally has been tremendously difficult,

primarily because memory traces are stored diffusely, making precise measurements of trace formation essentially impossible. In 1973, Bliss and Lomo devised a method that allowed Hebb's assertion that pre- and post-synaptic co-activation strengthens synaptic connections to be tested. They lowered stimulating electrodes into the perforant paths of anesthetized rabbits. The perforant path is a large bundle of axons which carries information from numerous regions of the brain to the dentate gyrus of the hippocampus, a structure known to be involved in the memory process. By passing current through the electrodes, they were able to trigger action potentials in the perforant path axons and excitatory post-synaptic potentials (EPSPs) in their post-synaptic targets, the dentate gyrus granule cells. The intervening synapse utilizes the excitatory neurotransmitter glutamate. By delivering bursts of stimulation at a high frequency (the "conditioning" trains), Bliss and Lomo ensured that excitatory transmitter release from the stimulation was temporally coupled with a strong activation of the post-synaptic dentate gyrus neurons. This appeared to meet Hebb's criterion for synapse modification, and his theory would predict a strengthening of the connections between the perforant path and the dentate gyrus.

Bliss and Lomo monitored the strength of the synaptic connections by recording the sizes of the EPSPs evoked in the dentate gyrus by rectangular electrical pulses delivered to the perforant path. They used both *in vivo* and *in vitro* preparations (Bliss & Lomo, 1973; Bliss & Gardner-Medwin, 1973) and recorded both individual cell and population responses via recording electrodes in the dentate gyrus. By recording the

EPSP amplitude both prior to, and following the high-frequency stimulation protocol, they confirmed that the post-stimulation evoked potentials had increased in size. Responses in unstimulated control pathways in the same animals remained unchanged, which demonstrated that the response enhancement was specific to the activated pathway. This phenomenon is known as long-term potentiation (LTP) and is consistent with Hebb's law for strengthening synaptic connections.

Hebb outlined the memory store as one of a network of neurons, or a neural assembly. Studying the nature of the neural trace in a large and complicated matrix of neurons is extremely difficult. The importance of LTP is that, unlike neural changes which result from actual memory, it is amenable to precise manipulation. Researchers can spatially and temporally isolate the affected synaptic connections, something that is nearly impossible to do when investigating real memory. By using a model of memory in this fashion, it is possible to pursue the mechanisms of memory trace formation at the cellular level. Currently, LTP is by far the most widely used model phenomenon for the study of synaptic plasticity.

If LTP is to serve as a model of synaptic plasticity, then it must be confirmed that the response enhancement is, in fact, due to a synaptic mechanism. Synaptic involvement has been verified by showing the effect to be specific to the activated pathway. Input specificity has typically been studied in the hippocampal slices because of the experimental control this preparation affords. Using this preparation, Andersen and colleagues (1977; 1980) lowered stimulating electrodes into two independent pathways both of which

converge on the CA1 pyramidal neurons. Pulses delivered to either pathway produced a response in the target cells. Tetanus was delivered to one of the two pathways, but not both. Following this stimulation, only the stimulated pathway showed potentiation. In fact, the unstimulated pathway showed a brief depression effect in some of the slices. Input specificity has been frequently demonstrated and is often accompanied by a depression in unstimulated pathways (e.g. Lynch, Dunwiddie, & Gribkoff, 1977; Dunwiddie & Lynch, 1978; Abraham, Bliss, & Goddard, 1985). This effect is often referred to as heterosynaptic depression. Input specificity ensures that only information which activates the system will be encoded. The heterosynaptic depression effect may also reflect an information encoding mechanism. The Hebb rule does not work well unless a provision is added by which uncorrelated activation of pre- and post-synaptic elements leads to weakened synaptic connections. It is also known that LTP-inducing stimulation needs to reach a certain threshold in order to produce potentiation. This effect is known as co-operativity because it is thought that sufficient numbers of fibers need to be active before a system will potentiate (Lee, 1983).

Additional experiments were required to determine the underlying mechanisms of LTP and confirm that the connection strength enhancement followed Hebb's rule. LTP has been found in nearly every pathway tested in the brain, particularly those thought to be involved in memory. The regions supporting LTP include the amygdala (Maren & Fanselow, 1995), limbic forebrain (Racine, Milgram, & Hafner, 1983), the frontal cortex (Sutor & Hablitz, 1989; Racine, et al., 1995), the olfactory cortex (Stripling, Patneau, &

Gramlich, 1988) and the visual cortex (Kirkwood & Bear, 1994). There is even some preliminary evidence that the human hippocampus may support LTP (Babb, 1982; cf. Teyler & Discenna, 1987).

In pursuing the criteria of Hebbian synaptic modification, numerous attempts have been made to determine the consequences of altering the temporal relationship between pre- and post-synaptic neural activity. Kelso, Gagnon, and Brown (1986) delivered stimulation to Shaffer collateral/commisural fibers which project to area CA1. At the same time, they voltage clamped CA1 neurons in a depolarized state. Combining pre- and post-synaptic activity in this way induced LTP. When the two do *not* occur together, not only is there an absence of LTP, there is often a depression effect (Lisman, 1989). Malinow and Miller (1986) delivered high-frequency stimulation to the pre-synaptic cell while voltage-clamping the post-synaptic cell in a hyperpolarized state. This protocol, in which the pre-synaptic cell was active in the absence of post-synaptic activity, failed to produce synaptic enhancement.

Most forms of LTP are critically dependant on the N-methyl D-aspartate (NMDA) receptor, a type of glutamate receptor (Malenka & Nicholl, 1999). The responses recorded at excitatory brain synapses, however, are due primarily to activation of another type of ionotropic glutamate receptor called the AMPA receptor. Its activation opens channels for the flow of monovalent cations, resulting in post-synaptic depolarization. The NMDA receptor, however, requires more than just the binding of glutamate for its activation. The calcium ion channels to which they are coupled are normally blocked by

magnesium ions. These positively charged ions are released whenever the post-synaptic cell is in a state of depolarization. The requirement that glutamate release (pre-synaptic activity) be coupled with depolarization of the post-synaptic cell, gives the NMDA receptors the property of "coincidence detectors" (Coan & Collingridge, 1985). As such they are perfectly suited to detect the conditions that satisfy Hebb's rule.

Because the NMDA receptor seems to be an ideal candidate for a Hebbian mechanism, and because NMDA receptors are abundant in regions of the brain thought to be important for memory storage (Collingridge, 1985), they have been the focus of extensive research. Selective NMDA receptor antagonists have been used to demonstrate an NMDA-receptor dependency for LTP. Errington, Lynch, and Bliss (1987), for example, demonstrated that administration of the selective NMDA receptor antagonist D (-) aminophosphonovalerate (AP5), blocks the induction of LTP in the rat hippocampus. It has also been demonstrated that merely applying NMDA is sufficient to produce an LTP effect in the hippocampus (Kauer, Malenka, & Nicholl, 1988a). More recently, genetic engineering techniques have been used to create knockout mice with selective deficits of NMDA receptors in the CA1 region of the hippocampus, an area thought to be crucial in the acquisition of spatial information. These mice are highly resistant to LTP induction in CA1 (Wilson & Tonegawa, 1997). Non-NMDA glutamate receptor antagonists DNQX and CNQX have been used to demonstrate the importance of the AMPA receptor in the expression of LTP (Muller, Joly, & Lynch, 1988; Kauer, Malenka, & Nicholl, 1988b).

NMDA receptor dependencies have been repeatedly and reliably demonstrated

(e.g., Gustafsson & Wigstrom, 1988; Collingridge, Kehl, & McLennan, 1983; Morris, Anderson, Lynch, & Baudry, 1986). There are, however, examples of LTP which do not rely on NMDA receptor activation. One such incidence of non-NMDA dependent LTP is found in the hippocampus itself, in area CA3. Harris and Cotman (1986), for example, demonstrated that NMDA receptor antagonists do not block LTP in the CA3 region of the hippocampus. This area is thought to be important for certain types of learning (Jensen, & Lisman, 1996), but it has a relatively low density of NMDA receptors (Monaghan, & Cotman, 1985). This non-NMDA LTP may be mediated pre-synaptically (Johnson, Williams, Jaffe, & Gray, 1992). This is in contrast to NMDA receptor dependent LTP which is thought to be mediated primarily by post-synaptic alterations (Nicholl & Malenka, 1999), although there is some evidence of a presynaptic increase in transmitter release (Dolphin, Errington, & Bliss, 1982; Errington, Lynch, & Bliss, 1987). Other NMDA-receptor independent forms of LTP have been demonstrated in the visual cortex (Aroniadou & Teyler, 1992) and in the basal dendrites of CA1 pyramidal cells (Cavus & Teyler, 1998). Despite these exceptions, the NMDA receptor remains a crucial component in most forms of LTP.

Because ion channels coupled to the NMDA receptor mediate an influx of calcium, researchers have focused on the role of calcium in LTP induction. Radio-labeling techniques have been used to demonstrate that there is an increase in calcium post-synaptically following the application of LTP-inducing stimulation in area CA1 (Baimbridge & Miller, 1981). Transient increases in extracellular calcium (Turner,

Baimbridge & Miller, 1982) or intracellular calcium (Malenka, Lancaster, & Zucker, 1992) can induce an LTP-like phenomenon, whereas reducing extracellular calcium can prevent hippocampal LTP induction (Dunwiddie & Lynch, 1979). In another line of evidence implicating calcium in LTP induction, the calcium chelator EGTA was injected directly into CA1 pyramidal neurons (Lynch, Larson, Kelso, Barrioneuvo, & Schottler, 1983) or layer II/III neurons of the visual cortex (Kimura, Tsumoto, Nishigori, Yoshimura, 1990). EGTA blocked LTP in those neurons without otherwise disrupting their functioning.

Following its discovery (Lomo, 1966) and its more thorough exploration in 1973, LTP seemed to provide researchers with the tool they would require to delineate the neural mechanism of memory. Although it is not yet proven that strengthened synapses are the mechanism of memory storage, the existence of LTP satisfies the Hebbian criterion of concurrent pre- and post-synaptic activity.

LTP and Memory

To the extent that LTP and memory are based on the same neural mechanism, it would be expected that the two phenomena would share similar properties and mechanisms. Early LTP research was largely designed to assess those expectations, and the results largely supported a connection between LTP and memory. The resulting experimental approaches and attempts to draw comparisons between LTP and memory can be separated into two categories: Those which address the degree to which the two phenomena share similar properties and those which address the degree to which they rely

on a common mechanism.

The Properties Argument

This approach is based on the principle that if both LTP and memory are activating the same plasticity substrate, then the two phenomena ought to display many of the same properties.

One property of memory is that it can be rapidly induced by a transient stimulus. Similarly, LTP is induced by a burst of high-frequency stimulation with a duration measured in the order of milliseconds. Abraham, Gustafsson, and Wigstrom (1986) have demonstrated an extreme example of a transient stimulus used in LTP induction. They delivered a single volley, as opposed to a series of volleys of high-frequency stimulation to area CA1 of the hippocampus. Even this remarkably transient stimulation protocol produced potentiation provided the stimulation was delivered at a sufficiently high intensity.

As with memory, transient neural stimulation induces an enduring change which lasts long after the termination of the stimulation. LTP can last for at least days to weeks in most systems (Bliss & Gardner-Medwin, 1973; Racine, Milgram, & Hafner, 1983; Barnes, 1979), although the duration varies. Neocortical LTP, for example, appears to be more enduring than hippocampal LTP.

The difference between the hippocampus and the neocortex in LTP longevity is also consistent with theories about the role of these structures in memory. Damage to, or

complete removal of the hippocampus and surrounding temporal lobe structures renders organisms essentially incapable of acquiring certain types of new memories, particularly declarative memories (memories of facts and events) (Zola-Morgan & Squire, 1985; 1986). Such damage also leads to a graded retrograde amnesia. Recently acquired declarative information is lost while more remote memories remain intact.

Memory for events must be stored rapidly as the event may occur briefly and only once. The importance of the hippocampus, then, is that it has the capacity to rapidly acquire new information. With the exception of priming, however, the memory that remains intact following hippocampal damage requires multiple trials to become secure. The types of learning that occur slowly include most forms of conditioning, habit learning and motor learning. All of these are believed to be at least partially dependent on the neocortex and can develop normally in the absence of the hippocampus. Partially due to the differences in memory content and learning rates, the hippocampus and neocortex are thought to play different but complimentary roles in the memory process (McClelland, McNaughton, & O'Reilly, 1995). The hippocampus is involved in declarative memory and is believed to acquire information rapidly. Eventually, the information will be stored more permanently in the neocortex, the site of long-term storage (Murray & Bussey, 2001), not the hippocampus. This is why remote memories remain intact in subjects with hippocampal damage. The delay in acquisition by the neocortex is believed to be due to the slower learning rate, which, in turn, allows the neocortex to interleave old and new information and accommodate overlapping information that would otherwise cause

interference and memory failure (McClelland et al., 1995).

There is an analogous distinction between LTP in the hippocampus and the neocortex. The induction of LTP in the neocortex typically requires numerous stimulation sessions and these sessions work better when they are spaced far apart (Hodgson, Trepel, & Racine, 1999). Beiko and Cain (1998) demonstrated that a single stimulation session can induce LTP in the posterior parietal cortex, but that it still requires multiple sessions to reach asymptote. Hippocampal LTP typically asymptotes much more rapidly (e.g., Hoh, Beiko, Boon, Weiss, Cain, 1999). Once LTP has been induced in the neocortex, it is durable, lasting much longer than hippocampal LTP. The induction of LTP in the hippocampus, in contrast, is much more rapid, often requiring only a single session to reach asymptotic levels. It is not nearly as durable, however, often decaying to baseline often over a period of days or weeks. Repeating LTP sessions, required for neocortical LTP (Racine et al., 1995), has little effect on the duration of hippocampal LTP (deJonge & Racine, 1985) which typically shows almost full decay within a day.

There are also similarities in the effects of age on both LTP and memory. Barnes (1979) demonstrated that older animals acquire spatial information more slowly, and lose it more rapidly than younger animals. The older animals also took longer to potentiate to asymptotic levels and displayed a faster rate of LTP decay. Moving to younger animals, Perkins and Teyler (1988) demonstrated that neocortical LTP is generally easier to induce in very young animals aged less than 15 days. The impact of age on LTP may relate to critical periods for the acquisition of information during which brain systems demonstrate

enhanced plasticity. The fact that younger animals show both better LTP and better learning has been linked to the NMDA receptor. Younger animals have an abundance of the r1 subunit of the NMDA receptor which is associated with a calcium ion channel that remains open for a prolonged time when the receptor binds glutamate. The long open-time of the channel allows for an abundant influx of calcium. Calcium is an essential trigger for LTP induction and the amount of intracellular calcium correlates with the amount of LTP induced. Older animals, conversely, have more of the r2 subunit which has faster kinetics (Bliss, 1999).

It is well known that events that overlap temporally become associated with one another. This effect is demonstrated most compellingly in classical conditioning studies in which a weak (insufficient to induce LTP on its own) stimulus, the conditional stimulus (CS), is paired temporally with a biologically meaningful stimulus, the unconditional stimulus (US). After numerous pairings, an appropriate response develops to the CS which reflects that it has acquired predictive power. Levy and Steward (1979) demonstrated an analogous phenomenon with LTP. They chronically implanted rats with a unilateral recording electrode in the dentate gyrus and stimulating electrodes in the entorhinal cortices of both hemispheres. The entorhinal cortex sends projections to the dentate gyrus via the perforant pathway. Stimulation of the ipsilateral entorhinal cortex produced a strong input sufficient to induce LTP; stimulation of the contralateral entorhinal cortex produced a response that was too weak to induce LTP. However, when the ipsilateral and contralateral entorhinal stimulation trains were paired, LTP was induced

in the weak contralateral pathway. Associative LTP, in principle, appears to provide a mechanism for associative learning. Levy and Steward (1983) strengthened this analogy when they demonstrated that the same temporal constraints which bind associative learning also bind associative LTP. Specifically, the weak stimulus must coincide with, or precede, the strong stimulus for associative LTP to occur.

Memory is also subject to a period of consolidation during which newly acquired memories are vulnerable (Murray & Bussey, 2001). One example of this vulnerability comes from patients who undergo electroconvulsive shock as a treatment for depression. Following the treatment, they display a retrograde amnesia for information acquired shortly prior to the treatment, but more remote memories are unaffected (Glickman, 1961; McGaugh & Petrinovich, 1966). A similar retrograde amnesia has been demonstrated in rats; electrical stimulation to the hippocampus disrupts recently acquired spatial information (Knowlton, McGowan, & Olton, 1985; Barnes et at., 1994). In an analogous fashion, electroconvulsive shock delivered to rats following the LTP-inducing stimulation has been shown to block the induction of LTP in both the hippocampus (Hesse & Teyler, 1976) and the neocortex (Trepel & Racine, 1999).

The Mechanism Argument

Although the properties of LTP make it a strong candidate memory model, they do not prove that LTP and memory share mechanisms. This shortcoming has led to a second major approach to assessing the relationship between the two phenomena.

The most apparent commonality between LTP and memory mechanisms is the

dependence on the NMDA receptor. NMDA receptor activation has been shown to be necessary to induce LTP in the dentate gyrus (Morris, Anderson, Lynch, & Baudry, 1986), area CA1 of the hippocampus (Collingridge, et al., 1985), the amygdala (Maren, 1999); the somatosensory cortex (Kitagawa, Nishimura, Yoshioka, Lin, Yamamoto, 1997), the visual cortex (Kirkwood & Bear, 1994), the piriform cortex (Jung, Larson, & Lynch, 1990), as well as numerous other regions in the brain.

The NMDA receptor has been shown to play a vital role in certain forms of learning as well. Infusing the rat hippocampus with AP5, for example, can impair acquisition of a spatial memory task, the Morris water maze, but not a non-spatial task (Bannerman, Good, Butcher, Ramasy, & Morris, 1995). Bannerman et al. (1995) also reported that a mutation in the NMDA receptor subunit can disrupt spatial learning. Infusing the barrel cortex in the rat with AP5 disrupts the acquisition of a learned whisker discrimination (Rema, Armstrong-James, & Ebner, 1998). Tonkiss, Morris, and Rawlins (1988) showed that AP5 infused intra-ventricularly blocked the acquisition of a non-spatial operant task. In another study, AP5 infused into the basolateral amygdala blocks latent inhibition fear conditioning (Schauz & Koch, 2000). The doses required to impair the acquisition of novel information are comparable to the doses used to block LTP.

