BIDIRECTIONAL SYNAPTIC PLASTICITY WITHIN LAYER V OF THE SENSORIMOTOR CORTEX

Christine M. Werk

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Abstract

Bidirectional Synaptic Plasticity within Layer V of the Sensorimotor Cortex

Christine Werk, M.A. (PhD candidate)

Concordia University, 2006

The neocortex of awake animals requires spaced and repeated tetanization to successfully induce long-term synaptic potentiation (LTP) and long-term depression (LTD). Changes in synaptic strength can be promoted by synchronous neuronal activation, and the theta-rhythm (10 Hz) may be effective at inducing these changes. To investigate if theta-patterned stimulation is effective at inducing LTD in the sensorimotor cortex standard 1 Hz stimulation, pairs of pulses (with a 100 ms inter-pulse interval) delivered every 2 seconds, or 20-pulse 10 Hz trains were delivered daily for 10 days. Results were compared with control animals that received no stimulation. Prolonged 10 Hz trains were most effective at inducing LTD. To assess if 10 Hz activity in the form of large amplitude spindle waves are affected by plasticity of neocortical synapses, evoked spindle waves were recorded before and after LTP induction. The ability of single pulses to evoke spindle waves was increased following induction of LTP. The increased activation of spindle waves may have occurred due to strengthening of Layer V horizontal projections. To directly assess changes in the strength of these projections, LTP was induced and responses were recorded from a rostro-caudal array of five electrodes following corpus callosum stimulation as well as following direct stimulation of Layer V. Long-term potentiation of monosynaptic inputs to the cortex was found, but no significant increases in field potentials evoked by direct stimulation of the horizontal projections were observed. In addition, paired-pulse facilitation was observed at all electrode sites

indicating the ability of the recording array to reflect enhanced responses.

This research emphasizes the importance of theta-patterned activity in bi-directional synaptic plasticity in the sensorimotor cortex and suggests that LTP may occur primarily at monosynaptic inputs to the cortex rather than at synapses within the horizontal collaterals of Layer V.

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Contribution of Authors

1. **Long-Term Depression in the Sensorimotor Cortex Induced by Repeated Delivery of 10 Hz Trains *In Vivo*** by Werk, Klein, Nesbitt and Chapman

Christine Werk: Collected most of the data, performed all the data analysis and graphing, and was responsible for writing the paper.

Andrew Chapman: Helped with the theoretical design and the writing.

Hannah Klein: Collected data for some of the animals in the main experiment.

Cathy Nesbitt: Collected data for the animals in the pilot study.

2. **Induction of Long-Term Potentiation Leads to Increased Reliability of Evoked Neocortical Spindles *In Vivo*** by Werk, Harbour and Chapman

Christine Werk: Collected a most of the data, performed all the data analysis and graphing, and was responsible for writing the paper.

Andrew Chapman: Helped with the theoretical design and the writing.

Valerie Harbour: Collected part of the data.

3. **Effects of Long-Term Potentiation on Responses in the Sensorimotor Cortex Evoked by Direct Stimulation of Layer V Horizontal Projections** by Werk, and Chapman

Christine Werk: Collected most of the data, performed all the data analysis and graphing, and was largely responsible for writing the paper.

Andrew Chapman: Helped with the theoretical design and the writing.

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List of Abbreviations

AMPA	x-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
Ca^{2+}	Calcium
CC	Corpus Callosum
EEG	Electroencephalogram
GABA	Gamma aminobutyric acid
I/O	Input/output
LTD	Long-term depression
LTP	Long-term synaptic potentiation
Mg^{2+}	Magnesium
Na^{+}	Sodium
NMDA	N-methyl-*D*-aspartate
PP-LFS	Paired-pulse low-frequency stimulation
SWS	Slow-wave sleep
VL	ventrolateral
VPL	ventroposterior lateral

CHAPTER ONE

GENERAL INTRODUCTION

Bidirectional Synaptic Plasticity within Layer V of the Sensorimotor Cortex

General Outline.

Learning of motor tasks occurs on a regular basis, and motor performance is vital for daily functioning. The ability to pick up an object, use a screwdriver, or turn a page in a book all require some form of motor learning. The brain has a phenomenal capacity to acquire new memories, and a massive amount of research has been focused on the neurophysiological processes that underlie memory formation and consolidation. This thesis deals with cellular mechanisms for the consolidation of motor skills within the sensorimotor cortex and the focus of this thesis is on the rhythmic patterns of brain activity that may contribute to these cellular changes.

Memory consolidation is thought to require long-lasting changes in synaptic strength that lead to the establishment of novel networks of neurons that represent new memories (Hebb, 1949). Long-term synaptic potentiation (LTP) and long-term depression (LTD) are experimental models that have been used to study the cellular mechanisms that lead to increases and decreases in synaptic strength, respectively (Malenka & Nicoll, 1999; Kemp & Bashir, 2001). In these models, different patterns of strong synaptic activation lead to either increases or decreases in synaptic strength (Stanton, 1996). There is, therefore, great interest in the nature of endogenous patterns of neural activity that may contribute to naturally occurring changes in synaptic strength.

Rhythmic patterns of neural activity during sleep may be particularly important for consolidation of memories. This is because transient, or short lasting changes in synaptic strength that occur during rhythmic patterns of activity during sleep are likely to enhance the induction of long-lasting changes in synaptic strength (Sejnowski &

Destexhe, 2000). A great deal of previous research has indicated that strong stimulation patterned after the theta-frequency (4-12 Hz) electroencephalographic (EEG) rhythm in the hippocampus can promote the induction of long-term changes in synaptic strength (e.g.s, Huang & Kandel, 2005; Staubli & Lynch, 1987). The theta rhythm is thought to be effective because stimulation at this frequency leads to short-term synaptic facilitation effects that promote the induction of longer-lasting changes in synaptic strength (Rose & Dunwiddie, 1986).

The present thesis is focused on how rhythmic activities in the cortex can promote the induction of long-lasting changes in synaptic strength. Spindle waves are very large amplitude EEG events that occur at a frequency near 10 Hz in the neocortex during slow-wave sleep, and a series of experiments in this thesis points to their importance for inducing short-term changes in neuronal excitability that may contribute to longer-lasting changes in synaptic strength that may be related to consolidation of motor learning.

To investigate if stimulation at a frequency corresponding to both spindles and the hippocampal theta rhythm may promote changes in synaptic strength in the cortex, in the first set of experiments in this thesis, two types of prolonged theta-patterned stimulation were compared to standard 1 Hz trains of pulses in their ability to induce lasting synaptic depression. To further assess the relationship between spindle activity and synaptic plasticity, the second set of experiments assessed whether increasing synaptic strength in the sensorimotor cortex by inducing LTP could also promote the induction of neocortical spindle waves. In a final set of experiments, tests were conducted to determine if potentiation of monosynaptic inputs to the cortex, or potentiation of synapses within horizontal layer V collaterals might contribute most to the strengthening of the late

component of responses induced by theta-patterned stimulation. Overall, the results are consistent with the proposal that strong rhythmic synchronization at frequencies near 10 Hz contribute to the induction of lasting changes in synaptic strength that may contribute to motor learning.

The next portion of this Introduction is focused on research dealing with the idea that rhythmic EEG activities during sleep may contribute to memory consolidation. A review of the major characteristics of LTP and LTD with respect to the neocortex is also provided, and this is followed by a description of the circuitry within the sensorimotor cortex that generates the major components of the synaptic responses recorded here. The nature of the stimulation patterns required to induce either LTP or LTD is then discussed in the context of short-term synaptic facilitation effects observed in the cortex.

Memory Consolidation and Sleep.

Neurophysiological processes that occur during sleep are believed to contribute to the consolidation of long-term memories (Sejnowski & Destexhe, 2000; Steriade & Timofeev, 2003). There are multiple stages of sleep that are characterized by different patterns of EEG activity. Two of these stages have been shown to be well-correlated with memory acquisition and performance; rapid-eye movement (REM) sleep and slow-wave sleep (SWS). Rapid-eye movement sleep is the stage in which dreams occur and it is characterized by high-frequency beta and gamma EEG activities of 20 to 80 Hz. This pattern of EEG activity during REM sleep is similar to the pattern of EEG activity that is observed during waking. Slow delta (1-4 Hz) activity and large amplitude (~10 Hz) spindle wave oscillations are the main components of SWS (Kandel & Buzsáki, 1997; Sejnowski & Destexhe, 2000). It has been proposed that dreaming is the process during

sleep that contributes primarily to memory consolidation. Evidence for the role of dreaming and REM sleep in memory consolidation comes from research showing memory impairments following deprivation of REM sleep (for review see Pearlman, 1979; or Smith, 1985), increases in the amount of REM sleep observed in the night following learning of a task (Smith, Conway, & Rose, 1993; Smith, Kitahama, Valatx, & Jouvet, 1974), and increases in REM sleep following exposure to enriched environments (Kiyono, Seo, & Shibagaki, 1981; Tagney, 1973).

Although, REM sleep has been of interest with respect to mechanisms of memory consolidation, the role of SWS in the formation of memories has recently become a focus of study as well. Slow-wave sleep is a stage of sleep characterized by the transition from light stage 1 sleep into a deeper sleep with the presence of large amplitude spindle waves. Spindle waves are large amplitude ~10 Hz rhythmic oscillations that occur during SWS and awake immobility (Sejnowski & Destexhe, 2000). The role of SWS in memory consolidation has been investigated by measuring changes in spindle activity following learning. Spindle activity is enhanced following learning in both humans (Gais, Molle, Helms & Born, 2002) and rats (Schiffelholz & Aldenhoff, 2002). Spindle waves are believed to be a particularly important part of SWS for learning because they involve mass synchronization of neuronal firing across the neocortex, which can increase the excitability of cortical cells (Sejnowski & Destexhe, 2000; Contreras & Steriade, 1995). Increasing cellular excitability can promote changes in synaptic strength in activated pathways.

Reactivation of cells that were activated during learning is believed to be necessary for the consolidation of memory traces (Nadel, Samsonovich, Ryan, &

Moscovitch, 2000; Sejnowski & Destexhe, 2000). Repeated reactivation of developing memory traces during SWS could be particularly important for the slow consolidation of memory in the neocortex. Standard models of memory formation hold that the hippocampus is responsible for the initial processing and rapid acquisition of memories and it is also thought to be required for the recall of newly formed memories by reactivating cortical memory traces (Nadel, Samsonovich, Ryan, & Moscovitch, 2000). This re-activation of intra-cortical connections by the hippocampus is thought to be required during memory consolidation until intra-cortical connections are strengthened enough to support memory (Sejnowski & Destexhe, 2000, but see Nadel, Samsonovich, Ryan, & Moscovitch, 2000). The hippocampus could contribute to this reactivation both during waking recall of developing memories, and during sleep.

There is growing evidence that the hippocampus may contribute to reactivation of cortical circuits during SWS episodes. For instance, Siapas and Wilson (1998) found that there is a temporal correlation between neocortical spindles and hippocampal ripples-sharp wave episodes during SWS in which spindles are immediately preceded by sharp-waves. A fine time scale analysis of spindle waves and hippocampal ripple episodes, however, revealed that spindles likely activate hippocampal ripples (Sirota, Csicsvari, Buhl, & Buzsáki, 2003).

Additional evidence for the role of SWS in memory consolidation comes from research on hippocampal place cells. Place cells are neurons that fire preferentially when the animal is within a particular spatial location in the environment. To measure activity of place cells, multi-electrode arrays can be chronically implanted into the brain in order to record from many neurons simultaneously. Animals are then placed in an open field

and place cells are found as the animals explore the environment. During SWS following exposure to a new environment, place cells in the hippocampus have been shown to fire, or replay, in the same sequence that they fired in during the original exploration of the open field (for review see Sutherland & McNaughton, 2000). The strength of the relationship of firing between the original learning experience and the replay during sleep was shown to be greatest during sharp wave hippocampal ripple oscillations (Skaggs & McNaughton, 1996).

Spindle waves may be important for the reactivation of neocortical cell assemblies during SWS. Synchrony of reactivation of neuronal firing between neurons in the hippocampus and neocortex has been shown to occur during SWS (Qin, McNaughton, Skaggs & Barnes, 1997). Qin and colleagues recorded simultaneously from electrode arrays in the posterior parietal neocortex and in the CA1 area of the hippocampus during learning of a maze and during subsequent sleep sessions. The temporal patterns of firing of assemblies of cells that occurred during learning were also observed during slow wave sleep in both the hippocampus and the neocortex. This finding suggests that spindle waves may initiate coordinated interactions between the hippocampus and the neocortex during sleep, and emphasizes the potential importance of spindle activity for the consolidation of memories.

LTP and LTD.

Hebb (1949) proposed a mechanism in which memory traces could be formed through the development of "cell assemblies" which he conceived as networks of neurons linked together through the strengthening of synaptic connections. He proposed that such strengthening occurs when the presynaptic cell consistently takes part in causing the

firing of the postsynaptic cell. Repeated, concurrent intense pre-synaptic and post-synaptic activity is a condition that would be predicted to cause strengthening of a Hebbian synapse. This has since been confirmed empirically. One experimental demonstration that intense pre- and post synaptic activity can lead to the strengthening of real synapses is known as long-term synaptic potentiation (LTP; Bliss & Collingridge, 1993).

Long-term potentiation and its counterpart, long-term depression (LTD), are cellular models of memory formation. Long-term potentiation is a lasting enhancement of synaptic efficacy that can result from brief, high-frequency stimulation. Conversely, LTD is a lasting reduction of synaptic efficacy that can be induced by prolonged low-frequency stimulation. The induction of LTD by prolonged weak stimuli is consistent with an "anti Hebbian" rule that actively weakens the synapse following exposure to a prolonged weak tetanus (Stanton, 1996). This is because a prolonged weak stimulus can lead to presynaptic firing that is not consistently correlated with postsynaptic excitation. Bidirectional synaptic plasticity, defined by the potential for both increases and decreases in synaptic strength, is commonly believed to be important for learning and memory (Daoudal & Debanne, 2003; Froc & Racine, 2004, 2005).

Long-term potentiation is attractive as cellular models for memory, because it has the property of associativity (Bliss & Collingridge, 1993). Associativity can be demonstrated when weak synaptic inputs, that are not potentiated when stimulated alone, are potentiated in the presence of a tetanus (repeated stimulation trains) delivered to other stronger synaptic inputs. This feature of LTP is a cellular analogue of classical conditioning between a weak conditioned stimulus and a strong unconditioned stimulus

such that the weak input becomes more able to induce a postsynaptic response. This is consistent with the properties of the Hebbian synapse in which a weak synapse could be strengthened by increasing the degree of simultaneous pre- and postsynaptic activation.

Input specificity refers to a property of LTP in which the synaptic inputs that are strongly activated by tetanic stimulation become potentiated, while the synaptic inputs that were not activated by the tetanus do not express LTP. The property of input specificity means that strengthened synaptic responses following LTP can be specific to the tetanized input pathway, and that the potentiation of synaptic responses does not simply reflect an overall increase in postsynaptic excitability that enhances the strength of all synaptic inputs, or an overall and non-specific strengthening of synaptic inputs (e.g.s, Wang, Xu, Wu, Duan, & Poo, 2003; Wathey, Lytton, Jester, & Sejnowski, 1992). Long-term depression is also specific to activated synapses, so that activity-dependent weakening of stimulated pathways is not a result of a generalized, overall decrease in postsynaptic excitability or and overall, non-specific weakening of synapses (Linden, 1994). Input specificity makes LTP and LTD computationally more powerful because the roles of a neuron within multiple cell assemblies can be modified more selectively based on the patterns of input activation from other cells at a given time (McClelland and Rumelhart, 1986).