The evidence that NMDA antagonists block both learning and LTP in a similar manner remains controversial, however. This controversy is most notable in the hippocampus/spatial learning experiments. One of the most commonly used behavioral paradigms for assessing spatial learning is the Morris water maze. The Morris water maze

requires rats to find a platform submerged in an opaque liquid. They are placed in a large round pool of the liquid and must swim to the platform, the location of which must be determined on the basis of distal room cues. The animal learns where the platform is by using the relative position of the cues in the room. Thus there is extensive spatial processing which is thought to be mediated primarily by the hippocampus (Schenk, & Morris, 1985). Using this paradigm Saucier and Cain (1995) tested the hypothesis that both spatial learning and hippocampal LTP are critically dependent on NMDA receptor activation. They pre-trained rats on the non-spatial components of the water maze task, such as learning to swim and that there is a platform available for escape. They then trained the rats on the spatial component of the task – where the platform is consistently positioned relative to the spatial cues in the room. Prior to the training, they administered NPC17742 (2R,4R,5S-2-amino-4,5-(1,2-cyclo hexyl)-7-phosphonoheptano acid), which they described as a potent and specific antagonist of NMDA receptors. This antagonist completely blocked LTP in the dentate gyrus but failed to impair spatial learning in the pre-trained animals. It remains unknown if these animals are using an NMDA-independent form of LTP. Nevertheless, these results cast doubt on the hypothesis that NMDA-dependent LTP is necessary for spatial learning. A counter argument is that there are spatial processing demands in the pre-training. For example, learning the something about the features of the water maze environment may facilitate subsequent encoding of other spatial features. Perhaps this training made the animals more resistant to disruption on the subsequent task of finding the platform relative to the cues because it imposed a

relatively trivial amount of processing demand on the animals. Moreover, there were no comparable prior treatments for LTP making comparisons between LTP and memory difficult.

NMDA receptors mediate a post-synaptic influx of calcium, so a lot of attention has been paid to the role of calcium in both LTP and memory. Blocking voltage-dependant calcium channels impairs both spatial learning and hippocampal LTP (Borroni, Fichtenholtz, Woodside, Teyler, 2000). The use of calcium blockers disrupts the acquisition of passive avoidance learning in the rat (Izquierdo, et al., 2000; Nikolaev & Kaczamarek, 1994). Conversely, increasing the levels of extracellular calcium has been shown to facilitate performance on a memory task (Gibbs, Gibbs, & Ng, 1979).

There appear to be several downstream effector pathways involved in both memory and LTP including two calcium-dependent kinases, calcium/calmodulin dependent kinase II (CaMKII) (Tsien, Huerta, & Tonegawa, 1996) and protein kinase C (PKC) (Malinow, Madison, & Tsien, 1996). Several lines of research have implicated CaMKII in both memory and LTP. For example, a mutant mouse with a deficiency in CaMKII in the hippocampus shows a deficit in both spatial learning (Silva, Paylor, Wehner, & Tonegawa, 1992) and hippocampal LTP (Silva, Stevens, Tonegawa, & Wang, 1992) while normal synaptic transmission remains intact. Also Otmakhov, Griffith, and Lisman (1997) demonstrated that blocking the activity of CaMKII in area CA1 blocked the induction of LTP.

Kleschevinkov and Routtenberg (2001) showed that application of a PKC

activator (4-beta-phorbal-12, 13 dibutyrate) unblocks LTP induction in animals treated with the NMDA blocker, AP5. Compounds which activate PKC have been shown to facilitate the induction of LTP in the hippocampus (e.g., Routtenberg, et al., 1986; Malenka, Madison & Nicoll, 1986). Kawakami, Miyata, Tanaka, Ashida, and Yamaguchi (1995) demonstrated a similar facilitation effect in the somatosensory cortex. Lovinger, Wong, Murakami, and Routtenberg (1987) demonstrated that inhibiting PKC eliminated potentiation in the hippocampus, although it did not block its initial induction. Similarly, PKC has been implicated in the memory process. Wong, Murakami and Routtenberg (1989) demonstrated that increasing an animal's brain level of PKC enhances spatial learning ability. PKC inhibitors infused into the amygdala blocked a learned fear response (Goosens, Holt, & Maren, 2000).

Various structural synaptic changes have been associated with both LTP and learning as well (Geinisman, 2000). Electron microscopy experiments, for example, have shown that LTP induction leads to an increase in the number of dendritic spines in the dentate gyrus (Trommald, Hulleberg, & Andersen, 1996) and in area CA1 following the acquisition of spatial information (Moser, Trommald, & Andersen, 1994). As well, synapse density has been shown to increase in the dentate gyrus following LTP induction (Stewart, Harrison, Rusakov, Richter-Levin, & Maroun, 2000) and spatial learning (O'Malley, O'Connell, Murphy, & Regan, 2000).

Although the evidence implicating common mechanisms for LTP and memory is compelling, there are other interpretations of the data. The neural elements implicated in

memory and LTP are seen in concert with other behaviors and states. Shors and Matzel (1997), for example, have argued that the components of the LTP substrate make it suitable for an attentional mechanism. Increases in neural activation as a function of LTP increase the salience of an external stimulus. Stimuli which are more salient are more likely to be encoded into memory. Thus, an increase in attention would be expected to correlate positively with the formation of new memories which, according the Shors and Matzel, would explain the fact that LTP and memory tend to share a substrate. The time course, however, especially of neocortical LTP, does not seem appropriate for an attentional mechanism. Unless these attentional mechanisms are plastic, in which case LTP would necessarily be considered a memory mechanism for attention.

Taken together, experiments in which attempts have been made to implicate common mechanisms for learning and LTP have yet to make the case conclusively. The shared properties likely apply to numerous other functions as well. One problem with this research is that the conditions, manipulations and measures differ dramatically between memory research and LTP research. Memory manipulations involve the whole animal and leave all the supporting neural memory systems intact. LTP research, conversely, usually involves a slice of the brain and the manipulation is localized to a precise region and pathway. It is difficult to know how the two phenomena *should* relate.

Behavioral LTP

Barnes (1979) was one of the first to attempt to establish a link between LTP and memory by comparing their acquisition and decay properties in the hippocampus, which is

known to be required for spatial learning. She investigated the capacity both to learn a spatial task and to support hippocampal LTP within the same organism and found that rats that are good learners are more amenable to LTP induction. Barnes also found that animals that forget more quickly have a faster rate of decay of LTP. Although this was still a correlational approach, it was the first to bridge the gap between experimental learning and LTP paradigms.

The next step was to combine the experimental manipulation of memory experiments with the dependent measures of LTP research in a structure believed to be involved in storing task-specific information. In one such study, Sharp, McNaughton, and Barnes (1985) chronically implanted rats with stimulating and recording electrodes in the perforant path and dentate gyrus respectively. They then exposed the animals to a complex environment after having raised them in an impoverished environment. It was anticipated that exposure to an enriched environment would force the animals to acquire novel information. After exposure to the complex environment, there was a modest increase in the magnitude of the evoked field potentials they recorded. This response eventually decayed to baseline, but could be re-instantiated if the rats were exposed to a novel complex environment.

In another similar experiment, rabbits trained in a conditioned eye-blink paradigm displayed increased hippocampal field potential amplitudes following conditioning (Weisz, Clark, & Thompson, 1984). Training on a foot-shock avoidance task produced a similar effect (Reymann & Ott, 1982). Collectively, such effects have become known as

behavioral LTP (Teyler & DiScenna, 1987), and they seem to be consistent with a causal connection between learning and changes in synaptic connection strength. However, the behavioral LTP paradigms fail to provide the necessary control groups to rule out explanations other than learning for the increases in synaptic strength.

Other attempts to induce a behavioral LTP effect have been unsuccessful. Cain, Boon, and Dennison (1993) and Moser, Mathiesen, and Andersen (1993) found that training on the Morris water maze did not increase the amplitude of evoked hippocampal field potentials. Surprisingly, Moser et al. (1993) actually reported a depression effect after training on the water maze. Because it is a spatial task, the water maze would be expected to involve the hippocampus. Indeed, hippocampal lesions impair performance on the task (Moser, Moser, Andersen, 1993). If the training produced increases in the strength of connections, then they must have been diffuse and thus undetected by a focal electrode, or offset by decreases in connection strength in neighboring synapses.

Further research led to the conclusion that behavior alone, in the absence of learning, can produce a transient increase in hippocampal field potential magnitude (Green, McNaughton, & Barnes, 1990). Even in the absence of behavior, increases in hippocampal responding can be produced by merely heating an organism (Moser, et al., 1993; Thompson, Masukawa, & Prince, 1985). Subsequent research demonstrated that behaviorally trained animals maintained their increased field potential size, even after they were cooled with a fan, following exposure to a complex environment. This finding ruled out the interpretation that behaviorally induced modifications in field potential size were

purely a byproduct of the increased temperature of the animals following training. However, the issue of temperature-effect confounds undermined behavioral LTP experiments, and the uncertainty surrounding their validity continues.

A related problem is that the behavioral state of an animal can have an impact on the size of evoked field potentials. This finding has been demonstrated in both the hippocampus (Leung, 1980) and the cortex (Vanderwolf, 1988). Hargreaves, Cain, and Vanderwolf (1990) controlled for these effects of behavioral state and also failed to obtain behavioral LTP in a spatial task.

Another approach is to determine the impact of prior LTP stimulation on subsequent learning, commonly referred to as the saturation paradigm. These experiments are based on a questionable assumption: If learned responses are encoded in the brain via an increase in the strength of synapses, then it should be possible to block the acquisition of a specific skill by increasing all the synaptic strengths in the region responsible for that skill (i.e., by saturating the system). An attempt to employ LTP stimulation to "use up" the available plasticity in this manner was first tried by Castro, Silbert, McNaughton, and Barnes (1989). They delivered high-frequency stimulation bilaterally to the perforant path until asymptotic levels of LTP were achieved. They reported that this protocol blocked the capacity of their animals to acquire spatial information, and that this deficit was gone after the LTP had decayed. The assumption of saturation studies is questionable because it ignores the capacity of these systems to decrement, as well as increment, connection weights. These two processes are thought to act in a complementary fashion to encode

information (Martin, Grimwood & Morris, 2000). Thus, even when LTP is saturated, animals still have the capacity to employ decrements in synaptic strength as a mechanism for information storage. Perhaps for this reason, attempts to replicate the saturation-induced learning deficits have been unsuccessful (Jeffrey & Morris, 1993; Sutherland, Dringenberg, & Hoesing, 1993; McNamara, Kirkby, dePage, Skelton, & Cocoran, 1993; Korol, Abel, Church, Barnes, & McNaughton., 1993). However, the failure to produce the expected result has typically been attributed to the difficulty in stimulating all the fibers in the perforant path with a single focal electrode (e.g., Korol, et al., 1993). Saturation of LTP in only a selection of synaptic connections leaves the remaining connections available to encode the information necessary to learn the task. Moser, Krobert, Moser and Morris (1998) may have provided some support for this argument. They devised a novel stimulation protocol which allows them to stimulate more perforant path fibers than had been possible in the past. Using this procedure, they reported that saturation of hippocampal LTP blocked subsequent spatial learning. However, this effect was only apparent after a *post hoc* elimination of the animals that learned normally and in a later experiment animals pre-trained on the non-spatial components of the task learned normally despite saturation of dentate gyrus LTP (Otnaess, Brun, Moser, & Moser, 1999). Clearly the question remains unresolved.

Despite the fact that LTP has yet to be validated as a model memory mechanism, it retains a dogma-like status in the field. Although a case could be made for a lack of competing models as the main contributor to LTP's popularity, it is more likely due to the

fact that there is a wealth of circumstantial evidence which supports the LTP hypothesis that LTP-inducing stimulation trigger changes which are the same as those triggered when animals store information.

As mentioned above, part of the difficulty in attempting to validate LTP as a model memory mechanism is that it is not clear exactly how the two phenomena should relate. LTP is not the same as memory. It is a laboratory phenomenon induced by passing high frequency, and often high intensity, current through an electrode that has been surgically inserted to a specific point in the brain of an animal. This stimulation pattern is typically simple and rhythmic, and it would be expected to induce a highly synchronized pattern of discharge in the activated pathways. LTP is most commonly studied in the slice preparation which further deviates from the natural neuronal environment. Memory is a real world phenomenon with complex and distributed patterns of activation interacting across numerous regions of the brain. LTP, conversely, only models local synaptic mechanisms, Consequently, a number of assumptions must be made when parallels are drawn between LTP and real memory. It should not be surprising that the behavioral LTP paradigm has so far failed to provide compelling evidence substantiating the LTP hypothesis. It is not even clear that the present techniques would be adequate to reveal a training-induced LTP effect. If the information is dispersed such that encoding within any specific location is sparse, then it may be undetectable. If the approach is to work, then a system must be chosen where local effects are large enough to detect.

The aforementioned behavioral LTP experiments had mixed success in producing

measurable changes in neuronal response. None of them provided compelling evidence that such changes were caused by learning (as opposed to changes in activity level, due to arousal, attention, emotional state, etc.), nor have they shown the effects to occur at the level of the synapse.

A recent study, though, has provided the most compelling support to date of the LTP hypothesis. Rioult-Pedotti, Friedman, Hess, and Donoghue (1998) trained rats on a simple motor learning task, arm reaching for a food reward. The animals were placed in a rectangular box with a platform on the front. In order to obtain a food pellet, they were required to reach through a small opening with one forelimb. The movement of the arm and the grasping behavior were unfamiliar to the animals and they were unskillful with initial attempts to reach for the food. The training was done unilaterally and the trained forelimb displayed a clear acquisition of the necessary motor skills over the seven days of training. The authors examined the effect of training on layer II/III connections in the forelimb region of the somatomotor cortex. These connections are known to be plastic (Castro-Alamancos, Donoghue, & Connors, 1995; Hess, & Donoghue, 1996) and are thought to be involved in activity-dependent neural re-organization (Hess, & Donoghue, 1994). Consequently, the authors reasoned that layer II/III connections may be the site where the modifications underlying motor learning occur. Twenty-four hours following the training, the rats were sacrificed and sections of the cortex were placed in a slice chamber. Evoked field potentials recorded from layer II/III horizontal connections were larger in the trained hemisphere. To ensure that the effect was due to an LTP-related

mechanism, LTP was subsequently induced in both hemispheres. The untrained hemisphere supported greater LTP than the trained hemisphere. This rules out most non-specific changes.

This experimental paradigm has two advantages over previous behavioral LTP designs. Most prior LTP research exposed animals to spatial tasks and took recordings from the hippocampus. There was no way to restrict activation to a single hemisphere; presumably both hippocampi acquired the spatial information. Thus, control data were obtained from different groups of animals that necessarily had different behavioral experiences, different emotional states, and different stress levels as well as a series of other confounds which made interpretation difficult. By employing a simple, lateralized motor skill task and using the animals as their own controls, many of these confounds were eliminated. Another advantage of this paradigm is that the neural site of learning can be pinpointed with more precision. Enriched environments and spatial learning tasks are complex, allowing for multiple strategies, and the extent of involvement of any given system is difficult to predict. The simpler motor skill task relies on layer II/III connections. Although there are likely additional structures involved, as well as inter-animal variability associated with this task, it appears to provide a better-defined relationship between the skill acquisition and resultant neural modifications.

Nevertheless, there are still alternative explanations for the synaptic efficacy changes reported in the reaching-task experiment. One problem is that rats, like humans, demonstrate a preference for one forelimb. It is conceivable that this limb preference

corresponds with inherent differences in connectivity. Because the measures were taken only *following* the training protocol, it is impossible to rule out these inherent differences as the source of the effect.

Another problem is that the unilateral training design leads to an inter-forelimb imbalance in overall motor activity. The untrained limb is likely to have engaged in less total motor activity than the trained limb. Any neural alterations as a result of mere activity would confound the results.

An additional problem is that the recordings were made in the slice preparation, and thus could be taken only at one time point. By examining this phenomenon in the chronic preparation, it would be possible to identify the time course of acquisition and decay of the synaptic changes. In the chronic preparation it is also possible to determine if the hemispheric differences are driven by an increase in synaptic strength in the trained hemisphere or a decrease in the untrained hemisphere.

This thesis is designed to advance the behavioral LTP paradigm. The first step is to replicate the slice-preparation experiments of Rioult-Pedotti, et al. (1998). Subsequent experiments explore this phenomenon in the intact animal using both acute and chronic preparations. The final experiment is designed to assess both the effects that stimulation that induces LTP and long-term depression (LTD) has on learning. Collectively, this body of research provides a rigorous assessment of the training-induced potentiation phenomenon and its relationship to LTP.

Chapter 2

Training-induced potentiation in the cortex: Slice and acute preparations

Introduction

Ultimately, LTP must be either validated or invalidated as a memory model. Neither will likely be achieved until the critical molecular pathways for the two phenomena have been thoroughly explored, a chore that is easier for LTP than for memory. Meanwhile, other tests of the mechanistic relationship can be performed. Currently, the best way to establish such a relationship may be via behavioral LTP paradigms, in which animals are exposed to situations demanding the processing and storage of information and the brain pathways necessary for processing the information are monitored for any synaptic modifications resembling LTP (or LTD). Furthermore, these synaptic modifications must be causally linked to the learning component of the experiment. Few behavioral experiments have been designed to address the first criterion, and even fewer have addressed the second.

In 1998, Rioult-Pedotti, et al. attempted to demonstrate behavioral LTP in the forelimb region of the cortex. Their forelimb motor task provided an apparently simpler and more direct relationship between the learned behavior and the modifications that were recorded in the brain. An additional advantage was that the task demands could be

lateralized; restricting training to only one forelimb ensured that only one brain hemisphere was completely engaged in the task. Analogous manipulations are not possible with hippocampus-dependent tasks.

Rioult-Pedoitti et al. (1998) trained animals until they demonstrated clear unilateral acquisition of a forelimb motor skill. Following the training, the animals were sacrificed and coronal slices were taken through the forelimb regions of both motor cortices. The cortical slices were kept alive in warmed oxygenated artificial cerebrospinal fluid. Field potentials were evoked in layer II/III by stimulation of the horizontal connections in both hemispheres. The responses recorded in the trained hemisphere were greater than those in the untrained hemisphere. This finding is consistent with the hypothesis that learning induces increases in synaptic efficacy. The animals served as their own controls, which strengthens the interpretation that the synaptic modification was learning dependent. Other variables, such as level of stress, presumably act equivalently on both hemispheres and would not account for a unilateral phenomenon. After the recordings had been made, the researchers delivered high-frequency stimulation to the horizontal connections and found that the untrained hemisphere supported greater LTP. Because the training occluded subsequent LTP induction, it appears that the two phenomena may rely on the same underlying mechanism.

Given the distributed encoding presumed to underlie information storage in the cortex, it is surprising that the training effects were large enough to detect. Moreover, it is possible that memories are encoded by the use of both increments and decrements in

synaptic weight. Although the system may be biased toward increments, the combination of increments and decrements would collectively be expected to minimize any increase in recorded field potential size.

The results reported by Rioult Pedotti et al. were surprising and potentially important, so the first experiment in this chapter was designed to replicate their research using a similar training protocol. An additional group of animals was also included to address a potential confound. Like humans, rats have a preferred forelimb. All of the animals in the original study and in the experimental group of this study were trained on their preferred limb. Because the recordings were taken only once, it is possible that the difference between the trained and untrained hemispheres could be explained by inherent differences between the preferred and non-preferred limbs. In this experiment, a group was included that was treated exactly as the experimental group, except that the animals were not trained on the forelimb skill. Their preferred paw was assessed and a comparison was made between the size of evoked potentials in the preferred and non-preferred hemispheres. This procedure allowed for an assessment of differences due to paw preference.

The second experiment extends this line of research to the intact animal. In the slice preparation, responses were recorded in a damaged system; much of the normal input is necessarily removed In addition, horizontal cuts were made to ensure that interhemispheric signals did not interfere with the recorded signals. It is unclear how compromising the system in this fashion will impact neuronal function. To further assess

the training effect, recordings were made in the acute preparation. This additionally allowed for an evaluation of the impact training had on cross-hemispheric signals.

General Methods

Subjects

Twenty-two Long-Evans rats were used in the slice experiment, and 17 in the acute experiment. The animals were acquired from the Charles River Breeding colony and weighed between 225g and 300g when the experiment began. They were housed individually, kept on a 12 h on/12 h off light schedule and maintained on an *ad libitum* feeding schedule. Prior to training, the animals were food deprived to approximately 85% of their body weight and were maintained at this weight throughout the training procedure. The food deprivation was used to motivate the animals to reach for the food reward.

Training

During training, the rats were placed in a Plexiglas box which measured 14cm x 12cm x 6.5cm (Figure 1). A 2 cm vertical slit was cut in the front of the box. From inside the box, the animals were able to reach through this opening to retrieve food rewards which were placed on an external platform. The rewards were initially placed in the center of the platform such that they were accessible to either forelimb. Once an animal's forelimb preference was established, the reward could be placed to the side of the platform to ensure that only the preferred limb was used to retrieve it. In the experimental group

three animals displayed no obvious paw preference so their data were not included in the analysis.

The experimental animals were trained for forty-five minutes a day for seven days. During the training, the number of both successful and unsuccessful reach attempts was counted. A successful reach was defined as one in which the animal transferred the food reward from the platform to its mouth; an unsuccessful reach was defined as one in which the animal knocked the food reward off the platform. Reaching attempts in which an animal reached for the pellet and moved it slightly on the platform were not counted. Efficacy on this task was measured in two ways: The number of successful reach attempts, and successful reach attempts as a percentage of the total attempts.

Control animals received equivalent food deprivation and exposure to the training box. They also received the same number of food rewards as the experimental animals but did not have to reach for them. Instead, the food was placed directly in the mouths of the control animals by the experimenter. They learned the components of the task not directly involved with the skill such as where the food rewards were located and that they were edible.

Experiment 1: Slice Preparation

Methods

Twenty-four hours following the termination of training, the animals were sacrificed. Their brains were removed and immersed in artificial cerebrospinal fluid. A 400

μm coronal slice was cut roughly 1.2 mm anterior to Bregma such that the horizontal connections in the forelimb region of the somatomotor cortex remained intact. The ventral section of the slice was cut away. This cut was made dorsal to the corpus callosum, which ensured that the hemispheres were not connected by the corpus callosum during recording (Figure 2). The majority of animals provided two slices, and there was a total of 22 slices in each group from which recordings were taken. The experimenter was blind to both group assignment and handedness of the animals at the time of recording.

Stimulating and recording electrodes were lowered into the forelimb region of the somatomotor cortex in both hemispheres. The stimulation was delivered to layer II/III horizontal fibers through concentric bipolar stainless steel microelectrodes which were placed approximately 3mm lateral to the midline. The evoked field potentials were recorded using glass micropipettes, which were placed approximately 2.0 mm lateral to the midline. This was done in both hemispheres and the experimenter was blind to the condition of the animal at the time of recording. Evoked field potentials were generated with a 0.2 ms monophasic pulse delivered to the stimulating electrode. The pulses were delivered to alternating hemispheres with a 10 sec inter-pulse-interval. Five stimulation intensities (5, 10, 20, 30, and 40 μA) were used to evoke responses. Six recordings were taken at each intensity from each hemisphere and averaged.

After input-output (I/O) curves were constructed, intensities that elicited approximately 50% of the maximal response were determined and used to take baseline recordings. Once these baseline responses were stable in both hemispheres for at least 10

min, LTP was induced. Theta burst stimulation, which consisted of 10 trains at 5 Hz were delivered in an alternating fashion to the two hemispheres. The inter-train-interval was 10 sec which meant that there was an inter-train-interval of 20 sec in each hemisphere. The corpus callosum of each of the animals was not present in the sections so stimulation of one hemisphere did not interfere with recording in the other. Each train was composed of four pulses at 100 Hz. The inter-train-interval was 10 sec. The GABA receptor antagonist bicuculine was applied during the delivery of trains to reduce the level of inhibition and maximize the probability of LTP induction. Once stimulation had been delivered five times in this manner, recordings were taken for approximately 50 min to assess the amount and the stability of LTP.

RESULTS

Behavioral Data

For both groups, there was a roughly even distribution of animals with left and right paw preferences. Animals that consistently attempted to use both paws were excluded from the analyses. Early in the behavioral training, the animals performed poorly on the task. They tended to reach slowly for the reward and drag it back to their mouths on the platform as opposed to grasping it and carrying it back. Dragging it in this fashion was an ineffective strategy in retrieving the reward, because it was slow and allowed the food reward to fall off the platform easily. The animals also tended to keep their elbows close to their bodies which kept the movement restricted to the forearm region. When reaching for pellets placed far from them on the platform, they often reached short and

would knock the food off, and their reaches were executed slowly.

Over the course of the training, the experimental animals displayed improved efficiency on the task. Efficacy was assessed qualitatively in terms of the speed of the reaches, the willingness to extend the forelimb further out on the platform, and the accuracy of the reach attempts. The animals tended to reach for the pellet in what has been described as an arpeggio movement (Whishaw & Gornay, 1994). They tended to reach their paws out flat over the food with the digits spread apart. When the paw made contact with the food pellet, the digits were drawn together and toward the palm to grasp the pellet. Once the pellet was secured in the paw, the animal rotated the paw so that the palm was roughly facing up and brought the pellet to the mouth. The improved performance was measured both by the total number and percent of successful reaches. Measuring the total number of successful reaches captures a combination of improvements the animals made on the task, whereas the percent successful reaches confirms that the animals did not show improvement merely because they were reaching more often. On day 1 of training the animals successfully retrieved the food reward an average of 26.95 times. By day seven the mean numbers of successful retrievals increased to 119.21. On day 1 the animals were successful with only 9% of their reach attempts. By day seven they were successful an average of 52% of the time. A one-way repeated-measures ANOVA demonstrated that both number ($F(6,108) = 15.52$, $p<.001$) and percent ($F(6, 108) = 8.23$, $p<.001$) of successful reaches significantly improved over the days of training (Figure 3).

Electrophysiological Data

Evoked potentials were recorded in both hemispheres. For the experimental animals these were the trained and untrained hemispheres; for the control animals these were the preferred and non-preferred hemispheres to control for the confound of paw-preference in the experimental animals. Because both the recording and stimulating electrodes were located in layer II/III, the recorded field potential was thought to be driven primarily by horizontal connections in layer II/III. All the responses were negative relative to ground. The average onset of the responses was 2.0 ms in the trained and untrained hemispheres. For the control animals the average onset of the responses was 2.0 ms and 1.9 ms for the preferred and non-preferred hemispheres respectively. For the experimental animals the average latency to peak was 6.2 ms and 6.1 ms in the trained and untrained hemispheres respectively. For the control animals, the average latency to peak was 6.3 ms and 5.5 ms in the preferred and non-preferred hemispheres respectively. A three-way ANOVA (intensity x animal x hemisphere) revealed that the evoked potentials in the trained hemisphere were significantly greater than those in the untrained hemisphere ($F(4, 44) = 2.78$, $p<.05$). A three-way ANOVA (intensity x animal x hemisphere) did not reveal a difference between the size of the field potentials in the preferred and non-preferred hemispheres of the control animals ($F(4, 44) = 2.61$ $p > .05$) (Figure 4). Not all of the slices survived long enough to receive LTP induction. For those that did, there was a trend toward greater potentiation in the untrained hemisphere of the experimental animals, but it only approached significance ($F(1, 10) = 3.60$, $p = .08$) (Figure 5).

Experiment 2: Acute Recordings

Methods

Twenty-four hours following the termination of training, the animals were anesthetized and their heads were secured in a stereotaxic apparatus. Stimulating and recording electrodes were lowered into layer II/III of the forelimb region of the somatomotor cortex in both hemispheres. Pilot experiments were conducted to determine the electrode placements which produced the evoked potentials with the greatest amplitude. The animals were anaesthetized with sodium pentobarbital and their heads were restrained in a stereotaxic apparatus. The stimulating electrodes were placed 1.2 mm anterior to Bregma and 2mm lateral to the midline; the recording electrodes were placed 1.2 mm anterior to Bregma and 3 mm lateral to the midline. The electrodes were lowered under electrophysiological control to ensure that both were in layer II/III at depths yielding optimal responses. The experimenter was blind to the condition of the animal at the time of recording. Field potentials evoked by single biphasic stimulation pulses were recorded at five stimulation intensities (100, 250, 500, 1000, and 1260 μA). The pulse duration was 100 μs. Ten recordings were taken at each intensity and averaged. Unlike the animals prepared for the slice experiment, these animals had an intact callosum. It was thus possible to record evoked potentials from the homologous site in the hemisphere contralateral to the stimulation site.

Once the baseline recordings had been taken, high-frequency stimulation was delivered to induce LTP. The pulse frequency was 300 Hz, the train duration was 24 ms,

and the pulse intensity was 1260 μA. Sixty of these stimulating trains were delivered to each hemisphere in an interleaved pattern. This stimulation protocol has been shown to induce cortical LTP in an intact animal (Racine, et al., 1995). To assess the amount of LTP, 40 min following the termination of the trains another set of recordings was taken using the same protocols described above. Changes persisting longer than 10 min have been used as a criterion for LTP (Brown, Chapman, Kariss, & Keenan, 1988).

Histology

After completion of the LTP measures, the animals were sacrificed. They were perfused through the heart with a formal-saline solution, which was 4% formalin in 0.9% saline. Once the tissue was fixed, the animals were decapitated and the brains were removed and placed in the formal-saline solution for a period of not less than 36 hours. Twenty-fours hours prior to slicing the brains, they were placed in a 10% sucrose solution and kept cool in a 4 degrees C refrigerator. At least two 40 μm coronal sections were taken from each brain at a point where all four electrode tracks were visible in the same slice. Each slice was stained with Cresyl Violet and examined under an imaging microscope to confirm the electrode placements were in layer II/III.

Analyses

The peak amplitude of the evoked response was calculated at each intensity in each hemisphere. To assess the effect of training, a two-way repeated measures ANOVA was used to assess interhemispheric differences in both the experimental group (trained vs. untrained hemisphere across intensities) and the control group (preferred vs. non-preferred

hemisphere across intensities). To assess LTP, peak amplitudes of the pre-stimulation responses were subtracted from the peak amplitudes of the post-stimulation responses. This was done at all five intensities in both the trained and untrained hemispheres.

RESULTS

Behavioral Data

The performance of the animals on the task resembled those in the slice experiment (data not shown). Their performance improved over the seven days of training as measured both by the percent of successful reaches ($F(6, 60) = 8.24$, $p < .001$) and the number of successful reaches ($F(6, 60) = 15.53$, $p < .001$).

Electrophysiological Data

Evoked responses were recorded in both hemispheres as stimulation was applied both ipsilaterally and contralaterally to the recording electrode. Averaged across the experimental and control animals, responses evoked by ipsilateral stimulation had an average onset of 1.4 ms following stimulation and an average latency to peak of 8.1 ms; the responses evoked by contralateral stimulation had an average onset latency of 11.0 ms and average latency to peak of 18.2 ms (Figure 6). A two-way ANOVA (intensity x hemisphere) revealed that the evoked field potentials were significantly greater in the trained than the untrained hemisphere of the experimental animals ($F(1, 20)= 20.91$, $p < .001$). There was no similar difference between the preferred and non-preferred hemispheres in the control animals ($F(1, 10)= .009$, $p > .05$). A second two-way ANOVA was done on the measures of contralateral response amplitude from the experimental

animals. In this case, the magnitude of the field potentials was greater in the untrained hemisphere (with stimulation applied to the trained hemisphere) relative to the trained hemisphere (with stimulation applied to the untrained hemisphere) ($F(1, 20)= 8.66$, $p < .05$). Again no analogous inter-hemispheric differences were found in the control group ($F(1, 10)= .03$, $p > .90$) (Figure 6).

Of the five intensities used to generate an I/O curve, stimulation-induced LTP was only evident at the highest intensity. The degree of LTP was only assessed at this intensity. A paired t-test revealed that the untrained hemisphere supported more LTP than the trained hemisphere ($t(10) = 2.76$, $p<.05$) (Data not shown).

The high-frequency stimulation applied to the trained hemisphere seemed to produce a depression effect in the pathway from the trained hemisphere to the untrained hemisphere. There was a significantly a greater increase in field potential size in the pathway from the untrained hemisphere to the trained hemisphere than in the reverse pathway ($t(10) = 2.27$, $p < .05$) (Figure 8).

DISCUSSION

The results of both the slice (*in vitro*) and acute (*in vivo*) experiments are consistent with the results of the previous slice experiments of Rioult-Pedotti et al. Following unilateral training on a forelimb motor skill, evoked potentials are larger in the trained hemisphere in both preparations. Also, high-frequency stimulation produces greater LTP in the untrained hemisphere. It appears that the training occludes subsequent LTP by "using up" some of the potential for enhanced connectivity. LTP in the acute

preparation was small; no animals demonstrated more than 120% of baseline responding following the delivery of high-frequency stimulation. Such a small potentiation effect is consistent with previous work done in acutely-prepared animals (Racine, Wilson, Teskey, and Milgram, 1994). The small nature of the potentiation may have been due to the anaesthetic which has been shown to suppress responding in the cortex (Chapman, Trepel, Ivanco, Froc, Wilson, Racine, 1998; Racine, Hodgson, Plantinga, and Kim, 1998).

The mutually exclusive nature of both the training-induced and the stimulation-induced increases in field potential size suggests that the two effects are mediated by an increase in synaptic efficacy and not some other non-specific change in neuron responsivity. The results of these experiments support the conclusion that the neural representation of the motor skill is stored, in part, by increments in synaptic strength in the forelimb region of the cortex.

No difference was found between the preferred and non-preferred hemispheres of the control animals. This result was consistent across the two preparations which rules out the possibility that the trained effect simply reflects an inherent difference between the hemispheres.

In the acute preparation, the callosum was intact, which allowed the recording of responses triggered in the contralateral hemisphere. Contralaterally evoked potentials were larger in the untrained hemisphere than in the trained hemisphere. Callosal stimulation has been shown to induce a robust LTP in the neocortex (Racine et al., 1995). The training-induced alterations in callosal responses in the contralateral hemisphere are

noteworthy for two reasons. First, they confirm that there was not a systematic bias in the placement of the recording electrodes. Had the recording electrodes been systematically optimized for larger responses in the trained hemisphere, this effect would have likely been reflected in contralaterally-evoked responses, as well. This finding does not rule out the possibility that the placement of the *stimulating* electrodes was systematically optimized, but the experimenter was blind to the group designation of the animal when the electrodes were lowered, so such biases are unlikely.

The cross-hemispheric effect suggests an interactive information transfer between the hemispheres. One possibility is that there is a flow of information from the trained hemisphere to the untrained hemisphere, which would be consistent with the direction of the effect. The directional enhancement of information flow may represent a mechanism by which the skill is transferred to the untrained hemisphere. There is evidence from research with humans that once a motor skill is learned with one hand, it is transferred to the other hand (Parlow & Dewey, 1991; Marks, 1996). The animals in the arm-reaching task did tend to make more attempts with their untrained forelimbs toward the end of training. However, it is not clear that they would have demonstrated improved reaching performance with the untrained limb. More research is required to determine the extent of skill transfer and its relationship to the training-induced inter-hemispheric potentiation effect.

It is also possible that both limbs play a role in the task even when the reaching is restricted to one side. The untrained forelimb was necessarily involved in balancing the

animal's weight and maintaining the appropriate posture, which could be somewhat awkward and unnatural in the unilateral reaching task. There was likely an interplay between the two hemispheres to coordinate this balancing position. Such an interplay likely included information about the nature of the reach, such as angle rotation and distance sent from the reaching hemisphere to the balancing hemisphere. Again this explanation includes a flow of information from the trained to the untrained hemisphere and is consistent with the cross-hemispheric potentiation effect. More work is necessary to explore these, and other, explanations of the contralateral training effect.

Figure Captions

Figure 1. The apparatus in which the animals were trained. From inside the clear Plexiglas box, the animals were able to reach though a vertical slit **(A)** to retrieve a food reward located on a platform outside the box **(B)**.

Figure 2. Representation of electrode placements in the coronal slices. The coronal slices were cut such that the corpus callosum was removed. The stimulating and recording electrodes were 3 mm and 2 mm from the midline respectively.

Figure 3. Improvement in the reaching skill of the animals The skill of the animals improved across the 7 days of the training as measured both by the number **(A)** and percent **(B)** of successful reaches.

Figure 4. Differential field potential magnitude recorded from the animals in the slice experiment. A. Recordings were taken at five intensities. The experimental animals had larger evoked potentials in the trained hemisphere relative to the untrained hemisphere. **B.** Representative evoked potentials from a slice. The thick line is a recording from the trained hemisphere and the thin line is a recording from the untrained hemisphere. **C.** Control animals showed no systematic difference between evoked potentials recorded in the preferred and non-preferred hemispheres. **D.** Representative evoked potentials from

a slice. The thick line is a recording from the preferred hemisphere and the thin line is a recording from the non-preferred hemisphere.

Figure 5. **LTP-induction in the slice experiment. A.** In the slice experiment, Theta-burst stimulation (TBS) delivered following 10 min of baseline recording produced greater LTP in the untrained hemisphere than in the trained hemisphere of the experimental animals, although this difference was not significant. **B.** For the control animals, there was no difference in the magnitude of LTP induced in the preferred and non-preferred hemispheres.

Figure 6. **Acute experiment recordings. A.** Experimental animals. Responses were evoked at five intensities. The trained hemisphere had larger evoked potentials than the untrained hemisphere, on average. **B.** Representative evoked potentials from the trained hemisphere (thick line) and untrained hemisphere (thin line). **C.** Control animals. No difference was displayed between the preferred and non-preferred hemispheres. **D.** Representative evoked potentials from the preferred hemisphere (thick line) and non-preferred hemisphere (thin line).

Figure 7 **Recordings of field potentials evoked by cortical stimulation contralateral to the recording electrode. A.** Recordings taken from the untrained hemisphere were significantly larger than those taken from the trained hemisphere across the five

stimulation intensities. **B.** Representative evoked potentials taken from the untrained hemisphere (thick line) and trained hemisphere (thin line). **C.** For the control animals, there was no difference between the preferred and non-preferred hemispheres. **D.** Representative evoked potentials taken from the preferred hemisphere (thick line) and non-preferred hemisphere (thin line).