Long-term potentiation likely involves mechanisms similar to those involved in natural learning. If similar mechanisms do, in fact, underlie learning, then inducing LTP in a pathway should impair learning that requires use of the same pathway (Barnes, Jung, McNaughton, Korol, Andreasson, & Worley, 1994). In the hippocampal area there have been reports that saturating increases in synaptic strength with LTP can impair

subsequent learning (Barnes, et al., 1994) but this has not been a reliable finding (Moser & Moser, 1999). However, there is a report that olfactory memory can be disrupted by LTP induction. Stewart (2000) used an electromagnetic field to induce LTP before olfactory learning and found that potentiated rats explored a previously encountered juvenile rat longer than control animals. This suggests that the potentiated animals had impaired olfactory memory of the original encounter with the juvenile rat.

Motor learning of a skilled reaching task also depends on LTP-like mechanisms. Rioult-Pedotti, Friedman, Hess and Donoghue (1998) reported the first demonstration of naturally occurring LTP-like changes in the neocortex. The skilled reaching task they used requires fine motor control of forelimb reaching movements to reach for a pellet through a thin slot (Whishaw, O'Connor, & Dunnett, 1986). Animals were trained to reach with their preferred arm and strengthening of synaptic connections in the trained contra-lateral caudal forelimb area was expected. Following training, Rioult-Pedotti and colleagues (1998) sacrificed the animals and induced LTP *in vitro* in slices taken from both hemispheres. They found that less LTP was induced in the trained hemisphere versus the untrained hemisphere. This finding suggests that LTP-like synaptic changes had already occurred in the trained hemisphere during learning of the skilled-reaching task, and that the ability to induce further LTP was reduced. Monfils and Teskey (2004b) also used the skilled-reaching task to assess learning-induced changes in plasticity, except that they measured changes in synaptic plasticity *in vivo* during learning of the task. They found that LTP-like increases in field potential responses recorded from the caudal forelimb area occurred during a brief period during the acquisition of the task. These increases in synaptic strength could have contributed to the learning of the motor skill.

This adds to a reasonable body of evidence that suggests that LTP is as an appropriate cellular model for motor learning.

Changes in motor maps can also be used to assess neuronal plasticity during learning. The region of the brain responsible for forelimb motor activity, the caudal forelimb area, can increase in size following learning (Teskey, Monfils, VanderBerg, & Kleim, 2002; Monfils, VanderBerg, Kleim, & Teskey, 2004). The size of the caudal forelimb area of the sensorimotor cortex, assessed by mapping motor responses evoked by microstimulation, was also expanded following the induction of LTP (Monfils, et al., 2004). In addition, motor map expansion was found following epileptogenic stimulation of the cortex that also induces synaptic potentiation in the caudal forelimb area (Teskey et al., 2002). These changes in motor map representations provide further evidence that increased synaptic strength during motor learning on the skilled reaching task reflects alterations in the functional capabilities of the motor cortex.

Circuitry of the Sensorimotor Cortex in the Rat.

The sensorimotor cortex receives sensory inputs and plays a central role in generating voluntary motor behaviours. Somatosensory information from the posterior sensorimotor cortex is thought to help guide fine motor movements generated by commands within the anterior sensorimotor cortex (Rioult-Pedotti et al., 1998; 2000). In the experiments in this thesis, synaptic responses in the anterior sensorimotor cortex were evoked by stimulation of the corpus callosum in order to gain insights into synaptic plasticity that may affect motor behaviours. The components of the evoked synaptic field potentials recorded here must be interpreted in light of the circuitry of the sensorimotor cortex.

In the sensorimotor cortex, pyramidal cells are located in layers II/III, and in layers V and VI (see Figure 1, page 23; adapted from Figure 11, Chapman, Trepel, Ivanco, Froc, Wilson, & Racine, 1998). Layer IV is the main input layer for thalamic afferents in most primary *sensory* cortices, but layer IV is very thin in the sensorimotor cortex, and it is layers V and VI that receive the majority of subcortical inputs (Aroniadou & Keller, 1993). The main afferents to the sensorimotor cortex that are activated by stimulation of the corpus callosum arise in the ventroposterior lateral (VPL) nucleus of the thalamus and in the contralateral sensorimotor cortex. These afferents project mostly to layer V, but also to layers II/III (Aroniadou & Keller, 1993). In addition, stimulation of the corpus callosum activates layer V through antidromic activation of axons of Layer V pyramidal cells that send projections out of the cortex (Chapman et al., 1998). The synaptic inputs that mediate many of the features of responses recorded here are thought to be generated largely by activation of thalamic inputs from the VPL nucleus, activation of cortico-cortical inputs carried by the corpus callosum, and antidromic activation of layer V (Castro-Alamancos & Connors, 1996a; Chapman et al., 1998). In addition, stimulation of the corpus callosum is likely to activate cortico-striatal and cerebro-cerebellar circuits. Thus, in stimulating the anatomical structure of the corpus callosum which corresponds to subcortical white matter, stimulation pulses activate a variety of substrates in addition to transcallosal fibers that arise from the contralateral cortex. Sensory inputs from the somatosensory cortex can travel through layer I to the superficial layers of the anterior sensorimotor cortex, and plasticity in these inputs can contribute to motor learning (Rioult-Pedotti et al., 1998; 2000). However, layer I is not likely activated in the sensorimotor cortex following

electrical stimulation of the corpus callosum and is therefore not likely to be heavily involved in the experiments reported here.

Efferents from the sensorimotor cortex carry commands that generate motor output (Brooks, 1986). Sensorimotor cortex efferents project from deep layer cells to the contralateral cortex via the corpus callosum, to the thalamus, and to brainstem nuclei and the spinal cord. Corticospinal fibers pass through the midbrain and pons and decussate at the level of the medulla to descend to the lateral column of the spinal cord. These projections are the primary component of the lateral motor pathway that mediates voluntary movement under direct cortical control. The motor cortex also has projections to the reticular nucleus, superior colliculus and vestibular nuclei. This provides inputs to structures that lead to the ventromedial pathway which is responsible for involuntary control of movement. Because strengthening of synapses in layer V of the sensorimotor cortex during motor learning may affect outputs of layer V cells that contribute most directly to the lateral path, this type of plasticity could contribute most strongly to the regulation of voluntary, skilled motor behaviours.

This thesis deals with plasticity of evoked synaptic responses in the sensorimotor cortex generated by electrical activation of inputs from the corpus callosum. To quantify LTP and LTD in the sensorimotor cortex, synaptic field potentials evoked by brief pulses of electrical stimulation of the corpus callosum were recorded using a bipolar electrode with one tip located relatively superficially close to upper layer V or in layer III, and a second tip located deep in layers V or VI (Chapman et al., 1998). The origin of the evoked synaptic field potential responses are described here.

The electrode placements used in the experiments in this thesis were based on

results of a current source density (CSD) analysis obtained by Chapman and colleagues (1998) and are located close to the M1 hindlimb region of the motor cortex (Paxinos & Watson, 1998). Current source density analysis is a computational technique based on recordings of field potentials at multiple depths within the cortex, and it is used to obtain an estimate of the location of synaptic membrane currents within the cortex that generate evoked synaptic field potentials. They used CSD analysis to determine where the extracellular current sinks and sources are located for each of the components of the multi-component field potential responses recorded in the sensorimotor cortex following corpus callosum stimulation. Because of the lamina at which field potential components are well-known, it is possible to accurately place electrode tips by monitoring evoked responses during surgery (Werk & Chapman, 2003).

The earliest component of field potentials is an antidromically-activated spike with a latency of about 2 to 5 ms that is negative in the deep recording site (e.g., Figure 2, ♦, page 25; Chapman et al., 1998). The spike is generated by a current sink in the lower half of layer V that is thought to reflect the invasion of the cell bodies by the antidromic spike, and small matching current sources were also observed deep in layer VI and in upper layer V. The spike is observed most prominently in deep sites as a negative deflection in monopolar recordings. The initial spike component can be followed by two to eight other repetitive, small-amplitude spikes that suggest synchronous, repetitive firing in a large number of layer V pyramidal neurons (Chapman et al., 1998).

The next field potential component is an early superficial negative component which peaks at a latency of 6-8 ms (e.g., Figure 2, ●), and which is generated by an excitatory current sink in upper layer V (Chapman et al., 1998). It is referred to as a

superficial negative response because the component has a negative polarity at the more superficial electrode tip close to upper layer V. The negative early component reverses to a deep positive component and a current source deeper in layer VI. The early component is therefore thought to be generated by excitatory synaptic activation in upper layer V. Although the early component reflects monosynaptic activation, it can also be reduced in amplitude by spiking activity that can sometimes continue for latencies of up to about 10 ms to coincide with the peak of the early component (Chapman et al., 1998).

The later superficial negative component of field potential responses has a peak latency of about 14 to 20 ms and reverses to a positive polarity in upper layer V (e.g., Figure 2, ■). Thus, just as with the early component, the late component includes both superficial negative and deep positive responses. Current source density analysis showed that the late field potential component is generated by a current sink in layer V which suggests that it is mediated by excitatory synaptic activation within layer V (Chapman et al., 1998). This long-latency late component is thought to be largely due to polysynaptic activation of layer V neurons initiated by the monosynaptic activation of layer V neurons associated with the early component (Figure 1).

Previous experiments used "frequency of following" tests to help determine if the components of the field potentials are generated monosynaptically, polysynaptically or by antidromic activation (Chapman et al., 1998). Short trains of stimulation pulses are delivered at different frequencies in these tests, and a field potential component is said to successfully "follow" a stimulation frequency if each pulse in the train is able to evoke a distinct response. Monosynaptic components recorded from cortical and hippocampal areas follow frequencies up to about 100 Hz, polysynaptic components fail to follow at

much lower frequencies of near 40 to 50 Hz, and antidromic components typically follow high frequencies of up to 300 Hz (Berry & Pentreath, 1976; Laroche, Jay, & Thierry, 1990; Liu & Bilkey, 1996; Chapman & Racine, 1997). Chapman and colleagues (1998) conducted frequency of following tests in the sensorimotor cortex to determine the nature of the spike, early, and late components. They found that the spike followed frequencies of up to 320 Hz, the early component followed at 80 Hz, and the late component began to fail at 40 Hz and was completely eliminated at 80 Hz. These findings are consistent with the interpretation that the spike is generated by antidromic activation of layer V neurons, the early component is generated by monosynaptic activation, and that the late component is a polysynaptically activated synaptic response.

To record the early and late components effectively, and to monitor changes in these responses following induction of LTP or LTD, in the present studies, the superficial tip of the bipolar electrode was placed near upper layer V near the current sinks, and the deep tip was located in layer V and VI near the current sources (Figure 1). Differential recordings from the bipolar electrode reflects the voltage difference between the superficial and deep tips, and this recording method is useful to 1), eliminate noise common to both tips, and 2), amplify synaptic responses that reverse in polarity with cortical depth and have negative components near the superficial electrode tip and positive components near the deep electrode (Chapman et al., 1998).

Following LTP induction in the sensorimotor cortex, the bipolarly-recorded early component seems to *depress* as indicated by a reduction in the amplitude of the recorded field potential (Figure $2A_1$). Individual monopolar components from each tip of the bipolar electrode show, however, that the superficial negative responses typically become

larger, and the deep positive monopolar responses become smaller and less positive. The increase in the superficial negative response, close to the current sink, is consistent with LTP of monosynaptic inputs. The decrease in the response in deep sites accounts for the reduced bipolar recording and is thought to occur due to increases in simultaneous repetitive spiking in layer V neurons (Werk & Chapman, 2003). Therefore, reductions in the differentially recorded early component following LTP appear to reflect LTP of monosynaptic inputs that is masked by concurrent increases in cell firing.

In addition to changes in the early component, LTP induction leads to a striking increase in the amplitude of the late component (Figure $2A_1$). This increase in the size of the late component is thought to reflect an increase in the strength of polysynaptic activation of layer V neurons (Chapman et al., 1998).

Long-term depression is characterized by reductions in both the early and late synaptic components (Figure 2, A_2). However, the neuronal changes that lead to the reduction in the early component following LTD are thought to differ from those that produce the reduction in the early component following LTP. The induction of LTP is associated with a potentiation of the superficial negative response, and the reduced bipolar response results from concurrent increases in repetitive spiking in deep recording sites (Trepel & Racine, 1998; Werk & Chapman, 2003). Following LTD, however, there are reductions in the amplitudes of responses in both the superficial and deep recording sites (Froc, Chapman, Trepel, & Racine, 2000; Froc & Racine, 2005). The reduction in both superficial and deep responses observed following LTD induction is consistent with an active reduction in synaptic strength rather than increases in repetitive spiking.

Stimulation Parameters for LTP and LTD.

The timing and spacing of stimulation necessary for neocortical LTP (Figure 3, page 27) and LTD (Figure 7) is consistent with the idea that the neocortex requires repeated reactivations to support the slow consolidation of memory traces. Long-term potentiation is inducible in the sensorimotor cortex *in vivo*, but it requires repeated daily stimulation for a minimum of 5 days, and maximal effects are observable after 10 to 20 days (Chapman et al., 1998; Trepel & Racine, 1998). Neocortical LTD requires spaced and repeated daily stimulation as well. Effective stimulation trains have been delivered over a minimum of 4 to 5 days with maximal effects after 10 days of stimulation (Froc, et al., 2000). Therefore, spaced and repeated stimulation over many days is required to induce both LTP and LTD in the sensorimotor cortex in awake animals. It is useful to note, however, that a cholinergic potentiation of somatosensory evoked potentials can be obtained by pairing stimulation of the hindpaw and stimulation of the basal ganglia (Verdier & Dykes, 2001). These changes in somatosensory cortex in vivo are dependent on NMDA receptors and may therefore share mechanisms with LTP induced by intense stimulation.

The frequency and duration of stimulation determines whether LTP or LTD is induced. Long-term potentiation in the sensorimotor cortex is induced by short duration, high-frequency stimulation delivered to the corpus callosum daily (Trepel & Racine, 1998). High-frequency trains delivered at 300 Hz are typically used to induce LTP in the sensorimotor cortex, and are usually delivered in sets of eight pulses (Figure 3; Chapman et al., 1998; Trepel & Racine, 1998; Werk & Chapman, 2003). The minimum number of sets of pulses separated by 10 seconds and delivered daily is 10, but 20 sets of pulses

induce optimal amounts of LTP. Low-frequency stimulation used to induce LTD in the sensorimotor cortex is much more prolonged than high-frequency stimulation used for LTP induction. Fifteen minutes of daily low-frequency, 1 Hz, stimulation is typically used to induce LTD in the sensorimotor cortex (Froc et al., 2000).