Figure 8. Differential LTP-induction in the acute preparation. Following the initial recording, the high-frequency stimulation was delivered to the animals. This produced a small potentiation in the untrained hemisphere and a small depression effect in the trained hemisphere.

Figure 1

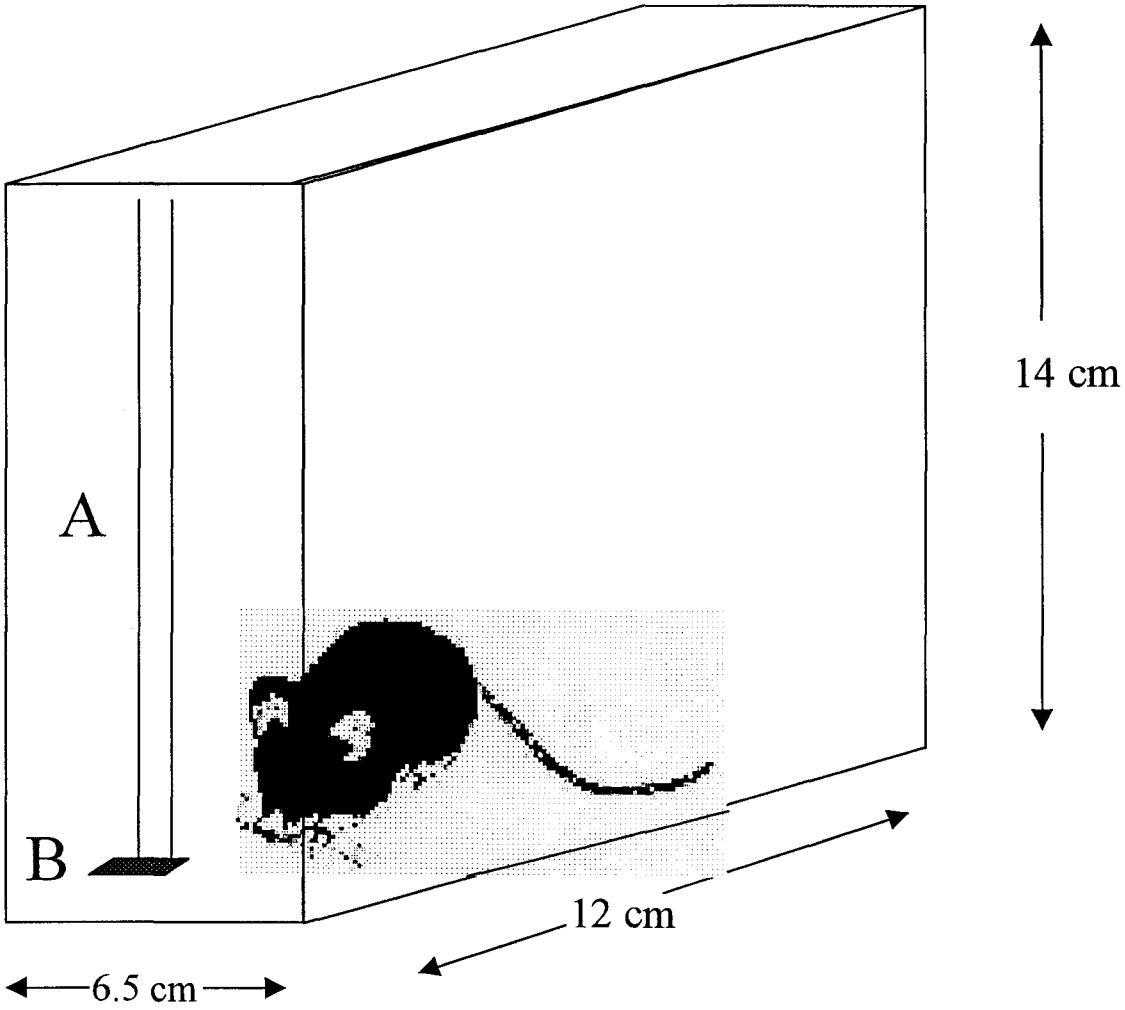


Figure 2

Coronal Section

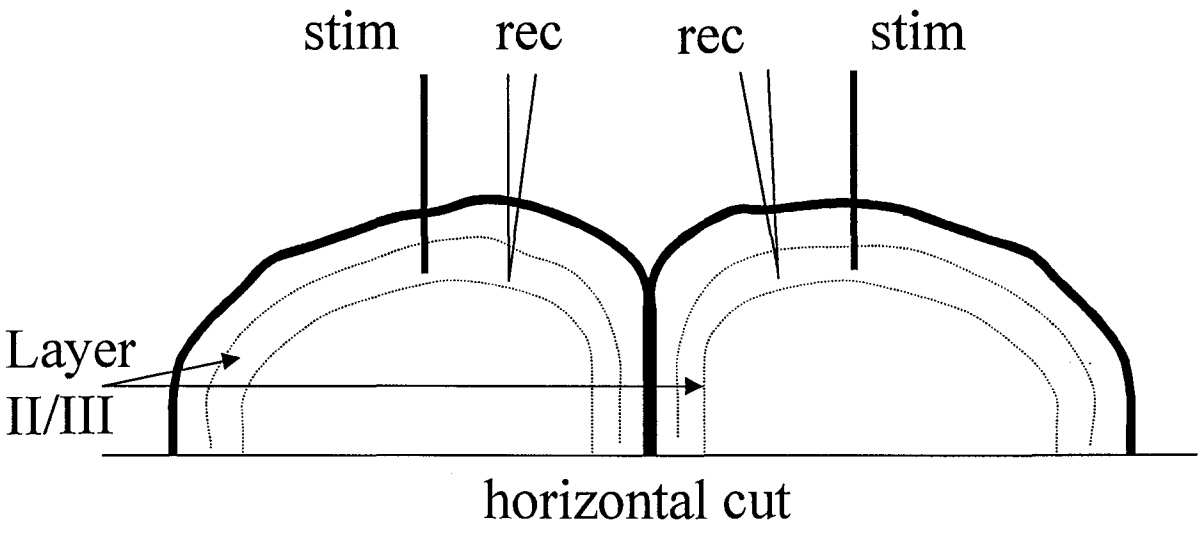


Figure 3

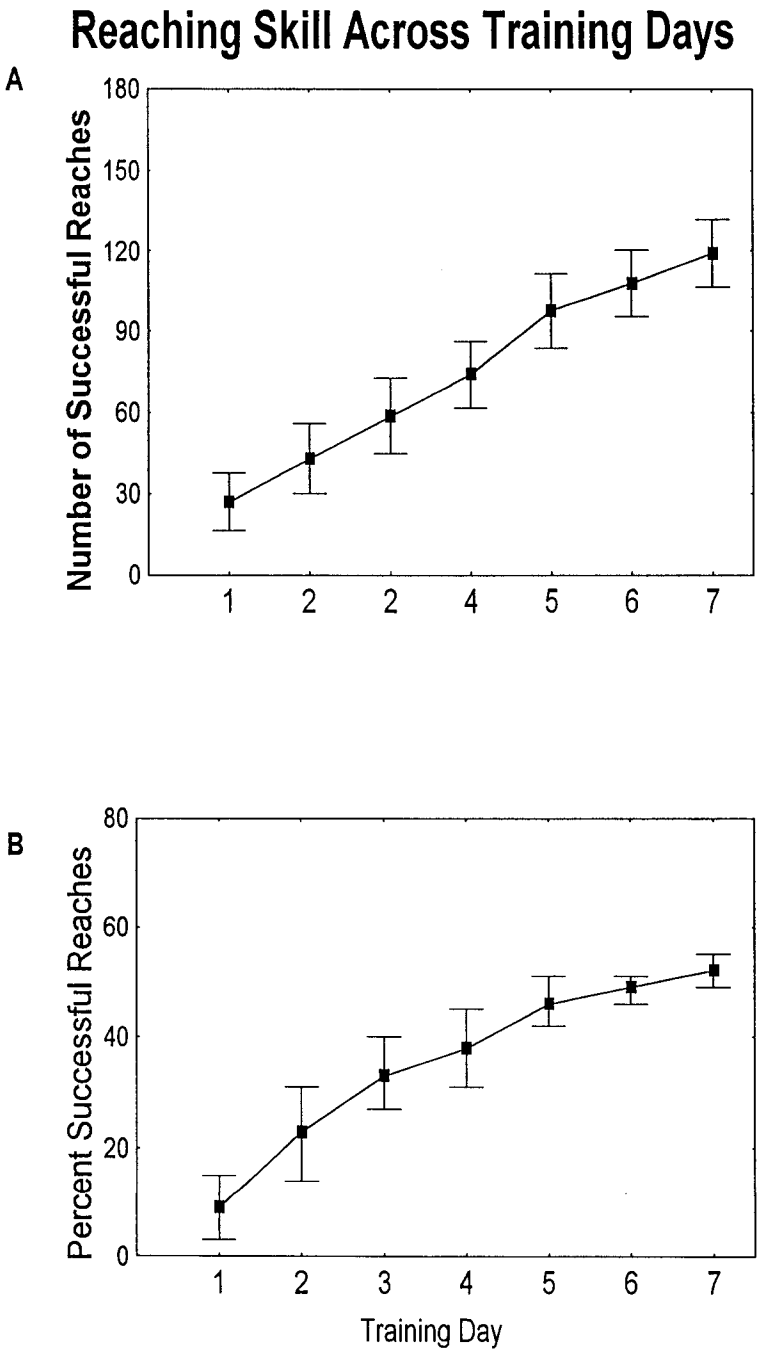

Reaching Skill Across Training Days
A
Number of Successful Reaches
180
150
120
90
60
30
0
1
2
2
4
5
6
7
B
Percent Successful Reaches
80
60
40
20
0
1
2
3
4
5
6
7
Training Day

Figure 4

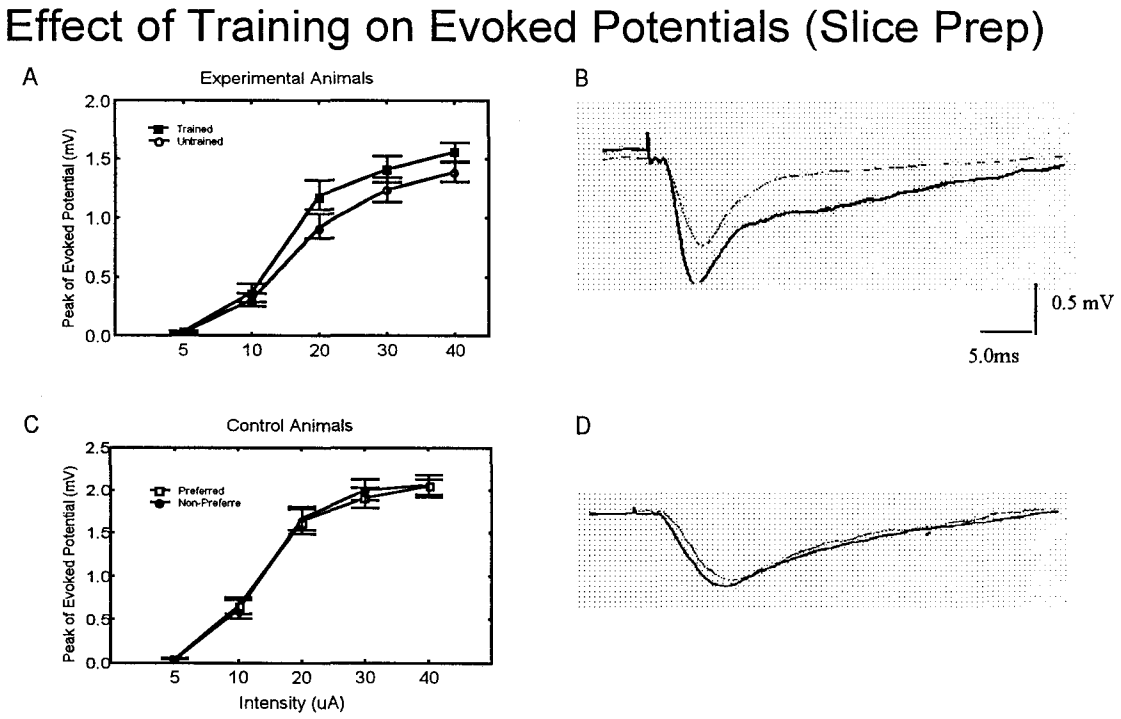

Effect of Training on Evoked Potentials (Slice Prep)
A
Experimental Animals
Trained
Untrained
Peak of Evoked Potential (mV)
2.0
1.5
1.0
0.5
0.0
5
10
20
30
40
B
0.5 mV
5.0ms
C
Control Animals
Preferred
Non-Preferre
Peak of Evoked Potential (mV)
2.5
2.0
1.5
1.0
0.5
0.0
5
10
20
30
40
Intensity (uA)
D

Figure 5

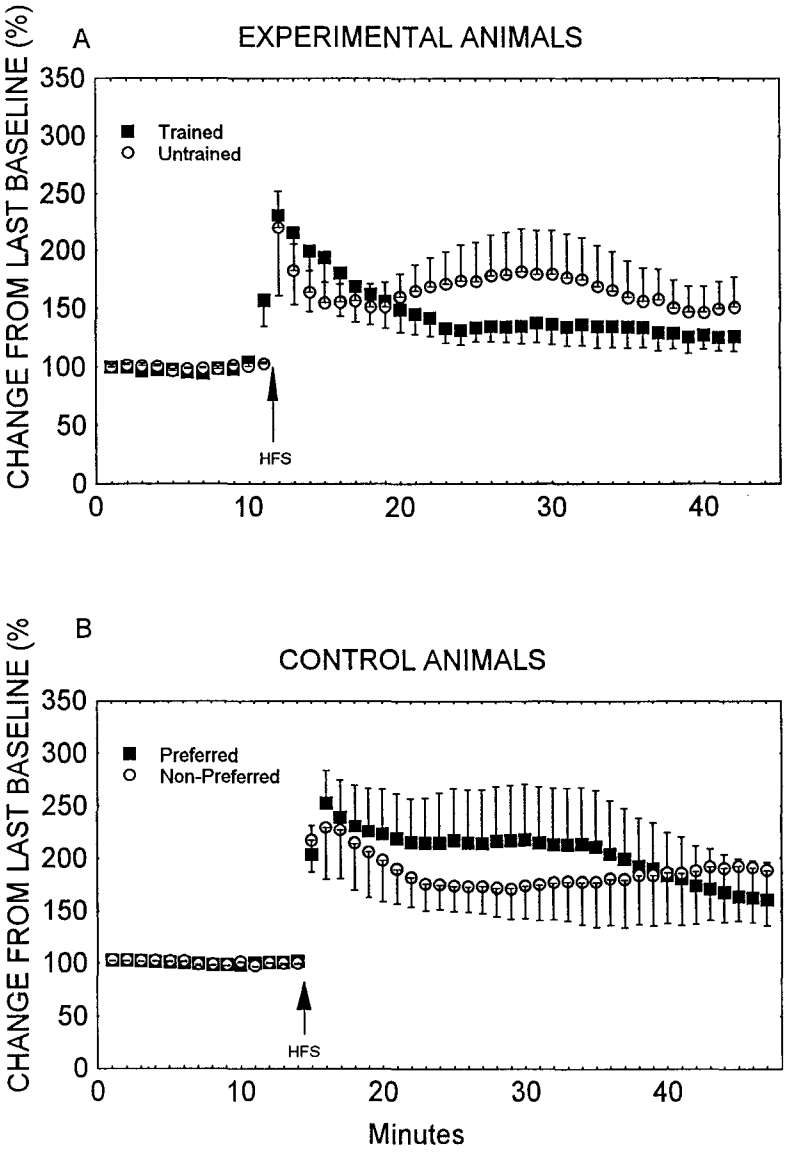

A
EXPERIMENTAL ANIMALS
CHANGE FROM LAST BASELINE (%)
350
300
250
200
150
100
50
0
Trained
Untrained
HFS
0
10
20
30
40
B
CONTROL ANIMALS
CHANGE FROM LAST BASELINE (%
Preferred
Non-Preferred
HFS
Minutes

Figure 6

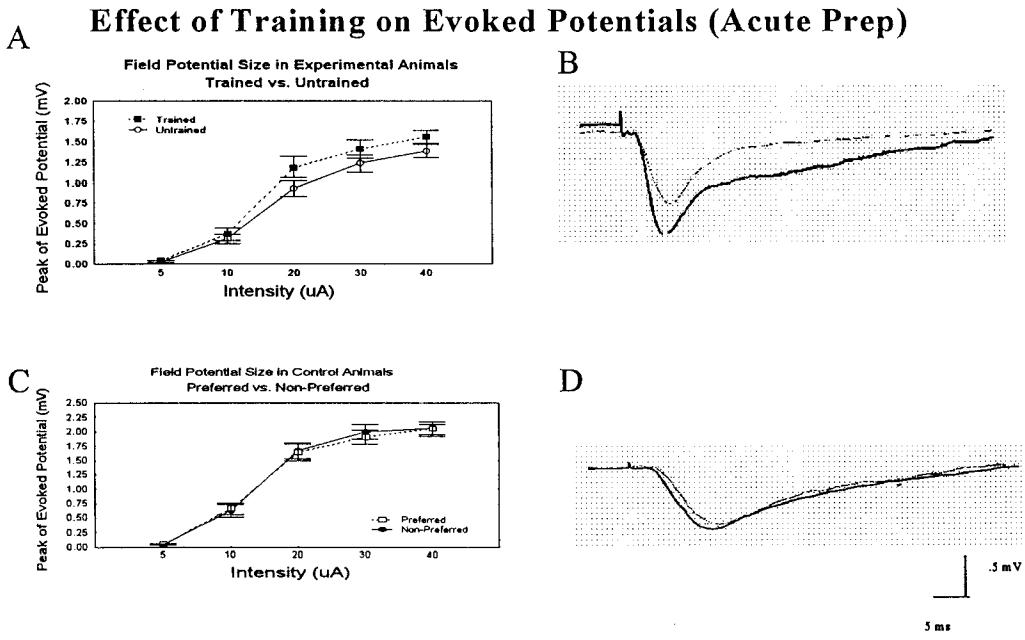
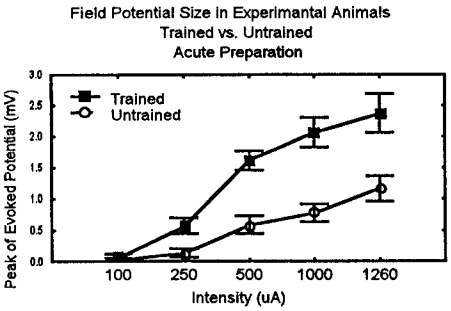

A
Effect of Training on Evoked Potentials (Acute Prep)
Field Potential Size in Experimental Animals
Trained vs. Untrained
Peak of Evoked Potential (mV)
Trained
Untrained
Intensity (uA)
B
C
Field Potential Size in Control Animals
Preferred vs. Non-Preferred
Peak of Evoked Potential (mV)
Preferred
Non-Preferred
Intensity (uA)
D
.5 mV
5 ms