Low-frequency and high-frequency trains activate different mechanisms which lead to either LTD or LTP induction, respectively (Stanton, 1996). The pattern of stimulation determines the amount of postsynaptic Ca^{2+} influx during synaptic activation, and the amount of Ca^{2+} influx determines whether LTP or LTD induction occurs. Calcium enters cells through voltage-gated Ca^{2+} channels and Ca^{2+}-permeable AMPA receptors, and also enters through N-methyl-*D*-aspartate receptors (NMDAR), which require both glutamate binding and depolarization of the postsynaptic cell (to remove the Mg^{2+}-block from the ionophore) to be opened. High-frequency stimulation used to induce LTP activates a large number of NMDAR's, which leads to high, but transient, Ca^{2+} entry. The transient Ca^{2+} entry leads to activation of protein kinases, which phosphorylate key sites on proteins. This phosphorylation changes the shape of the receptors and increases the receptor affinity for glutamate, and this enhances the strength of glutamate-mediated synaptic transmission. In contrast, low-frequency stimulation used to induce LTD activates a smaller number of NMDAR's, which leads to moderate Ca^{2+} influx prolonged over the duration of the trains. This prolonged and moderate Ca^{2+} influx leads to activation of protein phosphatases which cause de-phosphorylation, and reductions in synaptic strength (Stanton, 1996).

Rhythmicity and Short-Term Facilitation.

Theta activity (a quasi-sinusoidal rhythm of between 4 to 12 Hz) occurs in the

hippocampus during voluntary movement and in the neocortex in the form of large amplitude spindle waves during SWS and during awake immobility. Strong stimulation at this frequency effectively activates excitatory (Chapman, Perez, & Lacaille, 1998) and inhibitory circuits (Chapman & Lacaille, 1999; Perez, Chapman, Woodhall, Robitaille, & Lacaille, 1999) in the hippocampus. Theta-frequency activity may be important for activating cellular mechanisms that mediate learning in both the hippocampus and the neocortex because the synchronous activation of large numbers of neurons during each phase of the rhythm can promote synaptic plasticity. Synchronized activation of neurons during theta activity is thought to enhance synaptic plasticity by inducing strong postsynaptic depolarization which, in turn, enhances NMDAR-dependent Ca^{2+} influx in large numbers of neurons as described above (Stanton, 1996).

Stimulation patterned after the theta rhythm has been demonstrated to be particularly effective at inducing LTP. High frequency stimulation has traditionally been used to induce LTP in both the hippocampal formation (Lynch, Errington, & Bliss, 1985; Kairiss, Abraham, Bilkey, & Goddard, 1987; Weeks, Ivanco, Leboutillier, Racine & Petit, 2001) and the neocortex (Chapman et al., 1998; Monfils & Teskey, 2004b; Trepel & Racine, 1998). However, theta-patterned stimulation induces LTP effectively in many preparations (Kirkwood & Bear, 1994; Werk & Chapman, 2003), and is thought to be important for naturally-occurring synaptic plasticity. For example, high-frequency trains separated by a 100 ms inter-train interval, which is the period of the 10 Hz theta rhythm, are more effective at inducing LTP in the hippocampal formation (Hernandez, Navarro, Rodrigues, Martinez, & LeBaron, 2005; Huang & Kandel, 2005), and have also been shown to be more effective at inducing LTP in the visual cortex (Kirkwood & Bear,

1994) and in the sensorimotor cortex (Figure 2; Werk & Chapman, 2003) than high-frequency trains alone that are matched for the total number of stimulation pulses.

The thalamocortical augmenting response is an experimental phenomenon that also reflects the sensitivity of the sensorimotor cortex to theta-frequency activation. The augmenting response is a progressive enhancement of evoked potentials recorded in the sensorimotor cortex that occurs during theta-frequency stimulation of the ventroposterior lateral thalamic nucleus (Castro-Alamancos & Connors, 1996a, b). The response resembles naturally occurring spindle activity in both amplitude and frequency. Because spindle waves may be based in part on short-term synaptic facilitation effects that also mediate the augmenting response (Kandel & Buzsáki, 1997), it may be possible to use low-frequency stimulation of the corpus callosum or ventro-lateral thalamus to gain insights into spindle waves and into short-term facilitation effects that may contribute to learning and memory (Sejnowski & Destexhe, 2000; Gais et al., 2002).

Short-term facilitation that occurs during theta-patterned stimulation and the augmenting response is likely to increase activation of NMDARs and thereby promote synaptic plasticity (Addae & Stone, 1987; Castro-Alamancos & Connors, 1996a, b; Timofeev & Steriade, 1998; Trepel & Racine, 1998; Werk & Chapman, 2003). Long-term depression that is induced in the sensorimotor cortex with 1 Hz trains does not appear to be dependent on NMDAR activation (Froc & Racine, 2004). However, increased activation of NMDAR's during prolonged theta-patterned stimulation that causes short-term facilitation may induce greater LTD than 1 Hz trains and, therefore, enhance LTD induction.

Although 10 Hz activities in the frequency range of the theta rhythm and spindles

may play a critical role in endogenous learning, only 1 Hz trains have been used previously to induce LTD in the sensorimotor cortex (e.g.s, Froc et al., 2000; Froc & Racine, 2004, 2005). In the following chapter, three types of trains were compared in their ability to induce LTD. Because of increased short-term synaptic facilitation during the theta-patterned stimulation (Werk & Chapman, 2003), the two groups of animals that received theta-patterned stimulation trains were predicted to show greater LTD of both the early and late field potential components than animals that received conventional 1 Hz stimulation trains.

Figure 1. A schematic diagram of the major afferent and efferent pathways of the sensorimotor cortex adapted from Chapman and colleagues (1998; Figure 11). Excitatory synapses (◄) and inhibitory synapses (●) are illustrated by symbols. Afferent inputs project largely from thalamic and cortico-cortical afferents and outputs project to the motor cortex, thalamus and contra-lateral cortex. Electrode tip placements for the superficial and deep recording sites are illustrated by vertical bars. Bipolar recordings reflect the voltage difference between the superficial and deep recording tips.

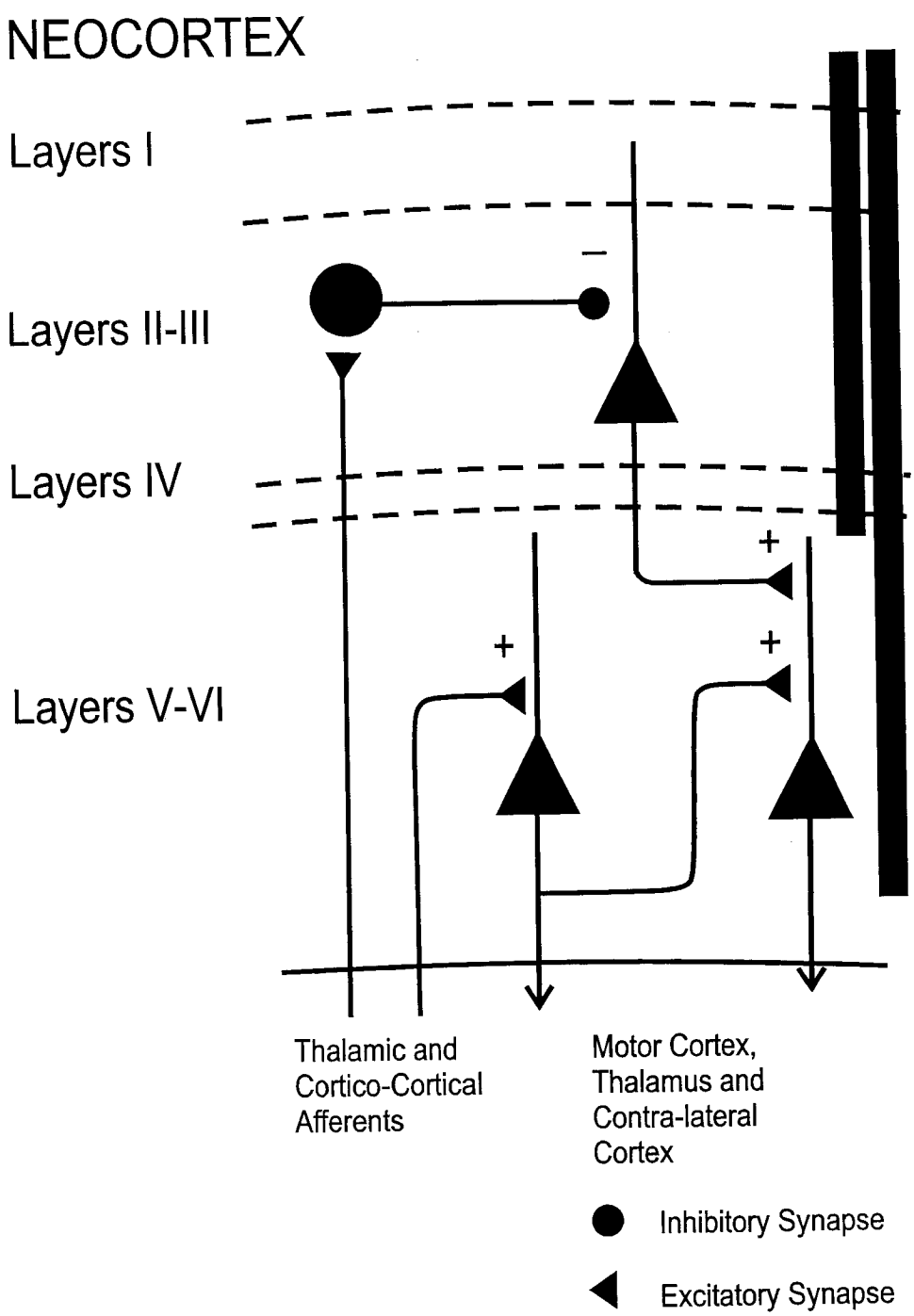

NEOCORTEX
Layers I
Layers II-III
Layers IV
Layers V-VI
–
+
+
+
Thalamic and Cortico-Cortical Afferents
Motor Cortex, Thalamus and Contra-lateral Cortex
Inhibitory Synapse
Excitatory Synapse

Figure 2. Representative sample traces of field potentials recorded before and after LTP (A_1) and LTD (A_2) of corpus callosum inputs to the sensorimotor cortex. Superficial, deep, and bipolar recordings are shown and latencies of the spike (♦) early (●) and late (■) components are illustrated by symbols. Traces recorded following LTP or LTD induction (dashed lines) are superimposed on baseline traces (solid lines).

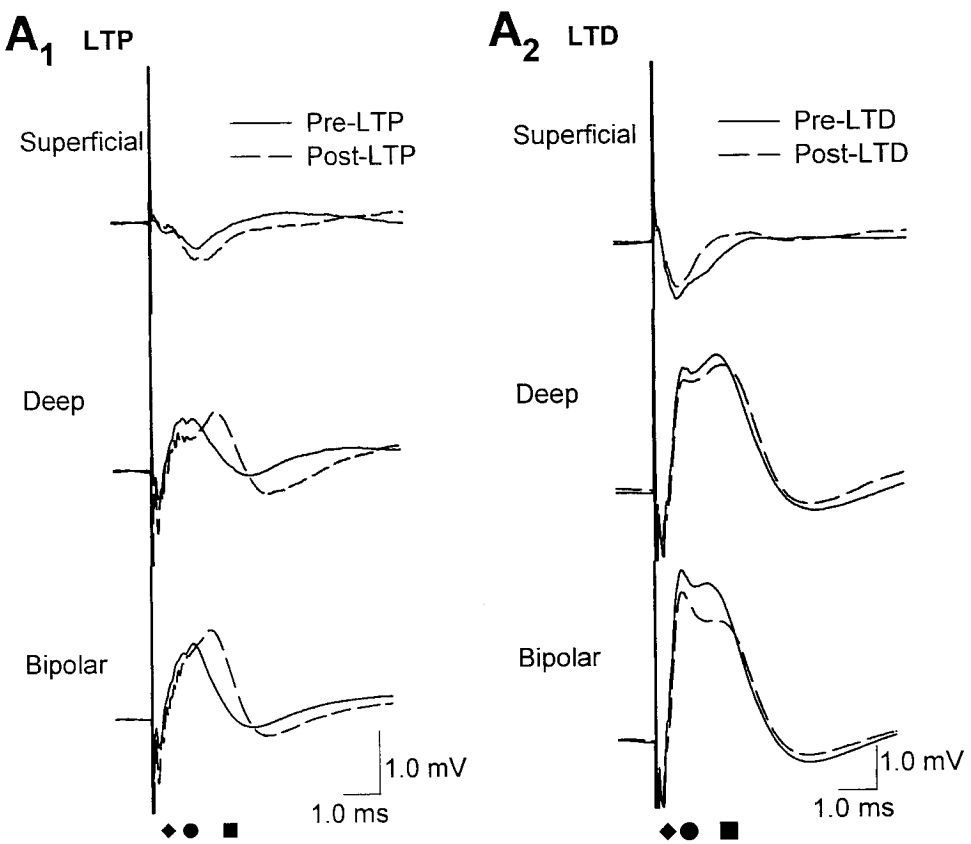
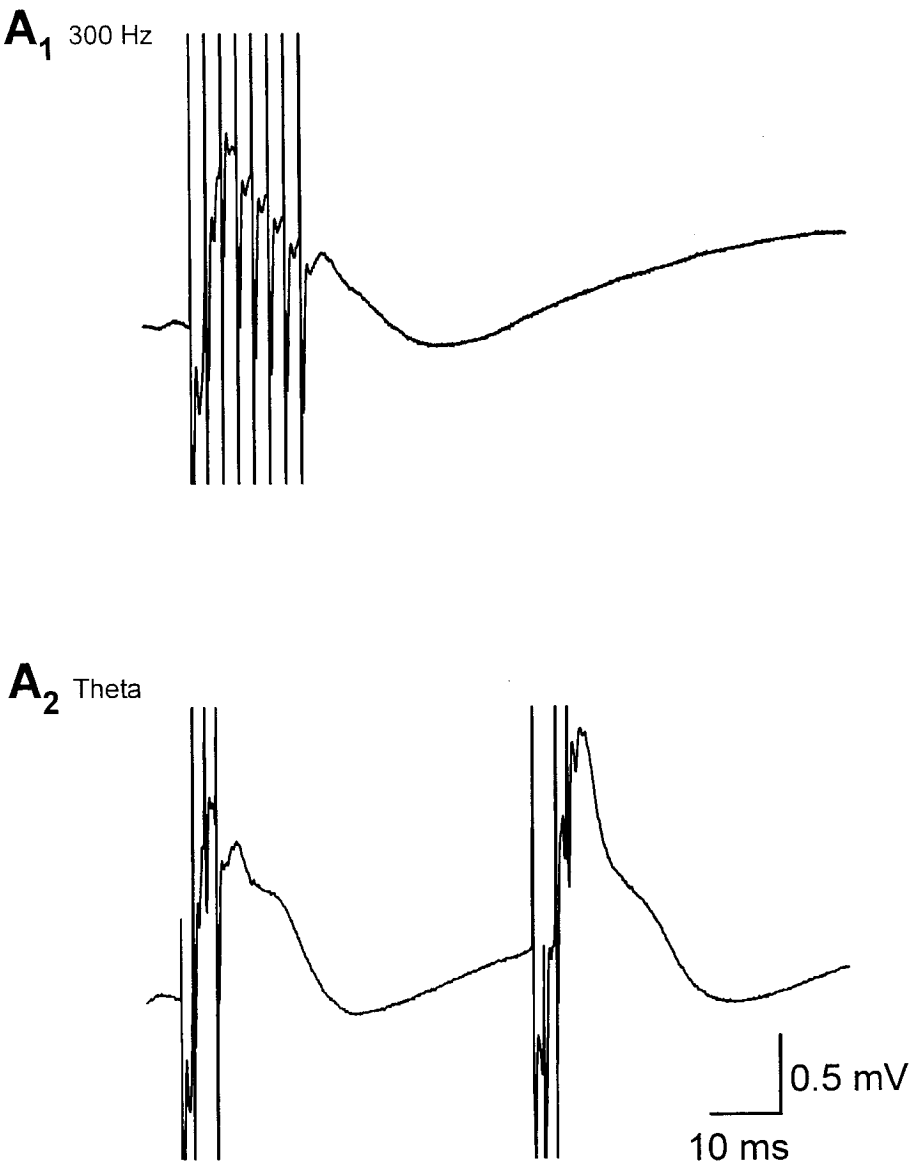

A1 LTP
Superficial
Pre-LTP
Post-LTP
Deep
Bipolar
1.0 mV
1.0 ms
A2 LTD
Superficial
Pre-LTD
Post-LTD
Deep
Bipolar
1.0 mV
1.0 ms

Figure 3. Examples of sensorimotor cortex responses evoked by patterns of high-frequency stimulation used to induce LTP. Field potentials evoked by 8-pulse high frequency trains (A_1), and by 8-pulse high-frequency trains separated by a 100 ms inter-train interval (A_2) are shown. Pulse intensity was 1000 μA.