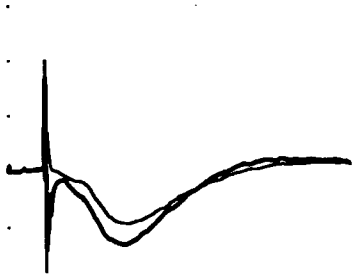
Figure 7

Cross-Hemispheric Evoked Potentials

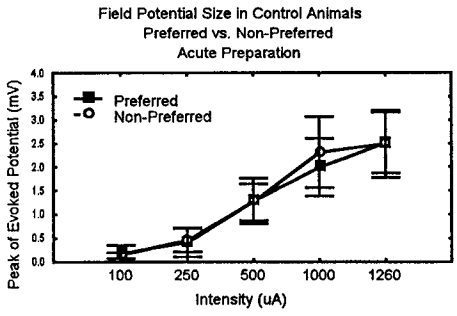
A



B



C



D

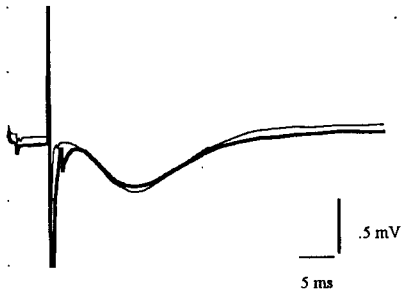
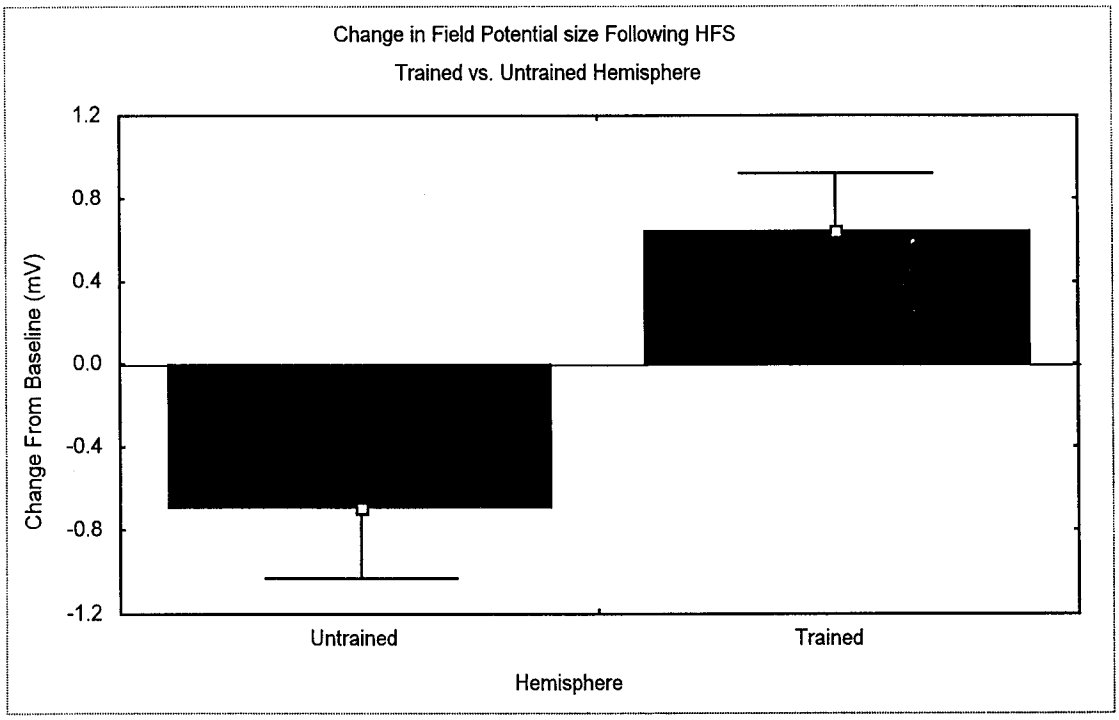


Figure 8


Change in Field Potential size Following HFS
Trained vs. Untrained Hemisphere
Change From Baseline (mV)
1.2
0.8
0.4
0.0
-0.4
-0.8
-1.2
Untrained
Trained
Hemisphere

Chapter 3

Training-induced potentiation in the cortex: Chronic preparations

Introduction

In the previous chapter, synaptic efficacy was shown to be enhanced in the forelimb region of the somatomotor cortex following the acquisition of a forelimb motor skill. Animals that were unilaterally trained on a forelimb reaching task had larger evoked potentials in the trained hemisphere than in the untrained hemisphere. These unilateral enhancements were found in both slice and acute preparations. Control animals not exposed to the training showed no systematic hemispheric differences in field potential size, which rules out inherent differences based on limb preference.

These results provide compelling evidence that neural representations of a motor skill are acquired by increases in synaptic strength. However, due to the nature of both the slice and the acute preparations, recordings could be taken at only one time point relative to the training protocol. This constraint meant that several questions necessarily went unanswered in the previous experiments. One such question is that of the direction of the synaptic modification. Given the LTP hypothesis, the most obvious conclusion to draw was that the hemispheric difference in field potential magnitude was a function of increases in the synaptic strength in the trained hemisphere. However, without assessing

the synaptic strength of the animals both before and after training, it is impossible to rule out the possibility that there was a decrease in synaptic efficacy in the untrained hemisphere, or a combination of increases and decreases in synaptic strength in the trained and untrained hemispheres, respectively. The animals may for example have learned to reach for the food reward with one forelimb while learning to inhibit responding with the other. The results of the experiments in chapter two were consistent with this interpretation. The magnitude of the responses in the control animals more closely resembled those in the trained, rather than the untrained hemisphere. This result may represent a sampling bias in the groups or it may be indicative of a decrease in field potential size in the untrained hemisphere as a result of training.

A second issue not possible to assess using either a slice or acute preparation is the temporal relationship between learning and modifications in synaptic strength. It is not known, for example, if the changes in the brain temporally map onto changes in the performance of the task. A temporal coupling of the neural modifications and the behavioral acquisition of the skill would provide further evidence that the two phenomena are linked. The present experiment avoids both of these shortcomings by employing chronically-prepared animals. Chronically implanting electrodes bilaterally in the cortex allows the effect of training on synaptic strength to be monitored during the course of training.

A separate issue not addressed by previous research was the possible contribution of motor activity in the modification of synaptic connections. In all previous research,

the forelimb which has been trained on the task has also been caused to move much more than the untrained forelimb. The forelimb movement is then confounded with the learning, and it is impossible to rule out a contribution of altered activity levels in the modification of the synaptic connection. The present experiment includes a group of animals who were enticed to move one forelimb in an inherent grooming motion. Because the experimental group experienced motor movement in the absence of training, it was possible to assess the relative contribution of movement in the field potential modification.

The acute experiment in chapter two suggested a role of cross-hemispheric communication. This possibility manifested itself as an increase in the amplitude in the potentials evoked in the untrained hemisphere by stimulation applied to the trained hemisphere. Although the callosal pathway is known to support LTP (Racine et al., 1995), the functional significance of this pathway in the acquisition of information is not known. To determine if this pathway is affected by the training, an additional group was added in which stimulating and recording electrodes were implanted in the corpus callosum and somatomotor cortex. Evoked field potentials were monitored daily during training on the reaching task to determine the impact of the skill acquisition has on synaptic efficacy in this pathway.

METHODS

Subjects

Forty-three animals were used in these experiments. Twenty-six were used to

assess the horizontal connections (8 experimental animals, 8 untrained control animals, and 10 activity control animals); six were used to assess the callosal-cortical connections. A control group consisting of eleven animals was used to obtain a baseline measure of forelimb movement in the absence of either training or elicited forelimb movement. The animals were acquired from the Charles River Breeding colony and weighed between 225g and 300g when the experiment began. They were housed individually, kept on a 12 h on/12 h off light schedule and maintained on an *ad libitum* feeding schedule. Both the testing and recording were done during the lights-on phase. Prior to training, the animals were food deprived to approximately 85% of their body weight and were maintained at this weight throughout the training procedure. Deprivation was used to motivate the animals to reach for the food rewards.

Surgery

The animals were anesthetized with sodium pentobarbital and their heads were immobilized in a stereotaxic apparatus. Twisted bipolar electrodes were made from Teflon-coated stainless steel wire. All animals had bilateral implants. For the callosal groups, the stimulating electrodes were chronically implanted into the callosum. The tips were separated by 0.5 mm to span the white matter of the callosum. The recording electrodes were lowered then chronically implanted into the forelimb region of the somatomotor cortex. The tips were separated by 1.0 mm so that they spanned the pyramidal cell dipoles across cortical layers II/III - V. For the horizontal groups the stimulating and recording electrodes were both implanted into layer II/III of the forelimb

region of the cortex. The stimulating electrodes were bipolar with a tip separation of .5 mm. The recording electrodes were monopolar. The stimulating electrodes were implanted 0.9 mm anterior to Bregma and 2 mm lateral to the midline. The recording electrodes were implanted 0.9 mm anterior to Bregma and 3 mm lateral to the midline. The electrodes were connected to gold-plated amphenol pins which were inserted into a 9-pin connector plug. Evoked responses were monitored during surgery to ensure that the electrodes were lowered to optimal depths.

Behavioral Training

During training, the rats were placed in a Plexiglas box which measured 14cm x 12cm x 6.5cm. A 2cm vertical slit was cut in the front of the box. From inside the box, the animals were able to reach through this opening to retrieve food rewards which were placed on a platform mounted on the outside of the box. The rewards were initially placed in the center of the platform such that they were accessible to either forelimb. Once an animal's forelimb preference was established, the reward could be placed to the side of the platform to ensure that the animal used only the preferred limb to retrieve it.

The experimental animals were trained for forty-five minutes a day for seven days to reach for and retrieve the food reward. Performance on this task was measured by dividing the number of successful reaches by the total number of reach attempts to get the percentage of successful reach attempts. Performance was additionally measured by counting the number of successful attempts during the session. A successful reach was defined as one in which the animal transferred the food reward from the platform to its

mouth; an unsuccessful reach was defined as one in which the animal knocked the food reward off the platform.

Control animals received equivalent food deprivation and exposure to the training box. They received an equivalent number of food rewards as the experimental animals but did not have to reach for them. Instead, the food was placed directly in the mouths of the animals by the experimenter.

The grooming animals had a foreign substance applied to one side of the face roughly every 7 - 10 min during the 45 min session. This application stimulated a unilateral grooming response in the animals. The number of forelimb movements made by this group was compared against a group of animals who were placed in the same testing apparatus but who were not subject to any experimental manipulation.

Stimulation and Recording

Once the animals had recovered from surgery, 3 sets of field potential measures, spaced 48 h apart, were taken to establish a series of baseline input/output (I/O) curves. Pulses of increasing intensity were delivered through the stimulating electrodes at a frequency of 0.1 Hz. High pass (.3 Hz) and low pass filters (3000 Hz) were used to filter out extraneous electrical noise. Ten field responses were evoked, amplified, digitized (at 10 kHz) and averaged at each of five intensities (100, 200, 500, 1000 and 1260 μA). Because it has been demonstrated that motor activity can impact evoked responses (Hargreaves, Cain, Vanderwolf, 1990), test pulses were delivered when the animals were in a non-active behavioural state. Training began twenty-four hours following the third

baseline I/O. Twenty three hours following each training session, an I/O measure was taken for each animal. Recordings were always made bilaterally and were taken in a room separate from the testing room.

LTP was induced with the application of 8-pulse trains with a frequency of 300 Hz and a duration of 24 ms. Sixty trains were delivered with an inter-train interval of 10 sec. Sixty trains were delivered to one hemisphere, then sixty to the other hemisphere. The order of delivery was randomized.

RESULTS

Horizontal Pathways

Repeated measures ANOVAs revealed that the animals demonstrated a significant improvement in reaching performance over the seven days of training. This was true both when using percent successful reaches, ($F(6, 42) = 3.36$, $p < .01$) and number of successful reaches ($F(6, 42) = 26.90$, $p < .001$) as the dependent measure (Figure 9). The observed behavior of the animals was consistent with the behavior of the animals described in chapter two.

The morphology of the responses in the chronically-prepared animals resembled that in the acute preparation. The latencies of both the onset and the peak of the response were similar.

To determine if the training induced a change in the synaptic efficacy of layer II/III horizontal connections, field potential measures were recorded from both the trained and untrained hemispheres. There was a significant interaction between day and

hemisphere for the experimental animals ($F(7, 98) = 2.58$, $p < .05$). The trained hemisphere showed a significant increase in field potential size relative to the untrained hemisphere (Figure 10). For the control animals, there was no such interaction between the preferred and non-preferred hemispheres ($F(7, 98) = 0.39$, $p > .05$) (Figure 10).

Stimulation of one hemisphere evoked field potentials in the homologous site of the contralateral hemisphere. The average latency to peak in the trained hemisphere was 11.2 sec. The average latency to peak in the untrained hemisphere was 10.9 sec. The magnitude of field potentials evoked by stimulation of the homologous site in the contralateral hemisphere revealed a similar effect to the one found in the acute preparation. Across the days of the training, field potentials recorded in the untrained hemisphere became larger than those recorded in the trained hemisphere ($F(9, 630) = 2.19$, $p < .05$). For the control animals, the average latencies to peak in the preferred and non-preferred hemispheres were 11.6 sec and 11.1 sec, respectively. The amplitude of these responses did not differ significantly ($F(9, 630) = 1.52$, $p > .05$) (Figure 11).

The response of one of the experimental animals became unusable during the LTP-induction phase of the experiment so its data were excluded. A three-way repeated-measures ANOVA (hemisphere x intensity x day) was conducted covering the last day of training and the ten days of LTP-induction. For both hemispheres, all field potential measures were taken as the difference from the last day of training. Neither hemisphere demonstrated significant LTP, but there was an interaction across days between the hemispheres in the anticipated direction ($F(10, 600) = 2.53$ $p < .05$) (Figure 12). The

potentiated response morphology indicated the emergence of a late component. This component did not overlap with the learning-induced changes in the response and may have represented potentiation in another layer so it was not analyzed.

It is possible that the reason for the failure to find a significant potentiation effect in the untrained hemispheres was because all the intensities were analyzed but changes only occurred at the high intensities. To assess this possibility, ANOVAs were redone isolating the top two intensities for both the trained and untrained hemispheres. This revealed a potentiation in the untrained hemisphere ($F(10, 120) = 2.21$, $p < .05$) but not the trained hemisphere ($F(10, 120) = 1.74$, $p > .05$).

In the control animals a three-way ANOVA (hemisphere x intensity x day) was run comparing the preferred and non-preferred hemispheres. There was no interaction between the groups across the seven days during which the experimental animals were trained ($F(40, 700) = .37$, $p > .05$). A repeated measures ANOVA indicated that field potential size in the preferred hemisphere increased in size across the days of LTP-inducing stimulation ($F(10, 350) = 2.23$, $p < .05$). A Tukey's LSD post-hoc test indicated that there was a significant difference between the last day of behavioral testing and the last day of high-frequency stimulation ($p < .05$). The non-preferred hemisphere also demonstrated a significant increase in field potentiation size ($F(10, 350) = 4.33$, $p < .05$). For the preferred hemisphere responses, an LSD post-hoc test indicated that there was a significant difference between the last day of behavioral testing and the last day of high-frequency stimulation ($p < .05$).

To assess the placement of the electrodes, the animals were monitored during high-frequency stimulation to the motor cortex. The stimulation elicited muscle movement in the forelimb. The movement was separated into three categories: 1) movement visible to the naked eye; 2) muscle contraction that could be felt when the animal's forelimb was held between the experimenter's fingers, and 3) no evident movement. The majority of animals displayed movement of both arms indicating that the electrodes in both hemispheres were in the forelimb region of the cortex. Those animals that did not display any movement were eliminated from the analysis.

For the activity control animals, the number of grooming arm movements was counted to ensure that one forelimb was consistently moving more than the other. The grooming forelimbs were moved an average of 102.19 times during the 45 min daily grooming session; the non-grooming forelimbs were moved an average of 24.19 times during the same period. There was a significant difference between the number of movements between the arms ($F(1, 18) = 14.89$, $p < .05$) during this seven-day period (Figure 13). A separate group of animals, placed in the reaching boxes for 45 min without any additional treatment, moved their right and left forelimbs in a grooming motion an average of 10.36 and 8.56 times respectively each session. The induced-grooming in the activity control animals was significantly more frequent for both the right ($F(1, 17) = 50.94$, $p < .05$) and left ($F(1, 17) = 48.39$, $p < .05$) paws. The mean number of daily grooming movements (102.19) made by the motor control animals was greater than the number of reaching movements made by the experimental animals (69.85) but

this difference was not significant ($F(1, 21) = 1.65$, $p > .05$) (Figure 13). A three-way repeated measures ANOVA (hemisphere x day x intensity) revealed that there was no significant difference in the size of the evoked responses in the grooming and non-grooming hemispheres ($F(4, 90) = 1.15$, $p > .05$).

Callosal-Cortical Placements

One-way repeated measures ANOVAs revealed that the skill level of the callosal animals also improved across the training days. Both the percent of successful reaches ($F(6, 30) = 3.10$, $p < .05$) and the number of successful reaches ($F(6, 30) = 6.16$, $p < .05$) showed significant improvement (Figure 14).

LTP was most evident in the responses evoked with the highest stimulation intensities, and many of the animals showed a net depression effect at the lowest stimulation intensities. This finding is consistent with previous work examining LTP in callosal-to-cortical connections in the chronic preparation. For this reason only the potentials evoked at 1000 μA were analyzed. The evoked potential in this pathway has two distinct components: an early, presumably monosynaptic component, and a late, presumably polysynaptic component. One-way repeated measures ANOVAs were conducted to determine if the magnitude of the field potentials changed across the three days of baseline and the seven days of behavioral training. In the trained hemisphere, both the early ($F(9, 45) = 57.59$, $p < .05$) and late components ($F(9, 45) = 67.58$, $p < .05$) increased significantly. Similarly, both early ($F(9, 45) = 9.19$, $p < .05$) and late ($F(9, 45) = 7.05$, $p < .05$) components were significantly increased in the untrained hemisphere as

well. There was no interaction between the hemispheres across days for either the early ($F(9, 90) = .26$ $p > .05$) or late ($F(9, 90) = .64$ $p > .05$) components suggesting that the changes in the untrained hemisphere resembled those in the trained hemisphere.

Discussion

This experiment is the first behavioral LTP reaching-task study to use behavioral tests to confirm that the electrode placements were actually localized in the forelimb region of the motor cortex. Microelectrical stimulation studies have demonstrated that there is inter-animal variability in the functional organization of the rat motor cortex and that this effect is greater once animals have recently learned a forelimb skill (Kleim, Barbay, & Nudo, 1998). In the slice and acute preparations it was possible to confirm the coordinates of the electrode placements, but there was no way to confirm that the electrodes were in the forelimb region of the cortex. Given the variable functional organization, some of the electrodes might have been misplaced.

The training induced a significant potentiation of the field potential magnitude in the trained, but not the untrained hemisphere of the experimental animals. This finding provides further support for the conclusion that motor skills are neurally instantiated with increases in synaptic strength. There is no clear time point when the animals reach an asymptotic performance level, but the skill improvement appears to show some leveling off around the third day of training. Similarly, field potential measures demonstrated a leveling off around the third day of recording, but again, there is no clear time point when they reach asymptote. The control animals demonstrated no change in field potential size.

However, responses in both the preferred and non-preferred hemispheres tended to drift upwards, raising the possibility that the trained hemisphere in the experimental group was drifting upwards while the untrained hemisphere was showing a depression effect.

Animals occasionally reach for the food rewards during the course of the training period with the untrained limb. These reaches are typically unsuccessful, because the placement of the reward is such that the untrained limb does not have easy access to it. Consequently, one aspect of a successful strategy is learning not to reach with the untrained forelimb. Such suppression may involve the recruitment of inhibitory interneurons. Stimulation of layer II/III neurons can produce inhibitory post-synaptic potentials (van Brederode & Spain, 1995). Perhaps the inhibitory connections are enhanced during the training, accounting for the differences between the trained and untrained hemispheres. More work needs to be done to determine whether the changes are due primarily to increases in field potential size in the trained hemisphere, decreases in the untrained hemisphere, or a combination of the two.

Animals that were induced to groom unilaterally did not display interhemispheric differences in field potential size. This result suggests that the interhemispheric difference in the experimental animals was not merely a consequence of the forelimb movement *per se*. There appeared to be a small downward drift in the size of the responses in the motor control animals. However, previous work done in this lab indicates that downward drifts are not uncommon in control animals. There is evidence that decorticate animals have essential intact grooming responses (Vanderwolf, Kolb, Cooley, 1978). This does not

mean that grooming does not engage the cortex in normal animals. There is also some evidence that animals with motor cortical lesions show a disruption in grooming (Berridge and Whishaw, 1992). More work needs to be done to assess the extent to which grooming is cortically driven in order to validate this group as a control for the cortical involvement in motor movement. However, it does provide an adequate control for the mere forelimb muscle movement which can effectively be ruled out at the cause of the effects.

Consistent with the acute preparation results, contralateral stimulation generated larger field potentials in the untrained hemisphere than in the trained hemisphere. Again appeared to be a temporal overlap between changes in field potentials and learning. Most of the increase in field potential size occurred in the first two days of training, which is also when the learning curve is the steepest. This is consistent with the interpretation from chapter two -- the trained hemisphere may be sending information about the reaching skill to the untrained hemisphere. There is evidence that interhemispheric manual skill transfer is impaired in acallosal humans (de Guise et al., 1999). Another possibility is that the reaching movement required to retrieve the food is unnatural and that the two hemispheres must communicate in order to coordinate their movements. This kind of communication between the limbs has been demonstrated (Jeeves, Silver, & Jacobson, 1988). This hypothesis can be tested by unilaterally training animals on the forelimb reaching task and then forcing them to switch and reach with the untrained side. If there is interhemispheric information transfer, the animal should perform better with

the new forelimb than naive animals because this hemisphere would have received information about the skill from the other, previously trained hemisphere.

Very little LTP was induced in the trained hemispheres with high-frequency stimulation. LTP induction was possible in the control animals so the stimulation protocol is an effective one. It is, therefore, not clear why both the trained and untrained hemispheres seemed resistant to LTP induction. The untrained hemisphere did show some LTP, but that was only evident when test pulses were delivered at a relatively high intensity. The difference between the groups is in the predicted direction; the untrained hemisphere appears to be more susceptible to LTP induction.

Animals with the callosal placements demonstrated increases in field potential size in both the trained and untrained hemispheres. This result was not expected. In the acute preparation, there was evidence of potentiation in the direction from the trained to the untrained hemisphere. This finding was also the case with the chronically prepared animals with horizontal-connection placements. For the callosal pathway there is evidence of potentiation in both directions. The primary difference between these animals and the previously described ones is the nature of the electrode placements. In particular, one tip of the recording electrode is in layer IV and activity in this layer presumably makes a contribution to the response. It is possible that the cells in this layer play a different role than those in layer II/III and the potentiation of the connections from the callosum represents something different from the cross-hemispheric potentiation previously demonstrated.

Collectively these data provide further evidence that motor learning is encoded by alterations in synaptic efficacy. It appears that there are increases in synaptic strength in the trained hemisphere which serve as the mechanism of the learning. Moreover, there is further evidence that there is communication between the hemispheres and that the direction of information transfer is from the trained hemisphere to the untrained hemisphere. Additional work is necessary to determine the functional significance of the interhemispheric communication.

Figure Captions

Figure 9. Improvement in reaching skill over training days. The skill of the experimental animals improved across the days of training as measured both by the percent of successful reaches **(A)** and the number of successful reaches **(B)**.

Figure 10. Field potentials recorded from the trained and control animals. A. The experimental animals showed increased field potential measures across the days of training in the trained hemisphere relative to the untrained hemisphere. Most of the changes were seen in the highest three intensities so only these three were averaged for the sake of this graph. **B.** Representative field potentials evoked with 1260 μA stimulation in the trained hemisphere taken on day 1 and day 7 of training. **C.** The control animals displayed no systematic difference between the preferred and non-preferred hemispheres. **D.** Representative field potentials evoked with 1260 μA stimulation in the preferred hemisphere taken on day 1 and day 7 of training.

Figure 11. Field potentials evoked by contralateral stimulation recorded from the experimental and control animals. A. The experimental animals showed increased field potential measures across the days of training in the untrained hemisphere relative to the trained hemisphere. Most of the changes were seen in the highest three intensities so only these three were averaged for the sake of this graph. **B.** Representative field potentials evoked with 1260 μA stimulation in the trained hemisphere taken on day 1 and day 7 of

training. **C**. The control animals displayed no systematic difference between the preferred and non-preferred hemispheres. **D.** Representative field potentials evoked with 1260 μA stimulation in the preferred hemisphere taken on day 1 and day 7 of training.

Figure 12. LTP induction across days of stimulation. Neither hemisphere showed reliable potentiation. **A.** There was an interaction between the trained and untrained hemispheres across the days of stimulation with the untrained hemisphere showing a greater increase in field potential size relative to the trained hemisphere. **B.** There was no systematic difference between the preferred and non-preferred hemispheres of the control animals.

Figure 13 . Movements made by the grooming-control animals. A. The animals that were enticed to groom unilaterally moved the grooming forelimb significantly more often than the non-grooming limb. **B.** Despite the fact that the grooming animals had no change in field potential size, they did not make fewer forelimb movements than the reaching animals.

Figure 14. Reaching skill in the callosal-cortical implant animals. The animals with callosal-to-cortical implants showed significant increases in skill across days of training as measured by number of successful reaches **(A)** and percent of successful reaches **(B).**

Figure 9

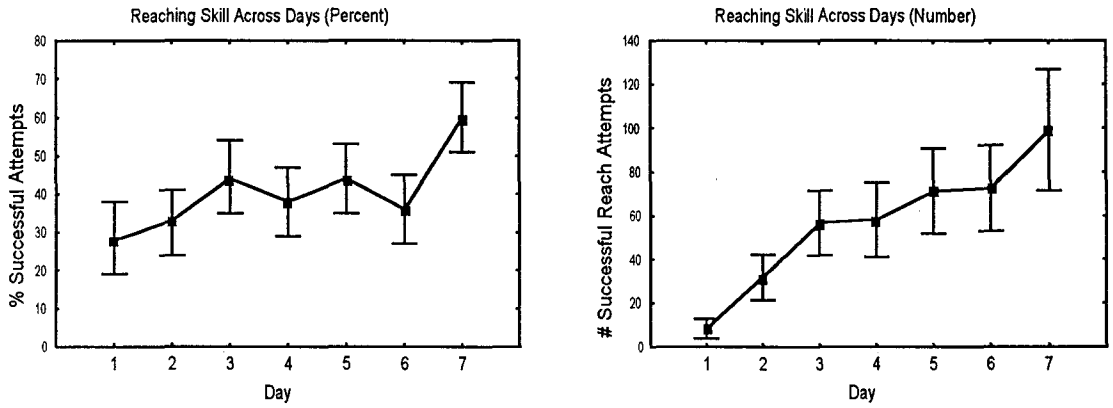

Reaching Skill Across Days (Percent)
% Successful Attempts
0
10
20
30
40
50
60
70
80
1
2
3
4
5
6
7
Day
Reaching Skill Across Days (Number)
Successful Reach Attempts
0
20
40
60
80
100
120
140
1
2
3
4
5
6
7
Day

Figure 10

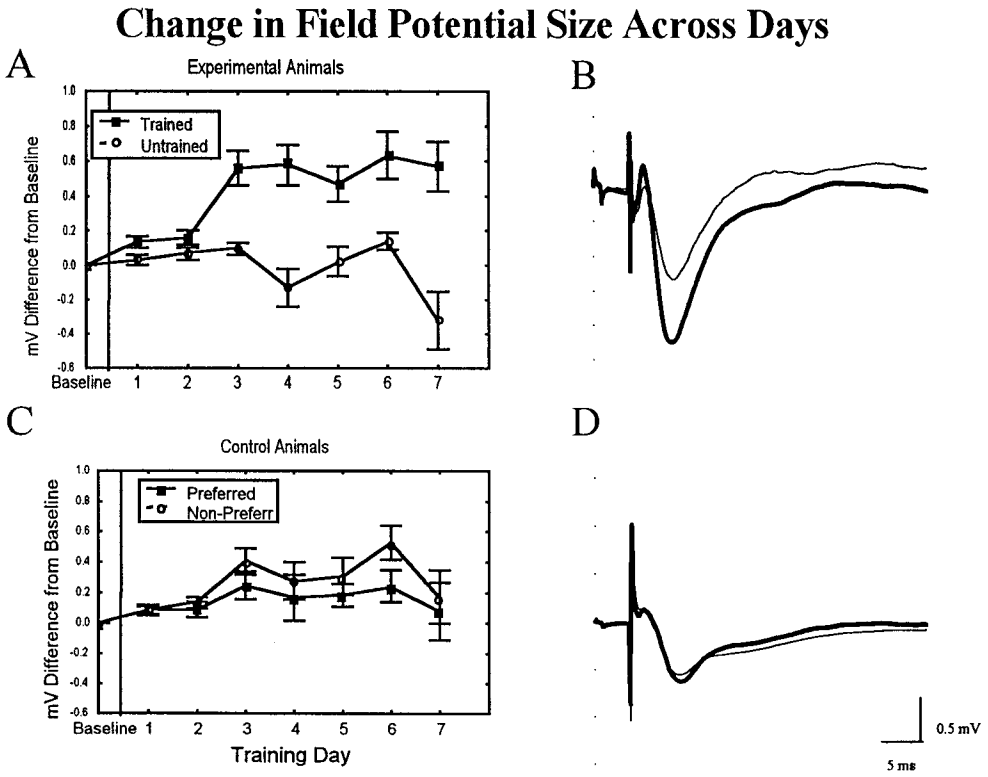

Change in Field Potential Size Across Days
A
Experimental Animals
Trained
Untrained
mV Difference from Baseline
Baseline 1 2 3 4 5 6 7
B
C
Control Animals
Preferred
Non-Preferr
mV Difference from Baseline
Baseline 1 2 3 4 5 6 7
Training Day
D
0.5 mV
5 ms

Figure 11

Change in Field Potential Magnitude
(Cross-Hemispheric Stimulation)

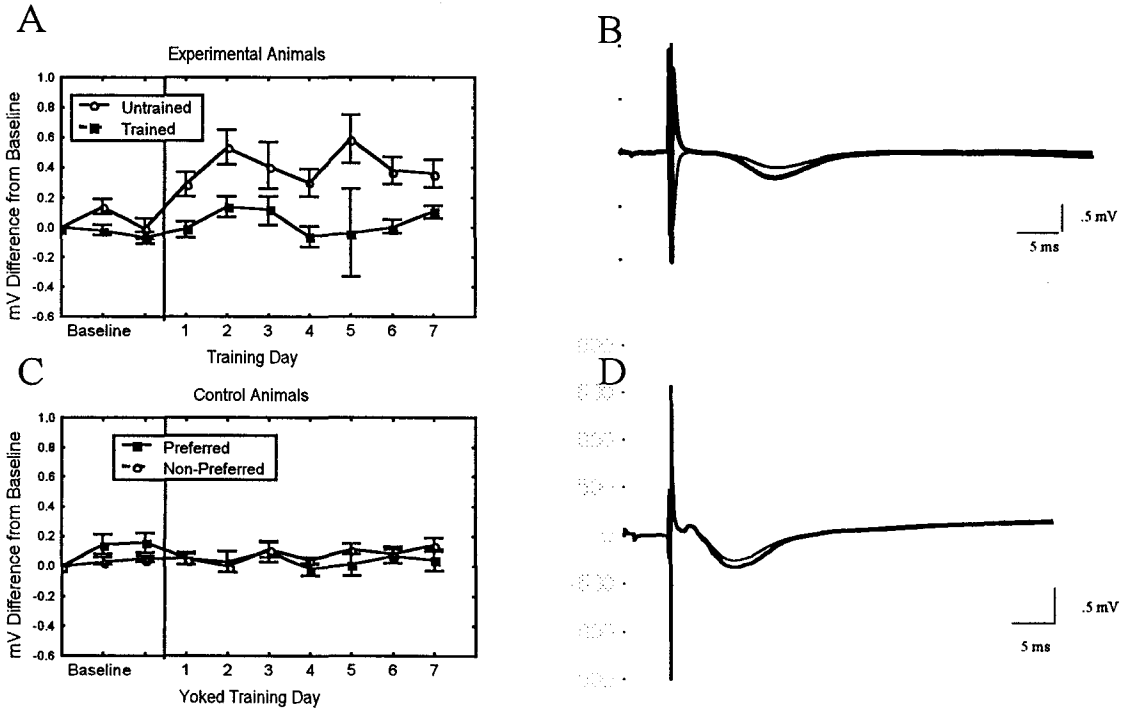


Figure 12

LTP Induction Across Days

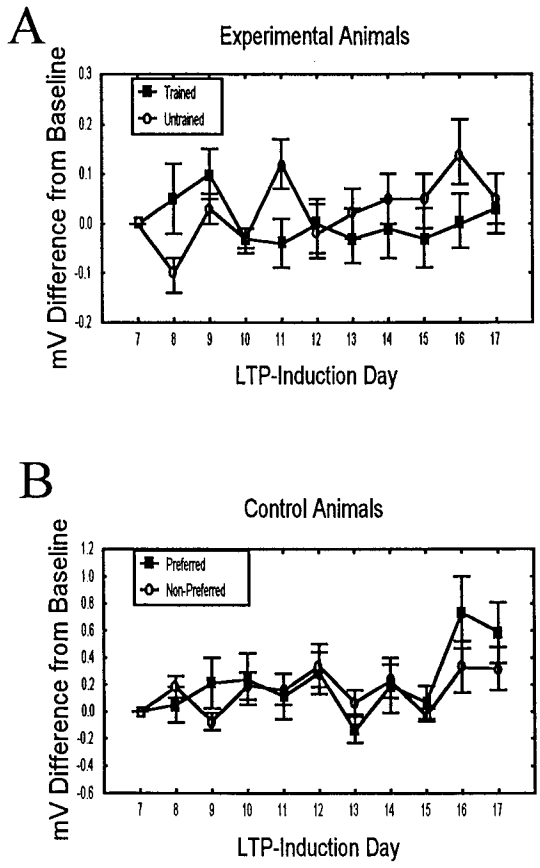


Figure 13

Forelimb Movements

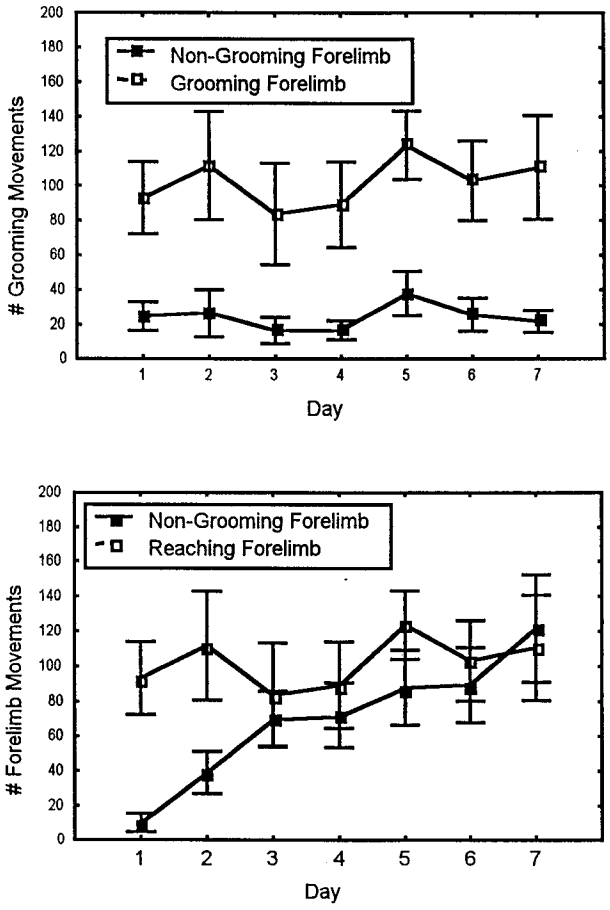
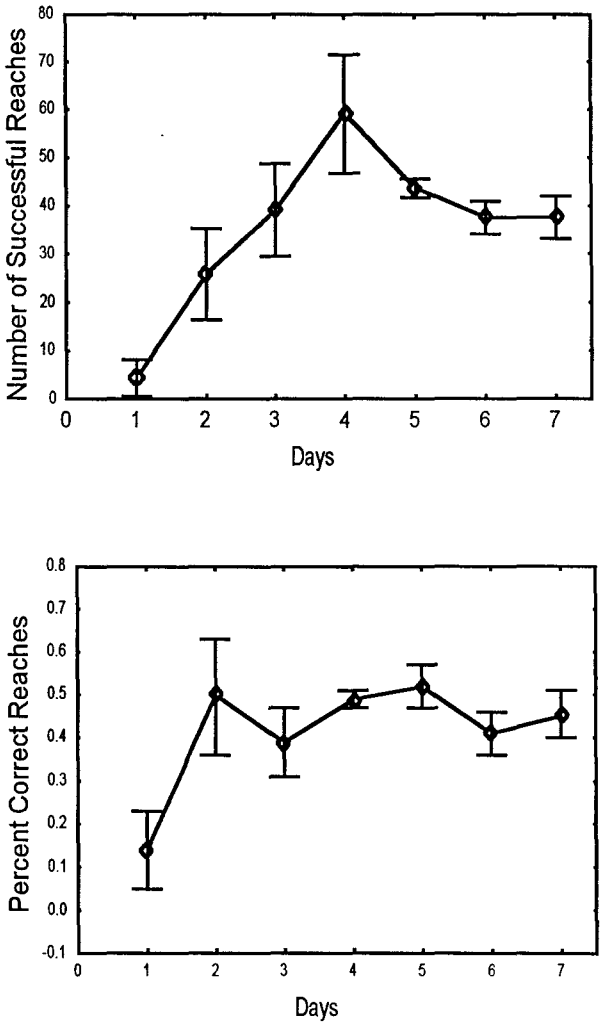


Figure 14

Reaching Skill Across Days



Chapter 4

Modification of synaptic weights disrupts memory

Introduction

Evidence was presented in the preceding two chapters that motor skill learning is mediated by an LTP-like (and/or LTD-like) mechanism. Following the acquisition of a reaching skill, recordings taken *in vitro* (slice preparation) and *in vivo* (acute and chronic preparations) indicated that the acquisition of the skill increased synaptic efficacy in the region of the brain responsible for the behavior. An alternate explanation is that synaptic efficacy was maintained relative to a decrease in the efficacy within the control site.