A1 300 Hz
A2 Theta
0.5 mV
10 ms

CHAPTER TWO

LONG-TERM DEPRESSION IN THE SENSORIMOTOR CORTEX INDUCED BY REPEATED DELIVERY OF 10 HZ TRAINS *IN VIVO*

Christine M. Werk, Hannah S. Klein, Catherine, E. Nesbitt, and C. Andrew Chapman

Abstract

Memory consolidation in the neocortex is thought to be mediated in part by bi-directional modifications of synaptic strength. The sensorimotor cortex shows marked spontaneous activity near 10 Hz during both waking and sleep in the form of EEG spindle waves, and is also sensitive to electrical activation of inputs at 10 Hz. Induction of long-term synaptic depression (LTD) in corpus callosum inputs to layer V of the sensorimotor cortex of the awake, adult rat requires repeated low-frequency stimulation over many days. To determine if 10 Hz stimulation may facilitate the induction of LTD, we compared the amounts of LTD induced by conventional 1 Hz trains, repeated delivery of 450 pairs of stimulation pulses using a 100 ms interpulse interval, and 45 short, 2 second, 10 Hz trains. Each pattern was delivered daily for 10 days and was matched for total duration and number of pulses. Changes in synaptic responses were assessed by monitoring field potentials evoked by stimulation of the corpus callosum. A facilitation of synaptic responses in layer V was observed during delivery of both paired-pulse trains and 10 Hz trains. There was no significant difference in LTD induced by 1 Hz stimulation and repeated paired-pulse stimulation, but 10 Hz trains induced significantly greater LTD than 1 Hz trains in both the early monosynaptic and late polysynaptic field potential components. The effectiveness of short 10 Hz trains for the induction of LTD suggests that synchronous population activity at frequencies near 10 Hz such as spindle waves may contribute to endogenous synaptic depression in sensorimotor cortex.

Key words: thalamus, spindle, LTP, LTD, rat.

Bidirectional modifications in synaptic strength are a major mechanism through which long-term memories are thought to be consolidated. Long-term potentiation (LTP) is a strengthening of synaptic connections that can be induced by tetanic stimulation (Bliss & Collingridge, 1993; Malenka & Nicoll, 1999), and long-term depression (LTD) is a weakening of synaptic strength that is typically induced by prolonged low-frequency stimulation (Christie, Kerr, & Abraham, 1994; Abbott & Nelson, 2000; Kemp & Bashir, 2001). Both LTP and LTD have been used as models to examine cortical network activities and intracellular mechanisms that regulate activity-dependent changes in synaptic strength.

Although the neocortex is thought to be the major site for synaptic alterations that support memory consolidation, both LTP and LTD can be difficult to induce in neocortical areas. Neocortical LTP has been studied successfully with acute preparations and in juvenile animals *in vivo* (Wilson & Racine, 1983; Crair & Malenka, 1995; Heynen & Bear, 2001) and in *in vitro* preparations (Kirkwood & Bear, 1994; Hess, Aizenman & Donoghue, 1996). In the awake adult animal, however, LTP of corpus callosum inputs to layer V of the sensorimotor cortex is only induced gradually and trains used to induce LTP must be spaced and repeated over multiple days (Chapman et al., 1998; Trepel & Racine, 1998; Teskey & Valentine, 1998; Monfils et al., 2004). In this same preparation, LTD is also induced gradually, and daily delivery of prolonged 1 Hz trains leads to maximal LTD after only 4 to 10 days (Froc et al., 2000; Monfils & Teskey, 2004a; Froc & Racine, 2004, 2005).

Although 1 Hz trains are able to induce LTD in the sensorimotor cortex and have also been successful in slices from juvenile animals (Dudek & Bear, 1992; Mulkey &

Malenka, 1992), other patterns of stimulation can be more effective. Prolonged 1 Hz trains do not induce LTD reliably in adult slices in the entorhinal cortex, dentate gyrus, or CA1 region *in vitro* (Dudek & Bear, 1993, Kemp, McQueen, Faulkes, & Bashir, 2000; Wagner & Alger, 1995; Kourrich & Chapman, 2002) or *in vivo* (Doyle, Cullen, Rowan, & Anwyl, 1997; Errington, Bliss, Richter-Levin, Yenk, Doyere, & Laroche, 1995; Staubli & Scafaldi, 1997; Bouras & Chapman, 2003). However, low-frequency delivery of *pairs* of stimulation pulses, that repeatedly evoke paired-pulse inhibition effects, does induce LTD in the CA1 and dentate gyrus *in vivo* (Doyere, Errington, Laroche, & Bliss, 1996; Thiels, Barrionuevo, & Berger, 1994; Thiels, Xie, Yeckel, Barrionuevo, & Berger, 1996). The LTD induced by this pattern of stimulation is consistent with anti-Hebbian rules for synaptic change because synaptic responses evoked by the second pulse in each pair occur when postsynaptic neurons are inhibited. A similar pattern of paired-pulse stimulation modified to repeatedly induce paired-pulse *facilitation* induces NMDA-receptor dependent LTD in the entorhinal cortex (Bouras & Chapman, 2003; Kourrich & Chapman, 2002; see also Kemp & Bashir, 1999), and this suggests that paired stimulation could enhance synaptic plasticity in neocortical areas resistant to LTD.

The synchronization of cortical population activities at frequencies near 10 Hz may contribute to learning-related bi-directional synaptic plasticity (Singer, 1993; Steriade, 2001). The sensorimotor cortex is particularly responsive to stimulation at frequencies near 10 Hz (Contreras and Llinas, 2001; Werk & Chapman, 2003; Werk et al., 2005), and paired-pulse stimulation with a 100 ms interval (the period of 10 Hz activity) evokes a marked facilitation effect that reflects widespread activation within layer V (Castro-Alamancos & Connors 1996a, b; Chapman et al., 1998). In addition to

low-amplitude EEG activities near 10 Hz that fall within the alpha- (8-13 Hz) or theta-frequency (4-12 Hz) bands, extremely large-amplitude ~7-14 Hz spindle waves also occur in sensorimotor cortex for many seconds during both waking and slow-wave sleep (McCormick & Bal, 1997; Werk et al., 2005), and there is a growing interest in the role that sleep spindles may play in memory consolidation during sleep (Sejnowski & Destesxhe, 2000; Steriade & Timofeev, 2003). Consistent with this idea, we have shown that delivery of intense paired-trains at the period of the theta rhythm induces strong facilitation effects, and is much more effective than single high-frequency trains in inducing NMDA receptor-dependent LTP in the sensorimotor cortex *in vivo* (Werk & Chapman, 2003; see also Rioult-Pedotti et al., 2000).

Our finding that synaptic facilitation effects, which strongly activate the sensorimotor cortex, can enhance LTP induction raised the possibility that more moderate and prolonged activation that activates similar facilitation effects may contribute to the induction of long-term synaptic depression. We have therefore used chronic field potential recording techniques in the awake animal to compare LTD induction following either 1 Hz stimulation, repeated paired-pulse stimulation (PP-LFS) that induces synaptic facilitation (Bouras & Chapman, 2003), and more prolonged and intense 2 sec trains of 10 Hz stimulation (Werk & Chapman, 2003).

Method

Surgery

Male Long-Evans rats (300-400 g) were anesthetized with ketamine and xylazine (90 and 8 mg/kg, respectively) and placed in a stereotaxic frame. Bipolar, Teflon-coated,

stainless steel twisted-wire electrodes (125 μm exposed tips) were implanted in the right corpus callosum (anterior, 2.0; lateral, 2.0; ventral, 2.6 mm from pia) and sensorimotor cortex (anterior, 2.0; lateral, 4.0; ventral, 1.8 mm from bregma). Tip separation was 0.5 mm for the corpus callosum electrode and 1.2 mm for the cortical recording electrode. Vertical placements were adjusted to maximize the early monosynaptic component of field potentials and to minimize current thresholds. A stainless-steel jeweler's screw in the left posterior parietal bone served as the ground electrode. Electrode leads were connected to gold-plated pins mounted in a connector, and the assembly was stabilized using dental cement anchored to the skull with screws. Animals were housed individually on a 12 h light-dark cycle and handled every 2-3 days during a ~2 week recovery period. All experimental testing was conducted during the lights-on period.

Stimulation and Recording

Animals were habituated to a 30 x 40 x 30 cm Plexiglas chamber, and recordings were collected while animals were in a quiet resting state. Biphasic, square-wave constant current stimulation pulses (0.1 ms duration) were delivered to the corpus callosum using a linear stimulus isolation unit (A-M Systems, Model 2200) driven by a computer DAC channel (50 kHz). Evoked field potentials were filtered (0.1 Hz to 5 kHz passband), amplified (A-M Systems, Model 1700) and digitized at 20 kHz (12 bit) for storage on computer hard disk (Datawave Tech.).

Input/Output Testing. Input/output tests were used to monitor field potential responses evoked by a range of stimulus intensities (0, 100, 200, 400, 500, 600, 800, 1000 μA) and to assess changes in responses induced by LTD induction. Eight evoked

potentials were recorded and averaged at each pulse intensity. The inter-pulse interval was 10 s and pulses were delivered in ascending order with respect to intensity. Input/output tests were conducted every 2 days during a 1 week baseline period, 1 day following the fourth and tenth trains to induce LTD, and up to 3 weeks following LTD induction.

LTD Induction. Animals matched for morphology and amplitude of evoked responses were assigned to a control group or to groups that received different patterns of daily stimulation to induce LTD. Stimulation patterns were matched for overall duration (15 min) and number of pulses delivered. In an initial pilot study, trains were delivered daily for 4 days, and animals received either a single train of 900 pulses at 1 Hz (n= 5), or paired-pulse low-frequency stimulation (PP-LFS) consisting of a series of 450 pairs of pulses delivered once every 2 sec (n= 6). A 100 ms inter-pulse interval was used for pairs of pulses to cause a strong paired-pulse facilitation (Werk & Chapman, 2003; Castro-Alamancos & Connors, 1996a), and a pulse intensity of 800 μA was used. Although others have observed LTD after as little as 4 days (e.g., Froc et al., 2000), we did not obtain LTD in these initial experiments. Later experiments therefore used a 10-day LTD induction period and a stronger pulse intensity of 1000 μA. Stimulation consisted of either 1 Hz trains (n= 13), repeated paired-pulse stimulation (n= 13), or a series of 45 short, 2-second 10 Hz trains of pulses (n= 12). Each 10 Hz train consisted of 20 pulses at 10 Hz, and there was a 20 sec interval between trains. Control animals were handled in the same manner, but received no low-frequency stimulation (n= 14).

Data Analysis

The amplitudes of field potentials were measured at the peaks of the early (6-8 ms latency) monosynaptic component, and the late (17-18 ms latency) polysynaptic component. Differential recordings provide a larger amplitude, lower noise signal for analysis than monopolar recordings (Froc & Racine, 2005; Monfils & Teskey, 2004a). Mean input/output curves for each group were obtained after normalizing amplitudes of field potentials to the average responses to 1000 μA pulses during baseline tests. The development of depression effects across days was plotted using responses evoked by 1000 μA pulses. The late polysynaptic component was too small to provide stable measures in 2 to 4 animals in each group, and these responses were omitted from analyses. Mixed-design ANOVA's were used to analyze changes in synaptic responses recorded during the last baseline versus the first post-LTD test. Group by Day interactions, rather than Group by Day by Intensity interactions, are reported for effects that were similar across stimulation intensities. Short-term synaptic facilitation effects evoked during delivery of trains of pulses were assessed using responses recorded on the first day of LTD induction.

Results

Standard histological analysis (Werk & Chapman, 2003) showed electrodes located in target areas of the corpus callosum and sensorimotor cortex and no systematic difference in placements between groups. Field potentials evoked by stimulation of the corpus callosum (e.g.s, Figure 4 A) contained an initial deep-negative spike (1.07 ±0.12 mV peak amplitude), an early surface-negative component (1.95 ±0.15 mV), and a late surface-negative component (1.81 ±0.14 mV). These components reflect, respectively,

antidromic activation of deep layer V cells, fast monosynaptic activation of superficial layer V, and later polysynaptic activation of layer V (Chapman et al., 1998).

In our initial pilot study, four days of low-frequency stimulation with either 1 Hz trains or PP-LFS was not sufficient to induce LTD of either the early or late field potential components (data not shown), and responses remained close to baseline levels on the first day after LTD induction for both the 1 Hz (early: 95.8 ±11.9% of baseline; late: 93.1 ±20.1%) and PP-LFS (early: 95.6 ±12.4%; late: 97.9 ±19.5%) groups. Four days of low-frequency stimulation at 800 μA was therefore not sufficient for the induction of long-term depression.

LTD was induced successfully when the induction period was extended to 10 days and more intense, 1000 μA, stimulation pulses were used. Each of the three stimulation patterns induced LTD of both the early and late synaptic responses (Figure 4). The early component was reduced significantly following 1 Hz trains (82.4 ±5.4% of baseline; $F_{1,25}= 9.54, p< 0.01, n= 13$), PP-LFS (66.0 ±9.4%; $F_{1,25}= 8.84, p< 0.01; n= 13$), and 10 Hz trains (55.6 ±10.5%; $F_{6,144}= 5.11, p< 0.01; n= 12$) relative to the control group (101.7 ±3.9%; $n=14$). The late component was also reduced by 1 Hz trains (91.8 ±5.9%; $F_{1,21}= 7.98, p< 0.05$), PP-LFS (83.1 ±9.6%; $F_{6,126}= 2.23, p< 0.05$) and 10 Hz trains (60.9 ±13.6%; $F_{6,102}= 7.80, p< 0.01$), relative to the control group (112.2 ±5.2%).