Because the training-induced modifications blocked subsequent LTP induction, it appears that the two phenomena rely on the same mechanism. Given this interpretation, a variety of predictions can be made regarding the interaction of learning and synaptic potentiation. If information is stored by incrementing synaptic weights, for example, then lowering the weights should erase the information and result in loss of the memory. Synaptic connection strength can be decreased by the application of low-frequency stimulation. This phenomenon is known as long-term depression (LTD). Depotentiation of previously potentiated synapses is also achieved with the application of low-frequency stimulation (Barrionuevo, Schottler, & Lynch, 1980; Staubli & Lynch, 1990). Saturating

LTP would also be expected to overwrite stored information and produce a memory loss. The reaching task offers a good paradigm to test these hypotheses because the cortical focus of learning is localized and unilateral.

A related argument is that prior saturation of LTP should "use up" the available plasticity in the affected synapses and interfere with or block subsequent learning (Castro et al., 1989). Counter arguments to this assumption were presented in the general introduction. Another problem is that tests of this hypothesis have used the hippocampus and tasks that engage the brain bilaterally (Bliss, 1998) which makes saturating LTP in the necessary systems twice as difficult as it would be when employing a unilateral task. The present experiment utilizes the reaching-task paradigm to determine if the delivery of LTP-inducing stimulation following training impairs the acquisition of the reaching skill.

Two logistic problems will make it difficult to test this hypothesis. The first is that it is difficult to saturate LTP or LTD over a sufficient number of synapses using a single bipolar stimulating electrode. Moser et al. (1998) devised a method to circumvent this problem. By implanting two bipolar stimulating electrodes parallel to one another, they dramatically increased the amount of neural tissue that they were able to activate. One bipolar stimulating electrode provides a single cathode-anode combination, whereas two electrodes provide six such combinations (Figure 15). Employing a similar electrode-placement protocol with the reaching task should dramatically increase the likelihood of activating a sufficient number of the synapses involved with the acquisition of the skill.

Another problem may result from how the information is stored. It is not likely to be stored in terms of absolute synaptic strength, but rather as a *pattern* of synaptic strengths. Some synapses will be strengthened while others are weakened. It is possible that a generalized depression of these connections would decrement the mean synaptic strength while leaving the relative pattern intact (Figure 16). If relative synaptic strength is the most critical information-encoding component, then anything less than a complete saturation of LTD could have little or no effect. One way by which this problem might be avoided is to start with the connection weights depressed, saturating the cortex with LTD prior to training. In order for the animals to encode the task, they will need to increment some of the synaptic connections to produce the necessary pattern of relatively strong and weak synaptic connections. Low-frequency stimulation every day following training can then more easily re-impose the presumably meaningless pattern of all synaptic connections at minimum strength.

An analogous design using high-frequency stimulation should also block memory storage. If LTP is saturated prior to learning, then the animals will necessarily have to decrement some synaptic strengths in order to encode the necessary information. The delivery of high-frequency stimulation every day following training would be expected to re-impose the meaningless pattern of fully saturated synaptic connections. It is, thus, expected that maintaining motor cortical synapses at maximal or minimal connectivity will disrupt the capacity of the animals to encode the necessary information to learn the reaching skill.

This expected result is based on the assumption that learning and LTP rely on the same neural mechanism. However, the expected result lends itself well to two other explanations. The manipulation requires a lot of activation of the cortex. There is the mechanical disruption required to implant three large electrodes and there is the abundance of stimulation that results from the elaborate stimulation protocol. It is possible that this heavy stimulation load on the cortex will cause tissue damage and disrupt memory accordingly. A simple way to test this would be to see if the animals are able to learn the task following the termination of the stimulation protocol. If cortical damage is the cause of a failed performance on the task, then these animals should not perform as well as controls, even when they are not receiving the stimulation. Otherwise, they would be expected to do as well as or better than unstimulated animals.

A second alternate explanation comes from studies which disrupt consolidation following learning. Animals that receive electroconvulsive shock following learning, for example, fail to retain the information. This failure could be due to the "overwriting" of the connection weights, as described above, or to some other process that is correlated with LTP. If the disruption of consolidation is due to a non-LTP mechanism, then other forms of post-trial stimulation might work by the same mechanism. For example, the post-training stimulation might deplete the metabolic reserves of the cells such that it is not possible for the cells to consolidate information. Although such a depletion would provide a different, but still compelling, link between LTP and memory, a group was added to at least partly counter this interpretation. These animals received the same post-

training stimulation as in consolidation experiments but were not previously saturated with LTP. If learning is blocked merely because the post-training stimulation disrupts consolidation in a manner other than by overwriting synaptic weights, then this control group should perform just like the experimental animals whose LTP is saturated prior to training.

METHOD

Subjects

Twenty-five animals were used in this experiment. The animals were divided into four groups: LTD, LTP, post-training stimulation and controls (see Figure 17 for a description of the manipulations). The animals were acquired from the Charles River Breeding colony and weighed between 225g and 300g when the experiment began. They were housed individually, kept on a 12 h on/12 h off light schedule and maintained on an *ad libitum* feeding schedule. The testing, stimulating and recording were all done during the lights-on phase. Prior to behavioral training, the animals were food deprived to approximately 85% of their body weight and were maintained at this weight throughout the training procedure. Deprivation was used to motivate the animals to reach for the food rewards.

Surgery

The animals were anesthetized with sodium pentobarbital and their heads were immobilized in a stereotaxic apparatus. Twisted bipolar electrodes made from Teflon-coated stainless steel wire were implanted unilaterally. Stimulating and recording

electrodes were both implanted into layer II/III of the forelimb region of the somatomotor cortex. The stimulating electrodes were bipolar with a tip separation of .5 mm. The recording electrodes were monopolar. Two stimulating electrodes were implanted in each animal. The first was implanted 0.9 mm anterior to Bregma, and the second was implanted 1.2 mm anterior to Bregma and 2.0 mm lateral to the midline. The recording electrodes were implanted 2.0 mm anterior to Bregma and 2.0 mm lateral to the midline. The electrodes were connected to gold-plated Amphenol pins which were inserted into a 9-pin connector plug. Evoked responses were monitored to ensure that the electrodes were lowered to optimal depths. Six cathode-anode combinations were possible with the two stimulating electrodes. Only animals that demonstrated evoked responses from at least five of the six combinations were included in the study.

Stimulating and Recording

Once the animals had recovered from surgery, 3 sets of field potential measures, spaced 48 h apart, were taken to establish a series of baseline input/output (I/O) curves. Pulses of increasing intensity were delivered through the stimulating electrodes at a frequency of 0.1 Hz. High pass and low pass filters set at 0.3 Hz and 3.0 kHz respectively. Ten field responses were evoked, amplified, digitized (at 10 kHz) and averaged at each of five logarithmically-spaced intensities (100, 200, 500, 1000 and 1260 μA). The training procedure began twenty-four hours following the third baseline I/O. No stimulation was delivered to the control animals. An additional I/O was recorded following the five days of LTP or LTD induction to ensure that the stimulation protocol

worked.

LTP was induced with the application of 8-pulse trains with a frequency of 300 Hz and a duration of 24 ms. Sixty trains were delivered with an inter-train interval of 1 sec. LTD was induced with the application of 900 single pulses delivered at a frequency of 1.0 Hz. This procedure was followed each day for five days with each of the six cathode-anode combinations for the LTP and LTD groups. Each day immediately prior to and following training the LTP or LTD stimulation protocol was delivered. For the post-training stimulation group the stimulation protocol was delivered only after training. The interval between training and stimulation was made as short as possible (never more than 2 min); depotentiation stimulation has maximal effect when performed shortly following the synaptic weights have been incremented (Martin, 1998). Sham implant animals were chronically implanted with electrodes but received no stimulation.

Both the LTP and the post-training stimulation groups received high-frequency stimulation, during which behavioral responses were monitored. Only animals with clear forelimb movement as a consequence of the stimulation were included in the study. This test provided a means of determining that the electrodes were placed in the forelimb region. For the LTD group, the low-frequency stimulation was not sufficiently intense to induce forelimb movement. Thus, high-frequency stimulation was delivered to the LTD animals following the study and again only animals who demonstrated forelimb movement as a consequence of the stimulation were included in the study. Because this stimulation was delivered only after all of the recordings had been taken and all of the behavioral

training had been conducted, it had no impact on the outcome of the experiment.

Behavioral Training

The behavioral training was conducted using the same apparatus and protocol outlined in the previous two chapters. An individual not training the animals placed them in the training apparatus to ensure that the experimenter was blind to the animal group at the time of behavioral testing. The control and post-training stimulation groups were trained for seven days. The LTP and LTD groups were trained for 12 days, the first seven with stimulation and the subsequent five without stimulation.

Histology

After completion of the behavioral measures, the animals were sacrificed and their brains fixed and removed. They were perfused through the heart with a formal-saline solution which was 4% formalin in 0.9% saline. Once the tissue was fixed, the animals were decapitated and the brains were removed and placed in the formal-saline solution for a period of not less than 36 hours. Twenty-fours hours prior to slicing the brains, they were placed in a 10% sucrose solution and kept cool in a 4 degrees C refrigerator. At least three 40 μm coronal sections were taken from each brain through the electrode tracks. Each slice was stained with Cresyl Violet and examined under an imaging microscope to confirm the electrode placements were located in layer II/III.

RESULTS

The LTD animals demonstrated a significant reduction in the size of evoked field potentials following the five days of low-frequency stimulation ($F(3, 15) = 7.10$, $p < .05$).

The LTP animals demonstrated a significant increase in the size of evoked field potentials following the five days of high-frequency stimulation ($F(3, 12) = 3.84$, $p < .05$) (Figure 18).

The same reaching skill measures employed in chapters two and three were used with these animals. Two-way ANOVAs were conducted to determine if the sham implant animals performed differently than the animals in the acute-preparation experiment from chapter 2. This was done to detect any possible impairments in performance caused by merely implanting multiple electrodes in the cortex. No difference was found between these groups in terms of percent of successful reaches ($F(1, 25) = 2.50$, $p > .05$). There was a difference, however with regard to the number of successful reaches ($F(1, 25) = 4.90$, $p < .05$).

Two-way repeated-measures ANOVAs were conducted to compare the performance of the animals across days. Performance was significantly different between the groups as measured both by percent of successful reaches ($F(3, 20) = 10.51$, $p < .05$) and number of successful reaches ($F(3, 20) = 3.44$, $p < .05$). Tukey HSD post hoc tests revealed that the sham implant control group performed significantly better than both the LTP and LTD groups on both these measures. No difference was found between the LTP and LTD groups on either measure. The post-training stimulation group performed significantly better than both the LTP and LTD group on the measure of percent of successful reaches and significantly worse than the control group. No difference was found between this group and any other group with regard to the number of successful

reaches daily (see Figure 19 for a summary of inter-group differences). Figure 20 depicts the performance of the groups across the days of training.

Qualitatively, the LTP and LTD animals appeared to behave differently than the control animals. They frequently came to the front of the reaching box and placed their noses out the slit in the front. This allowed them to retrieve some of the food rewards with their tongues, but they appeared to be reluctant to reach for the food. This is consistent with the behavior of most unstimulated animals on the first and second days of training after they have retrieved some of the food rewards with their tongues. The LTD and LTP animals behaved as if they had learned some of the non-reaching components of the task such as the location of the food rewards and that they were edible. It appeared that they were either lacking the capacity to reach or to learn that reaching was a successful strategy for acquiring the food reward.

The post-training stimulation groups displayed a deficit as well. They did not have the same fine motor skill as unstimulated controls but were able to adapt strategies to counter this problem. One strategy, for example, was to wedge the food pellet between two digits instead of grasping it. This meant that the animals were reaching frequently but not as proficiently. The animals appeared to have no other motoric deficits.

To ensure that the stimulation protocol did not damage cortical tissue, four of the animals from the LTP and LTD groups were trained on the reaching task for an additional five days without stimulation. The animals in both groups improved to the levels of the unstimulated animals (Figure 21).

DISCUSSION

Both the LTP and LTD stimulation protocols generated clear learning deficits in the animals. One possibility is that implanting the additional stimulating electrodes created a cortical insult which rendered the animals incapable of learning the reaching skill. However, the animals with sham placements performed as well as animals from a previous study that had no such insult, so this possibility is unlikely. They did not do as well as the unimplanted animals with regard to the number of successful reaches, so the implants may have reduced overall activity.

It is possible that the poor performance of the stimulated animals was caused by stimulation-induced cortical damage. This too seems unlikely because the skill of the stimulated animals improved dramatically following the termination of the stimulation. However, there is the possibility that a stimulation induced lesion is reversible and that the recovery of the animals skill following the termination of the stimulation was because the lesion was afforded time to recover. A control group that controls for stimulation will be necessary to rule out the effect of stimulation alone in the absence of modifications in synaptic strengths. For example, a group that receives the same number of trains as the LTP group but at a mid-range intensity which neither induces LTP or LTP would control for stimulation.

Following the termination of stimulation, the stimulated groups seem to surpass control animals with regard to the number of successful reach attempts (see Figure 21). Unfortunately, training did not continue with the control animals beyond day 7. The lack

of data from control animals beyond day 7 makes it unclear whether the controls animals had reached an asymptotic level of performance on this measure by day 7, or if they would have continued to improve in a manner consistent with the stimulated groups.

The effect does not seem to be entirely due to a disruption of consolidation resulting from post-trial disruption of activity patterns. Animals that received stimulation following the training, but not before training, did not experience as severe a learning impairment. They did, however, show some deterioration in their fine motor learning.

The data from this study provide further support for the conclusion that modifications in synaptic efficacy underlie the instantiation of learned motor skills. Moreover they suggest that the relative strength of synaptic connections is the necessary element in encoding information. This led to the hypothesis in chapter four; that modifying synaptic strength following learning would erase the previously learned information. The results were consistent with this prediction, the application of both high- and low frequency stimulation following learning disrupted acquisition. However, a great deal more work needs to be done to rule out the possibility of non-specific performance variables. The effects of only pre-trial stimulation must be tested, along with the effects of stimulation on previously acquired motor skills.

The animals that were stimulated showed no apparent impairment in motor skills which were already well established, despite the fact that a large number of synapses were affected by the stimulation. Once a memory is well consolidated, it may be very difficult disrupt the neural pattern of activation. Consistent with this idea is that older LTP is more

difficult to depotentiate than more recently induced LTP (Martin, 1998). This resistance of older LTP may be due to the fact that once a behavior is learned the synapses involved become less plastic. Rioult-Pedotti and Donoghue (1999), for example, found that animals that were trained on the reaching task for a long period of time (23 - 32 days) did not show an occlusion of LTP in the trained hemisphere. This finding suggests that there was a "resetting" of the plasticity. One way the brain might accomplish this would be to modify synapses involved in the storage of information so that they are less plastic and modify others to be more plastic so that new information can be encoded. It is known that NMDA receptors, like AMPA receptors, can be inserted into receptor membranes in an experience dependent fashion (Quinlan, Philpot, Huganir, Bear, 1999). If they can be inactivated or deleted from receptor membranes as well, this would provide a mechanism by which synapses involved in a previously learned skill become resistant to change. It may, therefore, be difficult to overwrite well-consolidated memories, creating a problem for this approach. Until a technique is discovered that modifies the synaptic strength of synapses involved in old memories, it will be difficult to confirm that modifying synaptic strength can overwrite or erase existing memories.

Figure Captions

Figure 15. Advantage of two stimulating electrodes. A. A single bipolar stimulating electrode, a field of stimulation can be produced surrounding the two poles (a and b). **B.** With two bipolar electrodes fields of activation can be produced around the poles of the two electrodes (a and b) and (c and d). Also fields of activation can be created around the horizontally aligned poles (a and c) and (b and d) and diagonally aligned poles (a and d) and (b and c).

Figure 16. Pattern of activity may be important. A. A certain pattern of activation may be required to produce a memory. **B.** The application of low-frequency stimulation (LFS), which has the effect of generally lowering the strength of synaptic connections may leave the pattern of activation intact.

Figure 17. Different treatments for the four groups in this experiment

Figure 18. LTP and LTD in the stimulated groups. Following three days of stable baseline recordings, HFS stimulation was delivered to the LTP group and LFS stimulation was delivered to the LTD group. Respectively the groups showed a significant increase and decrease in field potential magnitude.

Figure 19. Table of group differences. A Tukey's HSD was conducted to determine

group differences.

Figure 20. Reaching task skill. The LTP and LTD groups performed significantly worse than controls on the reaching task.

Figure 21. Skill after the termination of stimulation. Following the termination of stimulation, the LTP and LTD groups performed as well as or better than controls.

Figure 15

One vs. Two Stimulating Electrodes

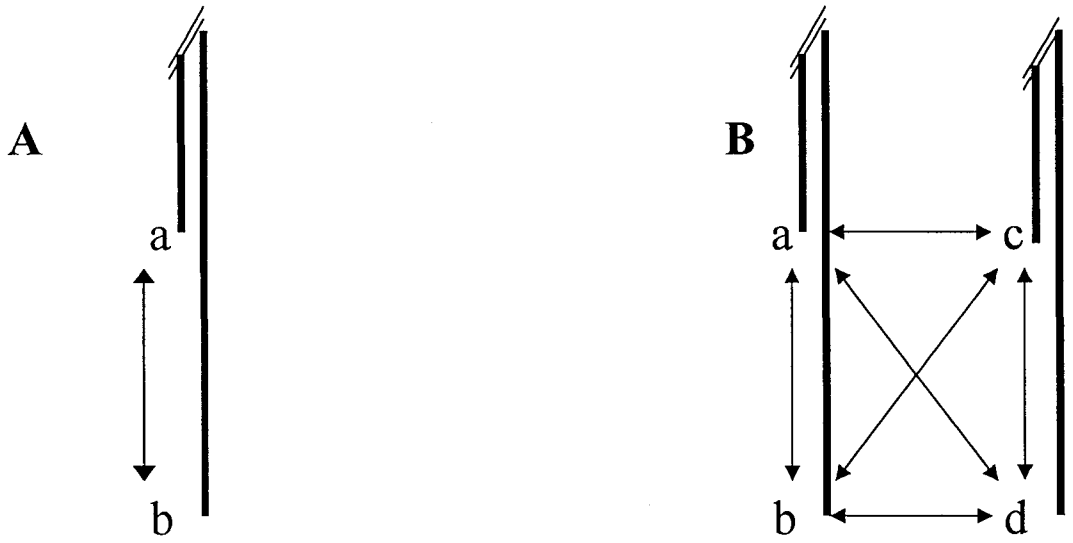


Figure 16

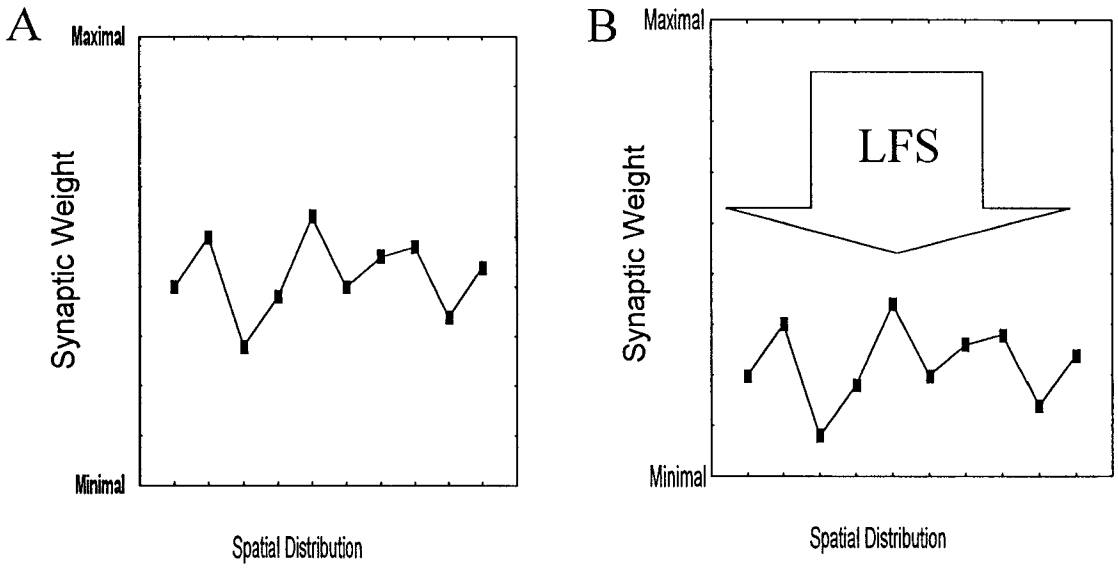

A
Maximal
Minimal
Synaptic Weight
Spatial Distribution
B
Maximal
Minimal
Synaptic Weight
LFS
Spatial Distribution

Figure 17

Group	Stimulation Pre-Training	Training	Stimulation on Training Days
LTD	Saturation of LTD (5 Days)	5 Days of Training on the Reaching Task	LTD Stimulation immediately before and following training
LTP	Saturation of LTP (5 Days)	5 Days of Training on the Reaching Task	LTP Stimulation immediately before and following training
Post-Training	No Stimulation	5 Days of Training on the Reaching Task	LTP Stimulation immediately following training
Controls	No Stimulation	5 Days of Training on the Reaching Task	No Stimulation

Figure 18

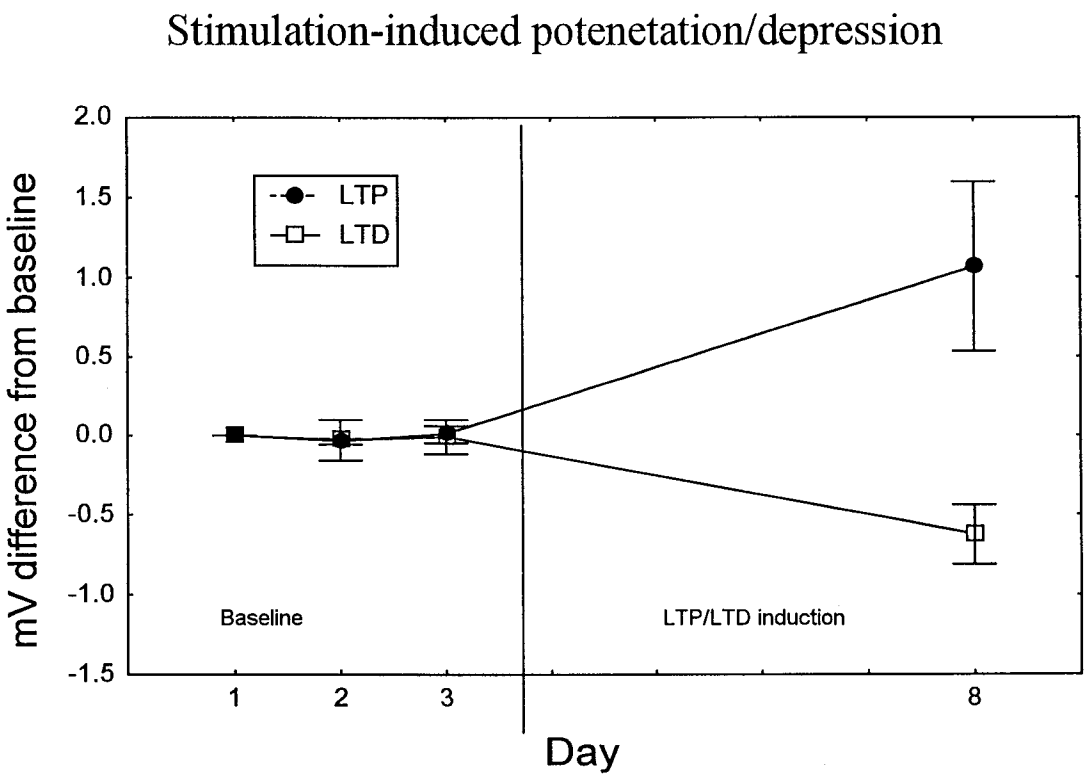

Stimulation-induced potenetation/depression
mV difference from baseline
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
LTP
LTD
Baseline
LTP/LTD induction
1
2
3
8
Day

Figure 19

Number of Successful Reaches

	LTD	LTP	Post-Stim	Control
LTD	******	.911	.074	.020 *
LTP		******	.070	.021 *
Post-Stim			******	.706
Control				******

Percent of Successful Reaches

	LTD	LTP	Post-Stim	Control
LTD	******	.984	.033 *	.002 *
LTP		******	.045 *	.005 *
Post-Stim			******	.000 *
Control				******

Figure 20

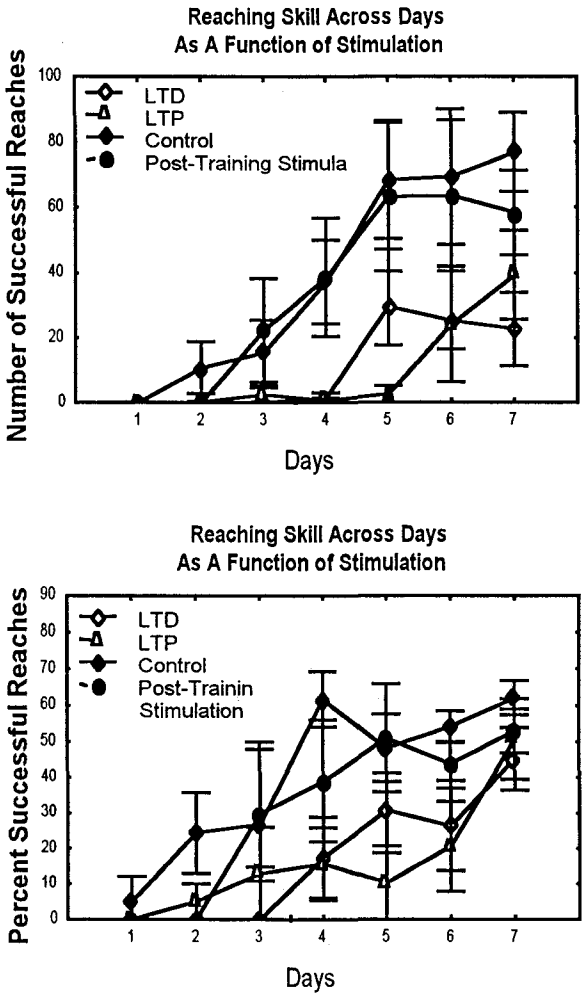
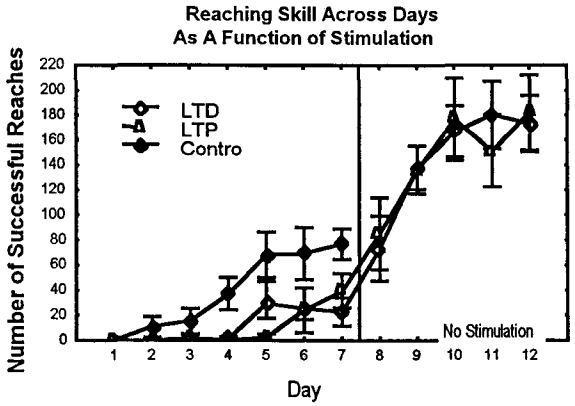
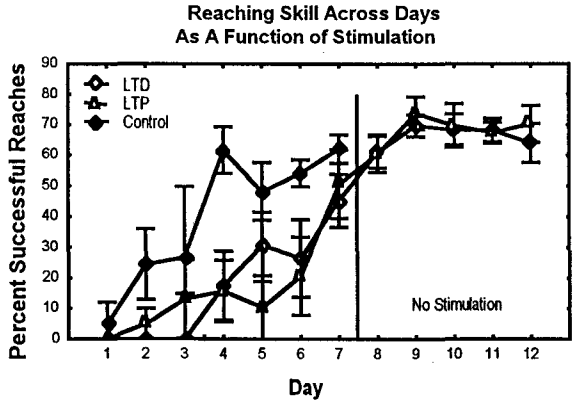

Reaching Skill Across Days
As A Function of Stimulation
Number of Successful Reaches
100
80
60
40
20
0
LTD
LTP
Control
Post-Training Stimula
1
2
3
4
5
6
7
Days
Reaching Skill Across Days
As A Function of Stimulation
Percent Successful Reaches
90
80
70
60
50
40
30
20
10
0
LTD
LTP
Control
Post-Trainin
Stimulation
1
2
3
4
5
6
7
Days

Figure 21


Reaching Skill Across Days
As A Function of Stimulation
Number of Successful Reaches
220
200
180
160
140
120
100
80
60
40
20
0
LTD
LTP
Contro
No Stimulation
1 2 3 4 5 6 7 8 9 10 11 12
Day


Reaching Skill Across Days
As A Function of Stimulation
Percent Successful Reaches
90
80
70
60
50
40
30
20
10
0
LTD
LTP
Control
No Stimulation
1 2 3 4 5 6 7 8 9 10 11 12
Day

Chapter 5

General Discussion

The most important question regarding LTP remains its relationship to memory. Because the phenomena are fundamentally different, it is difficult to know how to both conceptualize and investigate the relationship. A potentially effective way to do so is with behavioral LTP experiments, in which animals are exposed to a situation demanding the acquisition of new information while their neural circuitry is monitored for training-induced LTP-like changes. Chapters two and three of this thesis employ this approach. In chapter two rats trained unilaterally on a forelimb reaching task had larger evoked potentials in the trained hemisphere relative to the untrained hemisphere. This effect was consistent across the slice and acute preparations.

In chapter three, a similar effect was found in chronically-prepared animals. Those trained on the reaching task had larger evoked potentials in the trained hemispheres relative to the untrained hemispheres. Moreover, this inter-hemispheric difference appeared to be time-locked to the behaviorally-measured acquisition of the reaching skill. This finding is consistent with the hypothesis that the information is encoded with an LTP-like mechanism. However, it is likely that both increments and decrements are used in the

encoding of information. The results from both the slice and acute experiments in chapter one are also consistent with the hypothesis that there were decrements in synaptic strength in the untrained hemisphere accounting for the interhemispheric differences. The chronically implanted animals in chapter three, however, seemed to demonstrate both an increase in the trained hemisphere and a decrease in the untrained hemisphere. Because part of a successful strategy on the reaching task is to inhibit reaching with the forelimb that is not being trained, it is conceivable that there is a recruitment of layer II/III inhibitory connections, or decrements in excitatory connections, in the untrained hemisphere. Layer II/III is amenable to decrements in synaptic strength (Hess & Donoghue, 1996) and LTD is thought to play an important role in the encoding of information (Heynan, Abraham, & Bear, 1996; Scanziani, Malenka, Nicoll, 1996). Moreover, local inhibitory interneurons in layer II/III are hypothesized to play a role in certain forms of motor cortical plasticity (Jacobs & Donoghue, 1991). More work needs to be done to assess the relative contributions of excitatory and inhibitory connections and of increases and decreases in synaptic strength in these connections.

The research in chapters two and three also had the advantage of being able to verify that the electrodes were located in the forelimb region of the cortex. Previous work in this area (Rioult-Pedotti, et al., 1998) and the work in chapter one was unable to verify the location of the electrodes. The high-frequency stimulation delivered to the animals to confirm that the electrodes location of the electrodes stimulated movement in the forelimbs of the animals. Interestingly, this movement was typically in the shoulders and

elbows of the animals. In none of the animals was there stimulation induced movement of the digits such as movements in the digits.

Consistent with the interpretation that the learning-induced changes were using the same mechanism was that they impaired subsequent LTP induction. Rioult-Pedotti et al. (1998) reported the same result. The interpretation of this finding is that the learning "uses up" some of the available synaptic plasticity. The LTP that was induced in each of the preparations used was minimal, however. Perhaps this result is due to the fact that the stimulation parameters were different than the theta-burst stimulation used in the slice experiments. Neocortical LTP in the neocortex has a much different temporal dynamic in the chronic preparation than it does in the slice. In the slice, LTP is expressed within minutes of the stimulation; in the chronic preparation, the stimulation parameters that are effective for LTP-induction in the slice do not produce LTP (Racine, Teskey, Wilson, Seidlitz, & Milgram, 1994). Instead, neocortical LTP has been shown to rely on multiple stimulation sessions spaced over days (Racine, et al., 1994). Stimulation parameters were chosen which have been demonstrated to induce robust and reliable LTP in the neocortex in the chronic preparation. However, these stimulation parameters were used in callosal-to-cortical pathways as opposed to the horizontal connections stimulated in chapters two and three. These parameters may be non-optimal for horizontal pathway LTP induction.

One of the surprising results of this research was that there was a reliable learning-induced potentiation of the cross-hemispheric fibers carrying information from the trained hemisphere to the untrained hemisphere. This result was consistent across the acute and

chronic experiments. There are strong inter-hemispheric connections in this system, and Liang et al. (1993) demonstrated that stimulation of forelimb motor cortical neurons can elicit movement in the ipsilateral forelimb. Several explanations are consistent with a potentiation of information flowing from the trained hemisphere to the untrained hemisphere during training. One is that there is a transfer of the skill from the trained to the untrained paw. Observations of the animals indicated that they did use their untrained limbs more often later in the course of the experiment. Moreover, it is known that information is transferred from the trained to the untrained hemispheres in humans trained on manual motor skills (e.g., Marks, 1996). This effect is not seen in all human subjects and is thought to depend on a the degree of brain development (Uehara, 1998). A preliminary follow up on this possibility has so far failed to demonstrate that rats transfer the reaching skill to the untrained hemisphere, but these results are not yet conclusive.

Another possibility for the cross-hemispheric interaction is that the task itself involves both limbs and that they must co-ordinate a complex and unfamiliar body movement with one another. Acallosal humans tend to perform more poorly than normals on tasks that require bimanual co-ordination (Jeeves, Silver, Jacobson, 1988). Initial work from our laboratory, however, indicates that animals with a transected corpus callosum perform as well as normals on the task. This result is not consistent with the hypothesis that an interaction is required between the hemispheres, but the bi-hemispheric coordination of the callosally transected animals may be due to redundant systems (e.g., in the cerebellum) that support the cross-hemispheric communication. More behavioral

work needs to be done to assess the functional significance, if any, of the inter-hemispheric communication.

A potentially interesting finding from chapter four suggests that the learned reaching skill can be erased by post-training stimulation using either low- or high frequency stimulation. Previous attempts to erase stored information have been unsuccessful (Martin, Grimwood, & Morris, 2000). Those attempts have been tried in the hippocampus where taxing all of the interstructural circuitry participating in the acquisition of the information would be difficult.

This research was the first of its kind to electrophysiologically verify that the electrodes were implanted within the forelimb region of the cortex. Microstimulation of the rat motor cortex has demonstrated inter-rat variability in the map of the motor cortex (Liang, Rouiller, & Wiesendanger, 1993). Some of the animals in chapters 3 and 4 did not display forelimb movement as a result of the stimulation, which suggests that the electrodes placement in these animals was not in the motor cortical forelimb region. Interestingly, when forelimb movements were evoked, they appeared in the larger muscle groups controlling the shoulder and the elbow joints. None of the animals displayed a flexing of the finer muscle groups such as flexion of the digits. Presumably, however, most of the learning was happening in the finer muscle groups of the digits and wrists. Liang, et al. (1993) have demonstrated that it is possible to stimulate movement in these areas using micro stimulation of the motor cortex, although only in a limited subset (6%) of the animals. They used much more focal stimulation at much smaller intensities. When

they increased the stimulation intensity, the current spread caused activation over larger regions. Because of the high-intensity stimulation used in chapters three and four to confirm electrode placements, it is likely that there was current spread to regions encoding large, as well as fine muscle groups and the activation of the larger muscle groups may have overshadowed activation of the finer muscle movements. It is also possible that the larger muscle groups are activated more readily with the synchronizing stimulation patterns used here, whereas the digits need a finely patterned cortical activation that was not approximated by the high-frequency stimulation. Future research could be done to find stimulation parameters which will more precisely target the muscle groups primarily involved in the acquisition of the skill.

All of the recordings were temporally and spatially separated from which the training. Because the animals were tested 23 hours following training on the task, it is unlikely that the effects were due to any *short-term* modifications resulting from the training or the training-specific behaviors. When in the training environment, the neural representations related to the training context will be active, so it is likely that had the recordings been made in the training environment the hemispheric differences would have been more robust.

Because the rats served as their own controls, their untrained hemisphere was exposed to essentially the same stimuli as the trained hemisphere, minus the learning. One confound, however, was that the trained forelimb experienced more motor behavior than the untrained forelimb. To address this confound, an additional group was included who

was induced to groom unilaterally. The grooming motion was a well-established skill, so this group showed a similar lateralized bias in forelimb movement without the learning. These animals did not demonstrate inter-hemispheric differences in field potential magnitude which suggests that the differences found in the experimental groups was due to the learning and not merely movement.

That organisms use modifications in synaptic strength to encode information is often treated as a truism in the LTP literature. Taken together these experiments provide further support for the hypothesis that increments and decrements of synaptic strength underlying information storage. As well, the reaching task has proven to be a useful paradigm for investigating hypotheses regarding the relationship between LTP and memory.

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