All three types of stimulation induced LTD of both early and late components, but repeated stimulation with 10 Hz trains induced the largest LTD effects (Figures 4, 5). In comparison to LTD induced by 1 Hz trains, 10 Hz stimulation induced significantly greater LTD of both the early ($F_{6,138}= 4.04, p< 0.01$) and late ($F_{6,96}= 4.89, p< 0.01$) field potential components. There was also significantly greater LTD of the late component in

the 10 Hz group relative to the PP-LFS group ($F_{6,96}$= 2.64, p< 0.05), but there was no corresponding difference in the early component between these groups ($F_{6,138}$= 1.82, p= 0.10). The size of LTD effects in the 1 Hz and PP-LFS groups were quite similar, and showed no significant difference in LTD of either component (early: $F_{6,144}$= 0.62, p= 0.71; late: $F_{6,120}$= 1.41, p= 0.22).

Similar to previous results (Froc & Racine, 2004), the LTD effects observed here were very long lasting (Figure 6). Some recovery from LTD was observed after 2 to 3 weeks in groups that received PP-LFS and 10 Hz trains, but responses remained depressed three weeks following LTD induction in all groups. Responses were significantly depressed 3 weeks following 1 Hz trains (early: 77.2 ±7.9% of baseline; late: 77.3 ±10.9%), PP-LFS (early: 83.1 ±6.4%; late: 71.8 ±8.7%) and 10 Hz trains (early: 75.4 ±9.0%; late: 61.2 ±10.9%), and were stable in the control group (early: 102.3 ±6.5%; late: 110.9 ±7.5%) (early: F >2.32, p< 0.05; late: F >2.37, p <0.05).

Examples of responses evoked during stimulation to induce LTD are shown in Figure 7. Comparison of the first and last responses in 1 Hz trains reflected no significant change in the early or late components during the train (Figure 7A; t_{11}<1.13). Effects on both early and late components during paired-pulse stimulation (Figure 7B) were similar to those observed previously (Chapman et al., 1998) and showed a reduction in the early component (68.3 ±6.7% of the conditioning response, t_{11}=3.52, p<0.01) and a facilitation of the late component (201.1 ±32.7%, t_{11}=3.04, p<0.05). Responses evoked at the end of the 10 Hz trains (Figure 7C) also showed a reduction in the early component (46.5 ±8.8%, t_9=4.14, p<0.01) and a facilitation of the late component (197.1 ±53.1%, t_9=3.77, p<0.01), reflecting maintained effects throughout the 2 sec trains (see also Werk

& Chapman, 2003). The reduced early bipolar component results from increased repetitive spiking deep in layer V, and the enhanced late component reflects a facilitation of polysynaptic activation in layer V (Chapman et al., 1998). The PP-LFS and 10 Hz trains that are effective for inducing LTD are therefore also effective for inducing strong short-term changes in evoked responses.

Discussion

Recent findings have demonstrated that behavioural training on a skilled reaching task can strengthen synaptic responses in the sensorimotor cortex (Rioult-Pedotti et al., 2000; Monfils & Teskey, 2004b, Hodgson, Ji, Standish, Boyd-Hodgson, Henderson, & Racine, 2005; see also Cohen & Castro-Alamancos, 2005), and synaptic weakening is also thought to be critical for motor learning. The present study has used daily electrical stimulation of the corpus callosum in the awake rat to assess the relative effectiveness of stimulation at 10 Hz for induction of long-term synaptic depression in layer V of the sensorimotor cortex. The early component of field responses reflects monosynaptic activation of cortex, and the late component reflects spreading polysynaptic cortical activation mediated by horizontal layer V collaterals (Chapman et al., 1998). Plasticity in the late component reflects changes in long-range inter-columnar projections that may pay a central role in motor learning (Monfils & Teskey, 2004b). Similar to previous findings (Froc et al., 2000; Froc & Racine, 2004, 2005), daily delivery of 1 Hz trains for 10 days induced depression of both the early monosynaptic and late polysynaptic components of field potentials. Although repetitive paired-pulse stimulation is much more effective than 1 Hz trains at inducing LTD in both the CA1 and entorhinal cortex (Thiels et al, 1994, 1996; Doyere et al., 1996; Bouras & Chapman, 2003; Kourrich &

Chapman, 2003), the LTD induced here by PP-LFS was not significantly greater than that induced by 1 Hz trains. However, repeated delivery of 2-sec, 10 Hz trains induced significantly greater LTD of both the early and late components as compared to 1 Hz trains. We previously showed that paired *trains* delivered using a 100 ms interval (i.e., 10 Hz) that facilitates train-evoked responses induces LTP effectively (Werk & Chapman, 2003) and our present findings indicate that more moderate 10 Hz activity may contribute to endogenous mechanisms of lasting synaptic depression.

The sensorimotor cortex shows alpha activity at frequencies near 10 Hz, but spontaneous ~10 Hz spindle waves are much larger (1 to 5 mV), occur during both sleep and awake immobility, and can last for tens of seconds (Kandel & Buzsáki, 1997; Werk et al., 2005). Spindle waves are thought to contribute to memory consolidation during sleep (Sejnowski & Destesxhe, 2000; Steriade & Timofeev, 2003; Gais et al., 2002) by promoting corticohippocampal interactions (Siapas & Wilson, 1998; Buzsáki, 1998) and by enhancing synchronous activation of the cortex through powerful thalamocortical loops that activate the same layer V circuitry that has undergone LTD here (Kandel & Buzsáki, 1997; Chapman et al., 1998; Werk et al., 2005). The present findings support the idea that spindle waves contribute to lasting reductions in synaptic strength within layer V, and this is consistent with the sensitivity of the sensorimotor cortex to inputs at this frequency (Werk & Chapman, 2003; Contreras & Llinas, 2001). The 100 ms interval used here for PP-LFS corresponds to the period of 10 Hz and evokes a marked facilitation of layer V responses (Chapman et al., 1998). The 10 Hz trains used here also evoke a facilitation of layer V responses (Figure 7; Werk & Chapman, 2003), and increased activation of the cortex mediated by synaptic facilitation at 10 Hz may have

contributed to the LTD induced by paired-pulse and 10 Hz stimulation trains.

The receptor-mediated mechanisms that mediate the enhanced induction of LTD by 10 Hz trains are not known. In other pathways, PP-LFS induces greater LTD than 1 Hz stimulation and this has been attributed to increased NMDA glutamate receptor activation (Thiels et al., 1996; Doyere et al., 1996; Bouras & Chapman, 2003; Kourrich & Chapman, 2002). In sensorimotor cortex, paired-pulse facilitation in layer V is mediated in part by long-latency IPSPs that increase cortical excitability by activating a hyperpolarization-activated cationic current (Castro-Alamancos & Connors, 1996b). We have also shown previously that the facilitation during 2 sec 10 Hz trains is mediated in part by activation of NMDA receptors (Werk & Chapman, 2003). However, LTD in sensorimotor cortex induced by 1 Hz stimulation is not blocked by NMDA receptor antagonism, and this has led to the proposal that this form of LTD is dependent on activation of metabotropic glutamate receptors and/or voltage-gated calcium channels (Froc & Racine, 2004). Similarly Kemp & Bashir (1999) induced LTD in CA1 slices using PP-LFS that induces paired-pulse facilitation, as is the case here, and found that it is dependent on activation of AMPA and metabotropic glutamate receptors. It is not known, however, what receptor populations account for the enhanced LTD following 10 Hz versus 1 Hz trains.

It is not clear what mechanisms mediate the enhanced expression of LTD. The stimulation patterns were matched for total number of pulses and duration of stimulation, so differences between the LTD induced cannot be explained by an artefact related to total amount of stimulation such as tissue damage, and must be dependent on the temporal pattern of stimulation. It has been shown previously that LTD in this

preparation induced by 1 Hz stimulation is reversible by high-frequency stimulation, indicating that synapses remain viable and show continued capacity for plasticity (Froc & Racine, 2005). Morphological alterations in layer V neurons may contribute to the expression of LTD, however, and it has been shown recently that LTD induced by 1 Hz stimulation is associated with reduced dendritic length and spine density in layer V (Monfils & Teskey, 2004a).

We previously found that intense paired trains delivered at a 100 ms interval promotes LTP induction (Werk & Chapman, 2003), and we now show that more moderate and prolonged 10 Hz stimulation with single pulses enhances induction of LTD. This raises the question of how potentiation or depression of individual synapses may be regulated during endogenous 10 Hz rhythms. The induction of LTD versus LTP is thought to be determined by the intensity of postsynaptic activation, but the threshold for LTP induction can also be modified by the recent history of neuronal activation (Christie & Abraham, 1992; Bear & Abraham, 1996). Even though large areas of cortex are activated synchronously during thalamocortical spindle waves, there is likely to be substantial variability in the strength of synaptic inputs and recent histories of neuronal activation. Patterns of cortical activity during waking, and during cortico-hippocampal interactions in waking or sleep, could therefore affect the magnitude and direction of synaptic changes at individual synapses during 10 Hz rhythms such as spindle waves.

Figure 4. Repeated delivery of 10 Hz trains of stimulation led to the greatest LTD of both the early monosynaptic and late polysynaptic components of field potential responses in the sensorimotor cortex *in vivo*. Traces in A show field potentials recorded before (solid lines) and 1 day after (dashed lines) LTD induction in each group (calibration bars: 10 ms, 0.5 mV). Symbols indicate the latencies of the early (circle) and late (square) field potential components. Input/output curves are shown for the early (B_1) and late (B_2) components of responses recorded on the last baseline day (Pre LTD) and on the first follow-up day (Post LTD). Note the stability of responses in control animals (left panels).

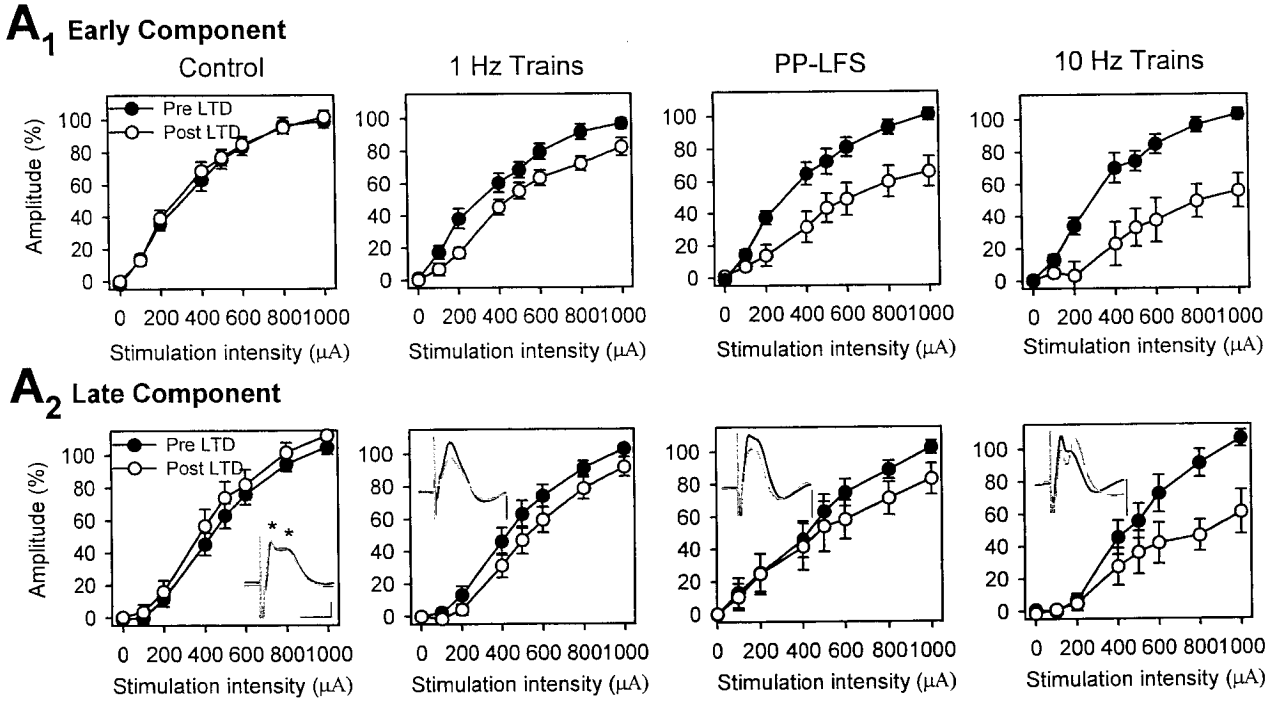

A1 Early Component
Control
1 Hz Trains
PP-LFS
10 Hz Trains
Pre LTD
Post LTD
Amplitude (%)
Stimulation intensity (μA)
A2 Late Component

Figure 5. Cumulative probability distributions show the range of LTD effects observed following repeated delivery of 1 Hz trains, low-frequency paired-pulse stimulation (PP-LFS) and 10 Hz trains in comparison to responses observed in the control group. Data reflect amplitudes of responses to the largest stimulation intensity on the first day following LTD induction, and are taken from the same groups as shown in Figure 4. Note the spontaneous increases in responses observed in several control animals. The relative size of the spontaneous increases is larger for the late component, but these relative increases may be exaggerated by the relatively small baseline amplitudes for these responses.

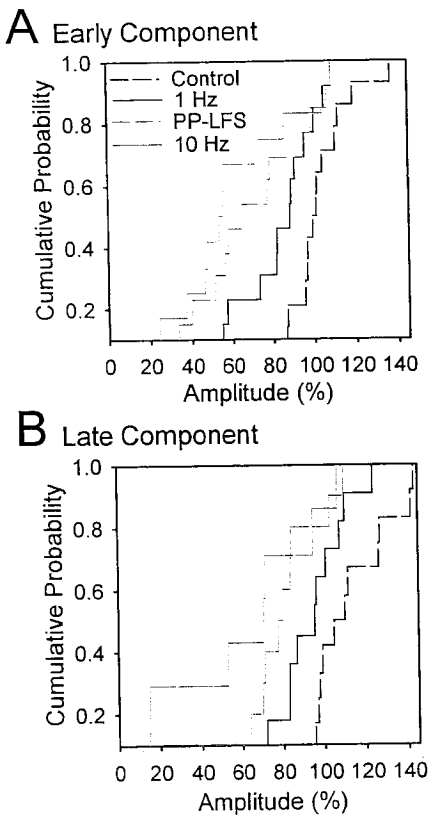

A Early Component
Control
1 Hz
PP-LFS
10 Hz
Cumulative Probability
1.0
0.8
0.6
0.4
0.2
0 20 40 60 80 100 120 140
Amplitude (%)
B Late Component
Cumulative Probability
1.0
0.8
0.6
0.4
0.2
0 20 40 60 80 100 120 140
Amplitude (%)

Figure 6. The development and maintenance of LTD in early (A_1) and late (A_2) field potential components in each of the four groups. Following a one week baseline period, daily tetanization using either 1 Hz trains, repeated paired-pulse stimulation (PP-LFS), or short 10 Hz trains led to lasting depression of both early and late synaptic components that lasted at least 3 weeks. Ten Hz trains induced the greatest amount of LTD of both the early and late components one day after the last train, but there were no significant differences between groups in the amount of LTD observed after 3 weeks. Data are shown for responses to 1000μA pulses, and vertical dashed lines indicate the duration of the LTD induction period.

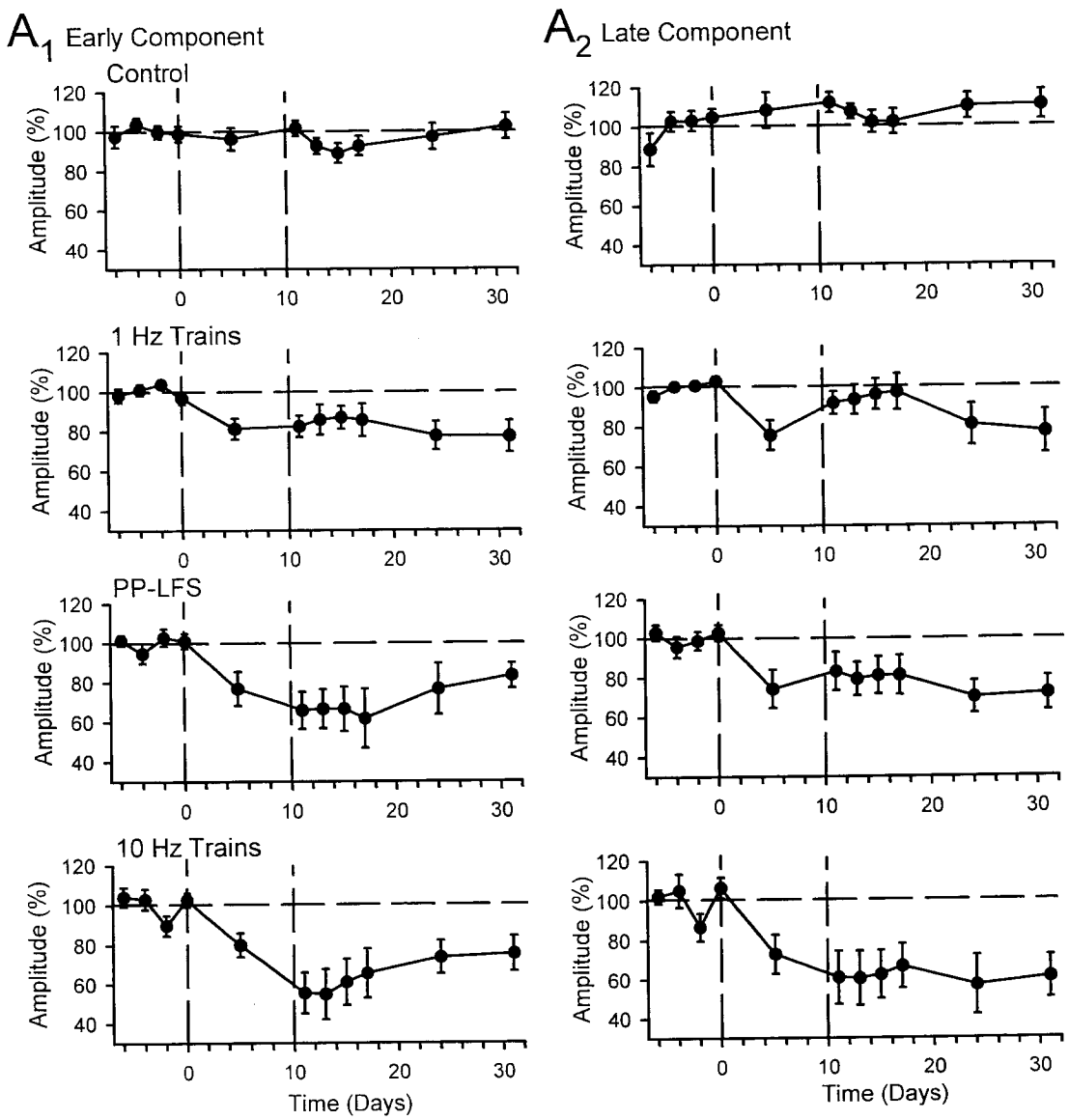
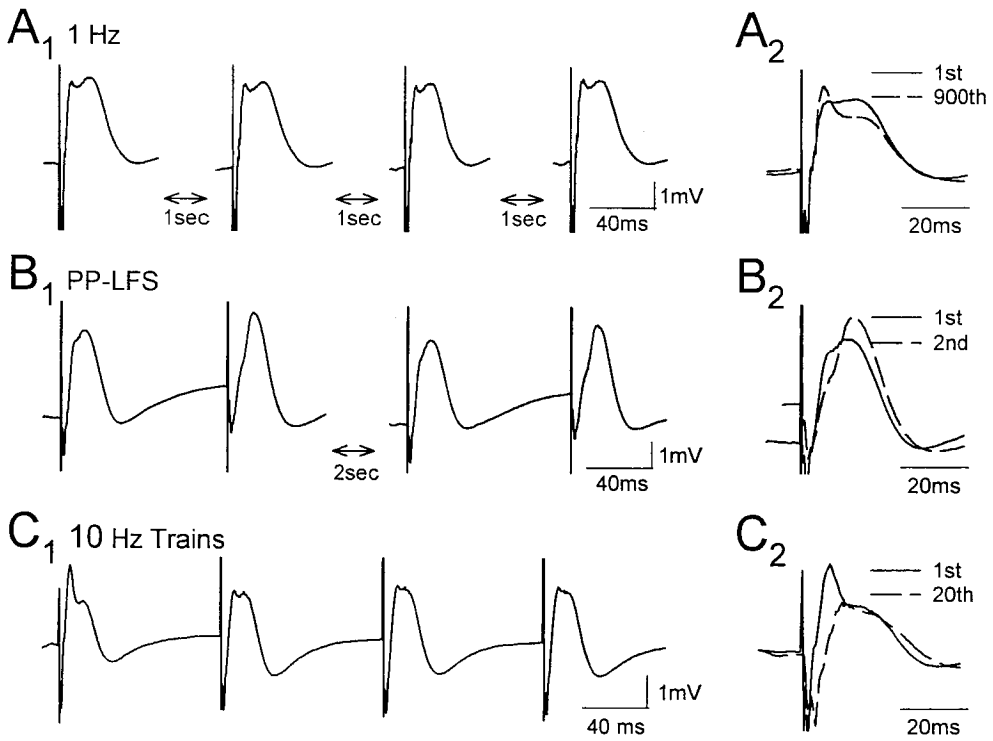

A1 Early Component
A2 Late Component
Control
1 Hz Trains
PP-LFS
10 Hz Trains
Amplitude (%)
120
100
80
60
40
0
10
20
30
Time (Days)

Figure 7. Examples of sensorimotor cortex responses evoked by patterns of low-frequency stimulation used to induce LTD. Field potentials evoked by the first four pulses in a 1 Hz stimulation train (A_1), the first and last pairs of pulses in a train of PP-LFS (B_1), and by the first 4 pulses in a 2-sec 10 Hz train (C_1) are shown. Comparison of responses to the first and last pulses in a 1 Hz, 15 min train reflects no significant facilitation of early or late responses during the train (A_1). In contrast, comparison of responses to the first and second pulses in the first pulse-pair during PP-LFS (B_2), and comparison of the first and 20^{th} pulses in 10 Hz trains (C_2) reflected significant reductions in the early component, and significant enhancements of the late component. Pulse intensity was 1000 μA.


A1 1 Hz
1sec
1sec
1sec
1mV
40ms
A2
1st
900th
20ms
B1 PP-LFS
2sec
1mV
40ms
B2
1st
2nd
20ms
C1 10 Hz Trains
1mV
40 ms
C2
1st
20th
20ms

CHAPTER THREE

INDUCTION OF LONG-TERM POTENTIATION LEADS TO INCREASED RELIABILITY OF EVOKED NEOCORTICAL SPINDLES *IN VIVO*

C.M. Werk, V.L. Harbour and C.A. Chapman

In the previous chapter, stimulation patterned after the theta rhythm was shown to be particularly effective at inducing LTD in the sensorimotor cortex *in vivo*. In particular, stimulation patterned after spindle waves (2 sec, 10 Hz trains) was more effective at inducing LTD than either standard 1 Hz trains or PP-LFS. Spindle waves are believed to contribute to memory consolidation (Sejnowski & Destexhe, 2000). Increases in spindle activity have been observed at the central scalp location Cz (which corresponds roughly to the motor cortex) during learning of paired-associates in humans (Gais, et al., 2002) and in the cortex following exposure to a novel object in rats (Shiffleholtz & Aldenhoff, 2002), however the mechanisms of learning-related changes in spindle activity are still unclear. Long-term potentiation and LTD are models of memory consolidation and provide a means by which to investigate the relationships between spindle activity and learning and memory.

Spindle activity may be affected by changes in synaptic plasticity, and LTP could therefore be used as a model for assessing the relationship between spindle activity and changes in synaptic strength that have been linked to motor learning. To test if spindle activity can be altered by LTP induction, the ability of single pulses to evoke spindle waves before, during, and after the induction of LTP in the sensorimotor cortex was assessed. An enhancement of spindle activity was expected following the induction of LTP because the generation of spindles is known to involve activation of layer V.

Abstract

Large amplitude electroencephalographic spindle waves (7-14 Hz) occur spontaneously in the neocortex during both sleep and awake immobility, and it has been proposed that synchronous neuronal activation during spindles may contribute to learning-related synaptic plasticity. Spindles can also be evoked in the sensorimotor cortex by electrical stimulation of cortical or thalamic inputs in the rat. To determine if strengthening cortical synapses can affect the initiation and maintenance of electrically evoked spindles, stimulation pulses were delivered at a range of intensities to the corpus callosum or ventrolateral thalamus in the awake rat before and after the induction of long-term potentiation (LTP) by tetanization of the corpus callosum. The morphology of evoked spindles was similar to that of naturally occurring spindles. Spindles were evoked less reliably during slow-wave sleep than during waking, and this was correlated with smaller synaptic responses during slow-wave sleep. Similar to previous findings, daily tetanization of the corpus callosum for 15 days decreased the early component and increased the late component of synaptic responses evoked by corpus callosum stimulation, but did not significantly affect synaptic responses evoked by thalamic stimulation. Similarly, LTP induction increased the reliability with which low-intensity corpus callosum stimulation evoked spindles, but increases in spindles evoked by thalamic stimulation were not significant. Synaptic potentiation and the increased reliability of spindles developed with a similar time-course over the 15-day LTP induction period. These results reflect strong correlations between the strength of cortical layer V activation and the initiation of spindles in the sensorimotor cortex, and support the idea that monosynaptic and polysynaptic horizontal collaterals of layer V neurons

play a significant role in the initiation of spindles.

Key Words: Sensorimotor cortex, layer V, thalamus, slow-wave sleep, high-voltage spindle, rat.

Rapid eye movement sleep has received much attention with respect to the neurobiological mechanisms through which sleep may contribute to memory consolidation (Smith, 1996; Graves, Pack & Abel, 2001), but there has been a growing interest in the role that sleep spindles and slow-wave sleep (SWS) may also play (Sejnowski & Detexhe, 2000; Steriade & Timofeev, 2003). Sleep spindles are large amplitude electroencephalographic (EEG) oscillations that occur during SWS at frequencies of ~7 to 14 Hz (Kandel & Buzsáki, 1997; Sejnowski & Destesxhe, 2000), and high-voltage spindles also occur at similar frequencies during awake immobility (Shaw, 2004). Both types of spindles are generated by thalamocortical interactions (Kandel & Buzsáki, 1997) but, while high-voltage spindles occur at amplitudes up to 5 mV and can last up to 60 seconds (Buzsáki, Bickford, Ponomareff, Thal, Mandel & Gage, 1988, Buzsáki, 1991), sleep spindles are usually smaller (~1 mV) and generally last 1 to 3 seconds (Steriade, McCormick, & Sejnowski, 1993).

Neocortical spindles promote cortico-hippocampal interactions during sleep that may contribute to memory consolidation. Spindle density was increased in humans following a declarative learning task and a spatial mapping task (Gais et al., 2002; Meier-Koll, Bussmann, Schmidt, & Neuschwander, 1999), and increases in spindles have been correlated positively with task performance (Gais et al., 2002). Exposure to a novel object also results in an increase in spindle activity in rats during the first two hours of sleep (Schiffelholz & Aldenhoff, 2002). Neocortical spindles tend to co-occur with hippocampal sharp wave-ripples during sleep (Siapas & Wilson, 1998; Sirota et al., 2003) and the timing of these events suggests that the output of deep layer neurons during spindles play a strong role in initiating hippocampal sharp waves (Sirota et al., 2003). In

turn, hippocampal output associated with sharp waves may modulate subsequent spindle waves, and contribute to a dynamic interplay between hippocampus and cortex during sleep.

Neocortical spindles are driven by thalamocortical neurons that fire bursts in response to rhythmic input from inhibitory thalamic reticular neurons. Both inhibitory reticular neurons and thalamocortical neurons can fire bursts driven by low-threshold calcium spikes, and reciprocal connections between these cells maintain rhythmic thalamic input to the cortex during spindles (Steriade, Deschenes, Domich & Mulle, 1985; McCormick & Bal, 1997; Buzsáki, 1998; Steriade & Timofeev, 2003). In addition to thalamic inputs, intracortical connections play a major role in cortical activation during spindles and, in addition to a prominent layer IV sink, current sinks and cell discharge are also observed in deep layers (Kandel & Buzsáki, 1997). Activation within layer V is strongly augmented by stimulation at spindle-frequencies (Castro-Alamancos & Connors, 1996a; Kohn, Metz, Buibrera, Tommerdahl, & Whitsel, 2000; Werk & Chapman, 2003) and feedback from the deep cortical layers to the thalamus is known to enhance the generation of spindles (Contreras & Steriade, 1996; Contreras et al., 1997).

In a previous study of LTP of corpus callosum inputs to the sensorimotor cortex of awake rats, we informally observed an increase in the amount of spindles evoked after LTP induction (Werk & Chapman, 2003). The induction of LTP in the sensorimotor cortex requires spaced and repeated stimulation over multiple days (Chapman et al., 1998; Teskey & Valentine, 1998; Trepel & Racine, 1998), and we found that pairs of trains delivered at a 100 ms interval were more effective at potentiating monosynaptic and polysynaptic responses in layer V than matched high-frequency trains (Werk &

Chapman, 2003). This finding is consistent with the idea that ~10 Hz spindle activity contributes to synaptic plasticity in horizontal layer V connections, and our informal observation that spindles were increased suggested that a potentiation of horizontal layer V collaterals could play a strong role in the initiation and propagation of spindles. The current study was aimed at determining if evoked spindles in awake rats are altered by the induction of LTP in the sensorimotor cortex. Spindles occur spontaneously during waking and sleep states, but single stimulation pulses delivered to the corpus callosum or ventrolateral thalamus can also be used to evoke spindles in a more controlled manner (Kandel & Buzsáki, 1997). Evoked spindles were recorded before and after LTP induction, and results were compared to recordings obtained from a control group. The induction of LTP is known to strengthen polysynaptic layer V responses, and a corresponding enhancement of the amplitude or reliability of spindle waves would support the idea that these connections likely play a major role in distributing cortical activation involved in spindles.

Method

Surgery

Male Long-Evans rats (300-400 g) were anesthetized with ketamine and xylazine (90 and 8 mg/kg, respectively) and placed in a stereotaxic frame. Bipolar, Teflon-coated, stainless steel twisted-wire electrodes (125 μm exposed tips) were implanted in the right corpus callosum (anterior 2.0, lateral 2.0, ventral, 2.6 mm from bregma), sensorimotor cortex (A 2.0, L 4.0, V 1.8 mm from pia), and the ventrolateral nucleus of the thalamus (A -2.3, L 1.8, V 5.4 mm from pia). Tip separation was 0.5 mm for electrodes in the corpus callosum and thalamus, and 1.2 mm for recording electrodes in the sensorimotor

cortex. Vertical placements of electrodes were adjusted to maximize the early component of field potentials and to minimize current thresholds. A stainless-steel jeweler's screw in the left posterior parietal bone served as the ground electrode. Electrode leads were connected to gold-plated pins, mounted in a connector, and the assembly was embedded in dental cement and anchored to the skull with screws. Animals were housed individually on a 12 h light-dark cycle and handled every 2 to 3 days during a ~2 week recovery period. All experimental testing was conducted during the lights-on period.

Stimulation and Recording

Animals were habituated to a 30 x 40 x 30 cm Plexiglas chamber with a wire grid floor. Biphasic, square-wave constant current stimulation pulses (0.1 ms duration) were delivered to either the corpus callosum or ventrolateral nucleus of the thalamus using a computer DAC channel (50 kHz) and a stimulation isolation unit (A-M Systems, Model 2200). EEG activity and evoked field potentials were filtered (0.1 Hz to 5 kHz passband), amplified (A-M Systems, Model 1700) and digitized at 20 kHz (12 bit) for storage on computer hard disk (Datawave Tech.).

Spontaneous intracorticographic activity, here called EEG activity, was recorded from the sensorimotor cortex in order to characterize spindles during awake and sleeping states. Three hours of EEG were recorded during awake immobility and again when the animal was sleeping on two separate occasions at least one week prior to LTP induction (n= 6). Animals were considered sleeping if they were immobile with head resting, eyes at least partially closed, with periodic large-amplitude low-frequency EEG activity. Spontaneous spindles recorded during awake and sleeping states were compared to

evoked spindles, and were assessed for the frequency, amplitude, and morphology of spindles in superficial and deep recordings sites.

To determine if spindles evoked during sleep differ from those evoked during waking, single stimulation pulses were also delivered while animals were asleep. Ten samples of evoked spindles were obtained using single stimulation pulses delivered to the corpus callosum and ventrolateral thalamus using a stimulation intensity adjusted to evoke a response of about 60% of the maximum (n= 6). Responses were compared to those evoked by the same stimulus intensity on the same day while the animal was awake.

Input-output tests were used to assess the strength of evoked synaptic responses and to monitor spindles evoked by various intensities of stimulation of the corpus callosum or ventrolateral thalamus. During each test, 10 evoked potentials were recorded and averaged at each of 7 test-pulse intensities (50, 100, 200, 400, 600, 800, 1000 μA). Pulses were delivered in ascending order with respect to intensity using a 20 s interpulse interval. The reliability with which each pulse intensity evoked spindles, and the duration of each spindle were measured. Input-output tests were conducted every 2 days during a one-week baseline period and every 5 days during a 15-day LTP induction period. Further tests were conducted every week for 2 weeks following LTP induction.

LTP Induction. Animals were matched for the amplitude and morphology of evoked responses and were assigned to either a control group or to an LTP group (n= 9/group). LTP was induced using 20 pairs of trains (pairs of 4-pulse, 300 Hz trains separated by 100 ms) for 15 days, with a pulse intensity of 800 μA and a 10 s interval between pairs of trains. Control animals were handled in the same manner, but received

no tetanization. Mean input/output curves were used to illustrate changes in field potentials and spindles as a function of pulse intensity during the last baseline day and one day after LTP. The development of potentiation effects across days was plotted using responses to 100 μA pulses in the corpus callosum, and 200 μA in the thalamus.

Data Analysis. Amplitudes of field potential components were measured at the peaks of the early monosynaptic (6-8 ms), and late polysynaptic (17-18 ms) components of field potentials in differential recordings (Chapman et al., 1998). Mean input-output curves were obtained after standardizing amplitudes of responses in each animal to the average baseline response to 1000 μA pulses. The reliability with which spindles were evoked was expressed as the percentage of trials that evoked a spindle at each stimulus intensity. Mixed-design ANOVA's were used to analyze synaptic potentiation effects, and to assess changes in the peak amplitude, duration and reliability of evoked spindles. Comparisons of the duration and reliability of evoked spindles, and the early and late synaptic responses evoked during awake immobility and SWS were compared using the dependent samples *t*-test.

Results

Standard histological analysis (Werk & Chapman, 2003) showed that electrode placements were located in target areas in the corpus callosum, sensorimotor cortex and ventrolateral thalamus. There was no systematic difference in electrode placements between groups. Field potentials evoked by stimulation of either the corpus callosum (CC) or the ventrolateral thalamus (e.g. Figure $10A_1$, inset) contained an initial deep-negative spike (CC, 0.90 ±0.18 mV; thalamus, 0.59 ±0.15 mV peak amplitude), an early surface-negative component (CC, 1.14 ±0.25 mV; thalamus, 1.74 ±0.58 mV), and a late

surface-negative component (CC, 1.50 ±0.26 mV; thalamus, 0.72 ±0.17 mV). These components reflect antidromic activation of deep layer V cells, fast monosynaptic activation in superficial layer V close to the superficial recording electrode, and later, polysynaptic activation that produce a current sink in superficial layer V (Chapman et al., 1998).

Spontaneous and Evoked Spindles

Spontaneous spindles recorded in the sensorimotor cortex during awake immobility and slow-wave sleep (SWS) were similar to spindles evoked by single stimulation pulses delivered to either the corpus callosum or ventrolateral thalamus (Figure 8). The morphology of spontaneous recordings in superficial and deep sites were consistent with previous findings (Kandel & Buzsáki, 1997) and superficial sites showed a large negative spike, and deep recordings showed a more sinusoidal waveform with a smaller spike component. Deep recordings were least sinusoidal during SWS in which there was typically a prominent positive spike (Figure 8B). Although the amplitude of spindles was somewhat larger during waking (1-5 mV) as compared to SWS (1-2 mV), the frequency of spindles was very similar between states (7-12 Hz).

Stimulation of the corpus callosum evoked spindles on a smaller percentage of trials during SWS (55.0 ±9.9%) than when animals were awake (79.2 ±6.2%; t_6 =2.62, p <0.05; Figure 9A). Similarly, the amplitudes of early and late synaptic responses were also smaller during SWS (early, 0.46 ±0.02 mV; late, 0.63 ±0.09 mV) than during awake immobility (Figure 9B; early, 0.78 ±0.12 mV; t_5 =2.71, p <0.05; late, 1.01 ±0.26 mV; t_5 =6.68, p <0.01). There was no difference, however, in the effectiveness of thalamic stimulation for evoking spindles (Figure 9A_2; SWS, 60.0 ±9.3%; awake, 50.0 ±17.4%).

In addition, SWS did not result in any significant reduction in the amplitudes of the early (SWS, 0.44 ±0.07 mV; awake, 1.17 ±0.48 mV) or late (SWS, 0.73 ±0.31 mV; awake, 0.43 ±0.07 mV) synaptic responses evoked by thalamic stimulation (Figure 9B). Although the mean duration of spindles was shorter during SWS than awake immobility, there was no significant difference between the duration of spindles evoked during SWS versus awake immobility (Figure $9A_3$). In summary, sleep reduced synaptic responses evoked by either corpus callosum or thalamic stimulation, but the changes were significant only for corpus callosum stimulation which also showed a significant reduction in the reliability with which spindles were evoked.

Potentiation of Spindles and Synaptic Responses

In responses evoked by corpus callosum stimulation, the induction of LTP caused a significant reduction of the early component (36.5 ±17.3% of baseline; Group x Day x Intensity interaction, $F_{48,768}$ =1.46, p <0.05), and a large increase in the late component (230.91 ±56.36% of baseline at 200μA; $F_{48,768}$ =1.97, p <0.001; n= 9). In contrast, there was no significant change in the early or late components evoked by thalamic inputs (n= 6) in potentiated animals, and no significant changes in responses evoked by either site in control animals (Figure 10A; n= 9).

The induction of LTP by corpus callosum stimulation led to an increase in the reliability with which low-intensity stimulation of the corpus callosum evoked spindles. The reliability with which single pulses evoked spindles increased with pulse intensity for both stimulation sites ($F_{6,102}$ =233.75, p <0.001), but stimulation of the corpus callosum was more effective than thalamic stimulation for inducing spindles; the maximal likelihood of evoking a spindle with high intensity (1000μA) pulses was 60.83 ±12.07%

for the corpus callosum and 30.00 ±9.97% for the thalamus (Figure 10B). The induction of LTP significantly increased the ability of low-intensity (100 to 200 μA) corpus callosum pulses to induce spindles ($F_{6,54}$ =8.13, p <0.001) but there was no such increase in the control group ($F_{6,54}$ =1.02, p =0.42). An increase in the ability of thalamic stimulation to evoke spindles in potentiated animals was not statistically significant (Figure 8B; $F_{6,48}$ =0.69, p =0.66). There was no change in the ability of thalamic stimulation to evoke spindles in control animals ($F_{6,54}$ =1.49, p =0.20). The frequency of spindles evoked by corpus callosum or thalamic stimulation did not change significantly with LTP induction (CC pre, 9.22 ±0.24 Hz, post 9.17 ±0.26 Hz; thalamus 9.32 ±0.67 Hz, post 8.87 ±0.35 Hz). Similarly, measures taken before and after LTP were also stable for spindle amplitude (CC pre, 1.82 ±0.36 mV, post 1.65 ±0.23 mV; thalamus 1.54 ±0.25 mV, post 1.79 ±0.25 mV) and duration (CC pre, 1.56 ±0.22 s, post 1.78 ±0.19 s; thalamus 3.04 ±0.67 s, post 2.12 ±0.47 s).

The increase in the reliability with which spindles were evoked showed a similar time-course over the 15-day LTP induction period as the changes in synaptic responses (Figure 11). Consistent with previous findings (Trepel & Racine, 1998; Werk & Chapman, 2003), LTP was slow to develop, and increases in synaptic responses that were observed after 10 days were further potentiated after 15 days (Figure 11A). The enhanced reliability of spindles was also first observed after 10 days, and was maintained throughout the testing period (Figure 11B).

Discussion

Similar to our previous findings (Werk & Chapman, 2003), we have found that intense daily tetanization of the corpus callosum patterned after the period of the theta

rhythm is effective at inducing LTP in the sensorimotor cortex. LTP induced changes in both the early and late components of synaptic responses (Figures 10 and 11). The reduction in the early component results from enhanced repetitive firing of layer V neurons that masks growth in the concurrent, monosynaptically generated current sink, and the increase in the late component reflects enhanced polysynaptic activation of layer V cells (Chapman et al., 1998; Trepel & Racine, 1998). Here, we have found that the induction of LTP in corpus callosum inputs to the sensorimotor cortex results in an increase in the reliability with which moderate-intensity pulses evoke spindles. Neocortical spindles are known to be mediated in part by layer V (Buzsáki et al., 1988; Kandel & Buzsáki, 1997; Steriade, 2001), and the potentiation of layer V responses here was associated with enhanced initiation of spindle waves. This is the first report we are aware of showing that the induction of LTP can alter the initiation of an EEG rhythm.

The effects of LTP on evoked spindles likely reflect alterations of the same circuitry that mediates spontaneous spindles. Spindles recorded during sleep and waking states show similar profiles in surface and deep recordings (Kandel & Buzsáki, 1997), and are also similar to electrically evoked spindles (Figure 8; Buzsáki, 1998). High-voltage spindles occur in seizure-prone areas of the brain (Clemens & Menes, 2000), and alterations of thalamic and neocortical circuitry that generates spindles can promote seizures (Drinkenburg, vanLuistelaar, vanSchaijk, & Coenen, 1993; Kandel & Buzsáki, 1993; McCormick& Bal, 1994; Steriade, 2001). In addition to a normal role in learning-related synaptic plasticity, then, changes in the generation of spindles could also contribute to cortical seizure activity. It has also been suggested that spindle-like oscillations in the rat may be analogous to the smaller amplitude *mu* rhythm in humans

(Wiest & Nicolelis, 2003), and alterations in evoked spindles could therefore reflect plasticity in cortical circuitry associated with behavioral readiness.

Potentiation of Spindles and Synaptic Responses

The reliability with which spindles were evoked was closely related to the amplitude of synaptic potentials evoked by the same pulses. Stronger stimulation pulses evoked larger synaptic responses, and also induced spindles more reliably up to a maximum reliability of approximately 60% of trials in which a spindle was evoked by each stimulation pulse (Figure 10). Similarly, stimulation of the corpus callosum induced a larger late component and was also more effective at inducing spindles than was thalamic stimulation. In contrast, the amplitude, frequency and duration of spindles was relatively unaffected by stimulus intensity. Therefore, although the initial activation of spindles is highly dependent upon stimulus intensity, once the circuitry that mediates spindles is activated, it appears to function very similarly whether activated by large or small pulses.

A major finding of this study was that synaptic potentiation increased the ability of corpus callosum stimulation to initiate spindles. The increased reliability of spindles occurred with a time-course that was similar to that of changes in synaptic responses over the 15-day LTP induction period (Figure 11), suggesting that the larger synaptic responses activated spindles more effectively. The effect occurred at the same moderate intensities for which potentiation of the late component was largest (compare Figure $10A_2$ and B). At higher intensities there may be a ceiling effect on the extent to which strong synaptic inputs induce spindles.

Although tetanization of the corpus callosum potentiated synaptic responses and enhanced spindles evoked by corpus callosum stimulation, changes in synaptic responses and spindles evoked by *thalamic* stimulation were not significant in the same animals. Both the corpus callosum and the ventrolateral nucleus activate layer V monosynaptically (Castro-Alamancos & Connors, 1996a; Chapman et al., 1998). This suggests that the enhancement of spindles evoked by corpus callosum stimulation may have resulted from an input-specific potentiation of monosynaptic inputs. In addition, stimulation of the corpus callosum leads to stronger antidromic activation, and stronger polysynaptic responses in layer V, as compared to thalamic inputs. The incidence of spontaneous spindles increases with age in the rat (Radek, Curzon, & Decker, 1994), but the increases observed here occurred within a two-week period, and did not occur in control animals (Figure 11).

Mechanisms of Enhanced Spindles

Tetanization of the corpus callosum may have altered the generation of spindles through a number of different sites. Spindles are maintained by an interplay between excitatory thalamocortical afferents and corticothalamic projections that terminate on thalamocortical cells and on inhibitory neurons of the nucleus reticularis. The timing of thalamocortical volleys and the frequency of spindles is regulated by rebound excitation of projection cells as they recover from synchronous inhibitory inputs from nucleus reticularis (McCormick & Bal, 1997; Steriade & Amzica, 1998; Sejnowski & Destexhe, 2000). The enhanced initiation of spindles observed here could be due to a potentiation of corticothalamic afferents or the synchronization of activity within nucleus reticularis

(Steriade et al., 1993; Contreras & Steriade 1996), but it is not known if these effects can be induced by tetanization of the corpus callosum *in vivo*.

Potentiation of corpus callosum inputs to the cortex is the most likely reason for the enhanced initiation of spindles, but activation of corpus callosum inputs leads to both monosynaptic and polysynaptic activation of layer V that may have promoted spindles. LTP was associated with a reduction in the early component associated with enhanced monosynaptic inputs, and with an enhancement of the late component that reflects polysynaptic activation of layer V (Chapman et al., 1998). Changes in both of these components were observed at the same moderate intensities at which increases in the initiation of spindles were observed. Potentiation of the monosynaptic response could increase synchronous firing within the cortex and increase the size of the resulting corticothalamic volley, and potentiation of polysynaptic responses could affect even larger areas of cortex. Both of these potentiation effects could contribute to enhanced spindles, and could be mediated in part by the marked increase in dendritic arborization within layer V neurons that occurs following synaptic potentiation in this preparation (Teskey et al., 2002; Monfils et al., 2004).

Theta-frequency EEG activity is observed in the hippocampus of the awake rat during exploratory behaviour, and neuronal synchronization during theta activity is thought to contribute to mechanisms of synaptic plasticity (Staubli & Lynch, 1987; Kahana, Seelig, & Madsen, 2001; Buzsáki, 2002). Similarly, it has been suggested that sleep spindles may synchronize cortical activity and contribute to learning-related synaptic plasticity during sleep (Sejnowski & Destexhe, 2000; Steriade, 2001) and layer V neurons may play an important role in this cortical plasticity. Layer V is activated

strongly during spindles (Kandel & Buzsáki, 1997), it contains axonal collaterals that project both within layer V and to thalamic and subcortical targets, and low-frequency stimulation with single pulses causes a potentiation of layer V responses that is at least partly NMDA receptor-mediated (Castro-Alamancos & Connors, 1996a; Werk & Chapman, 2003). Synchronization of layer V activation by sleep spindles may therefore contribute to long-range synchronization of cortical activation that could contribute to neural mechanisms that mediate memory consolidation during sleep. The present findings have shown that the circuitry that initiates spindles following corpus callosum stimulation can be modified by LTP induction. The amplitude and frequency of evoked spindles remained constant, but LTP increased the ability of stimulation to initiate spindles. This suggests that the circuitry mediating spindles is quite robust, but that synaptic plasticity within layer V can shape the subsequent timing and initiation of spindle activity.

Figure 8. Spontaneous spindles in the sensorimotor cortex recorded in the awake animal (A) and during slow-wave sleep (SWS, B) are compared to examples of spindles evoked by single stimulation pulses delivered to either the corpus callosum (C) or ventrolateral thalamus (D). Spindles were recorded using a bipolar electrode, and monopolar recordings from superficial and deep sites are shown together with the differential recording as indicated in A. The horizontal bar (-) indicates the region that has been expanded at the right of each trace. Evoked spindles were similar in frequency, amplitude and morphology to spontaneous spindles.

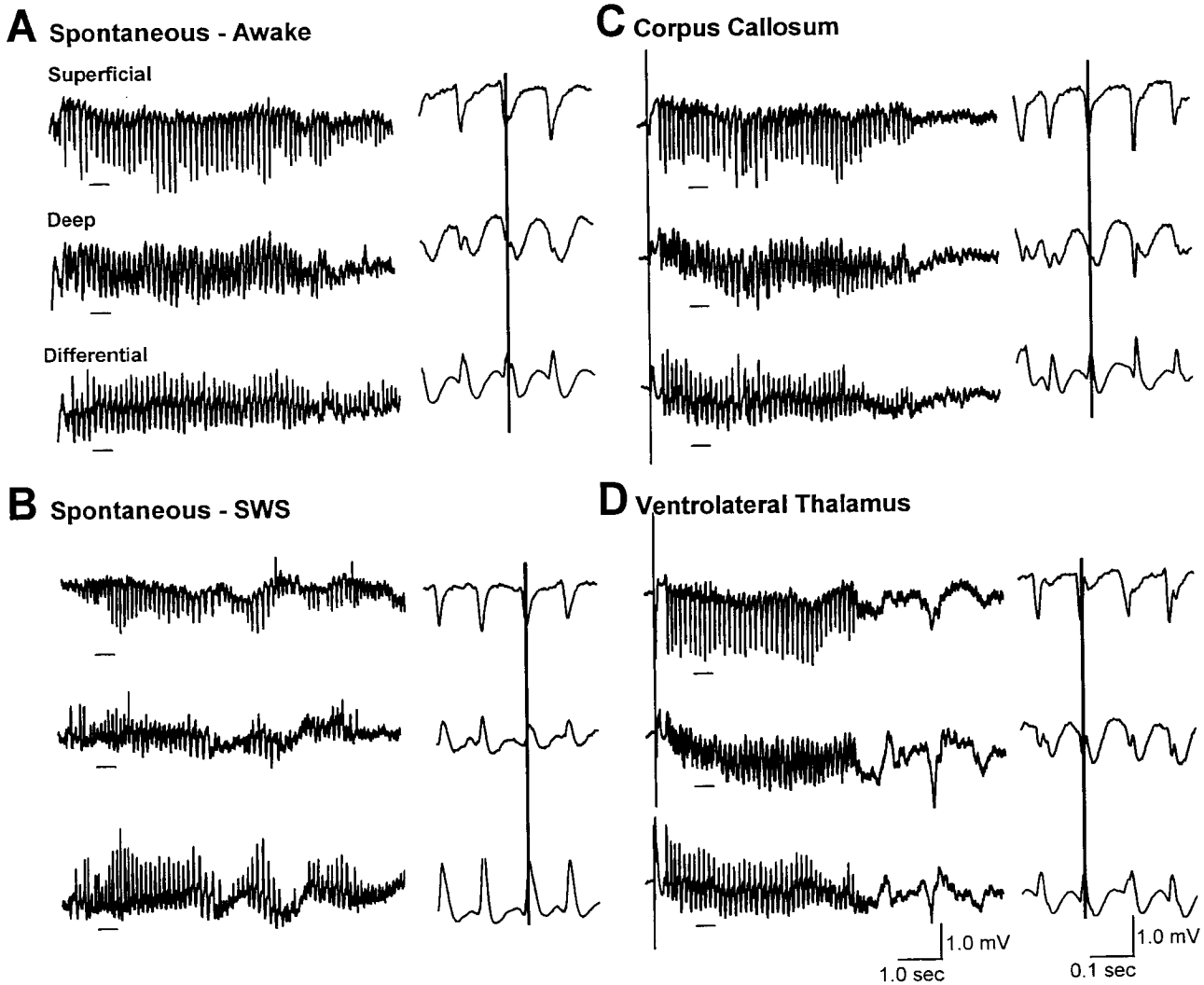
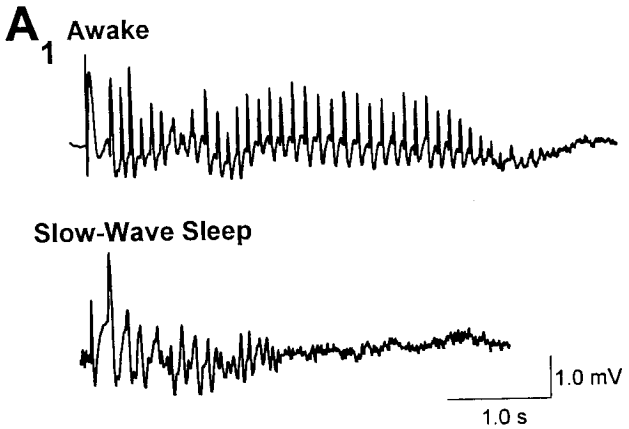
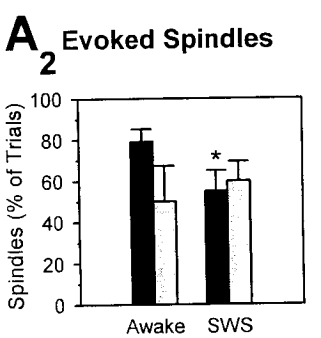
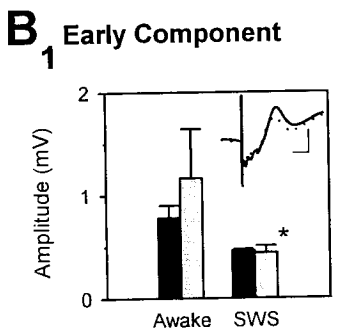
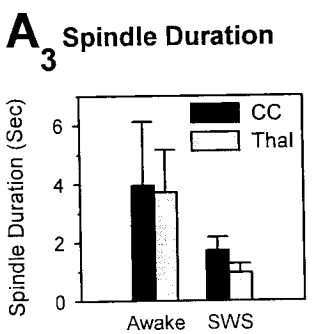

A Spontaneous - Awake
Superficial
Deep
Differential
B Spontaneous - SWS
C Corpus Callosum
D Ventrolateral Thalamus
1.0 mV
1.0 sec
1.0 mV
0.1 sec

Figure 9. Stimulation of the corpus callosum induces smaller synaptic responses during slow-wave sleep (SWS) than during waking, and also induces spindles less reliably. Spindles were evoked by single mid-intensity pulses during both awake immobility and SWS (A_1), but spindles evoked by corpus callosum stimulation occurred less reliably during SWS (A_2, black bars). Asterisks indicate significant differences from the awake condition ($p < .05$). The early (B_1) and late (B_2) synaptic responses evoked by corpus callosum stimulation were also significantly smaller during SWS. The inset in B_1 shows examples of responses evoked by stimulation of the thalamus and recorded while the animal was awake (solid line) and during SWS (dotted line; calibration 10 ms, 0.5 mV) For thalamic inputs (grey bars), there was no change in the reliability of evoked spindles (A_2) and reductions in synaptic responses were not statistically significant (B). Also note that, although the duration of spindles evoked by either pathway (A_3) was reduced during SWS, this reduction was not statistically significant.


A1 Awake
Slow-Wave Sleep
1.0 mV
1.0 s


A2 Evoked Spindles
Spindles (% of Trials)
100
80
60
40
20
0
*
Awake
SWS


B1 Early Component
Amplitude (mV)
2
1
0
*
Awake
SWS


A3 Spindle Duration
Spindle Duration (Sec)
6
4
2
0
CC
Thal
Awake
SWS

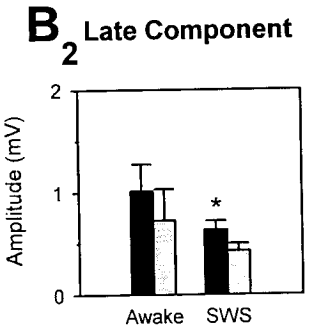

B2 Late Component
Amplitude (mV)
2
1
0
*
Awake
SWS

Figure 9. The induction of LTP leads to an increase in the reliability of evoked spindles. Mean input/output curves obtained before and after LTP induction are compared for the early monosynaptic (A_1), and late polysynaptic (A_2) components of field potential responses. Insets in A_1 show representative field potentials recorded before (solid lines) and after (dashed lines) LTP induction for both the LTP and Control groups (calibration: 10 ms, 1 mV). Induction of LTP caused a significant reduction of the early component and an enhancement of the late component evoked by corpus callosum stimulation (CC), but did not induce significant changes in responses to thalamic stimulation. Note the stability of responses in the control conditions. Low-intensity corpus callosum stimulation induced spindles much more reliably following LTP (B). The increase in the reliability of spindles evoked by thalamic stimulation was not statistically significant.

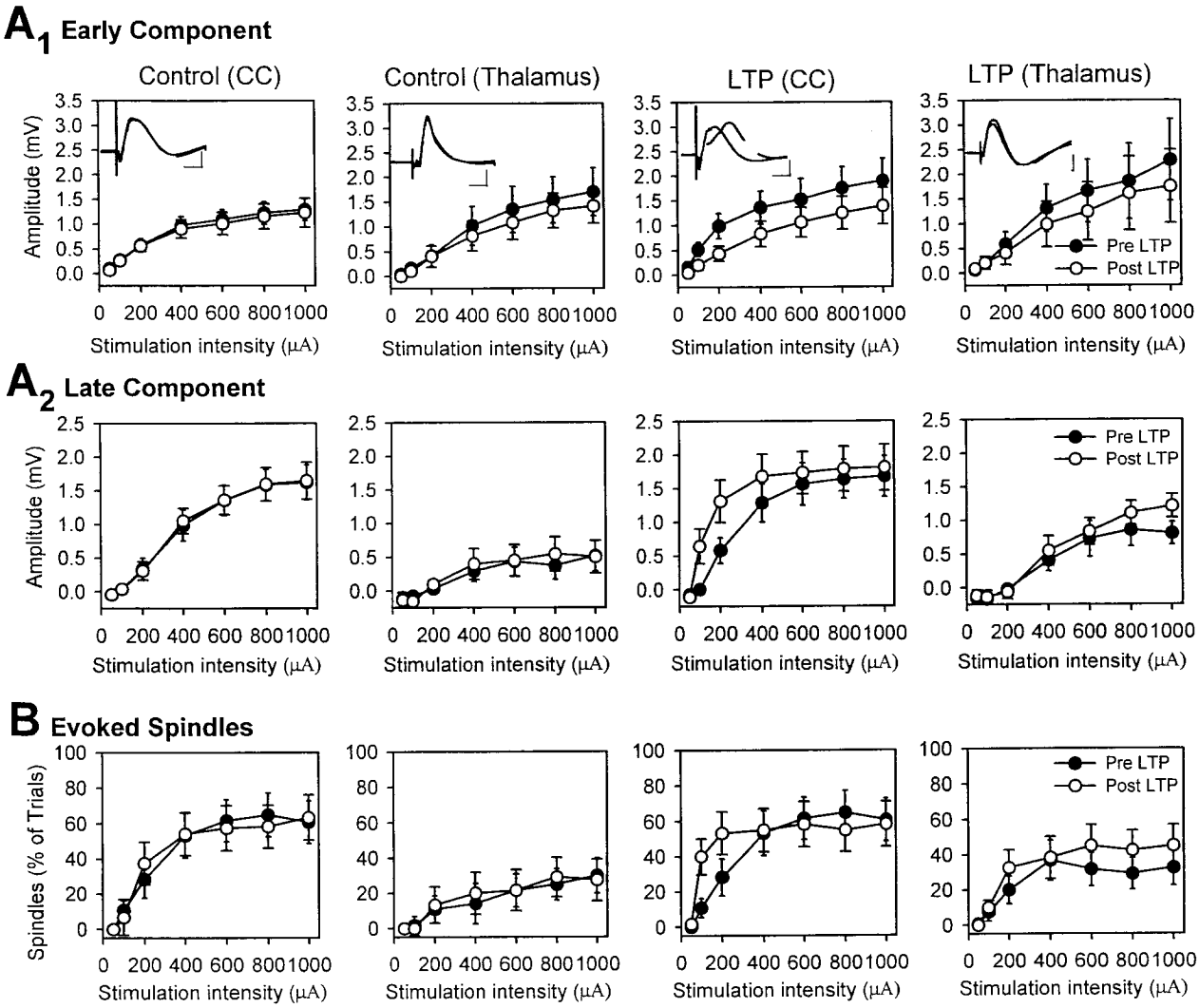
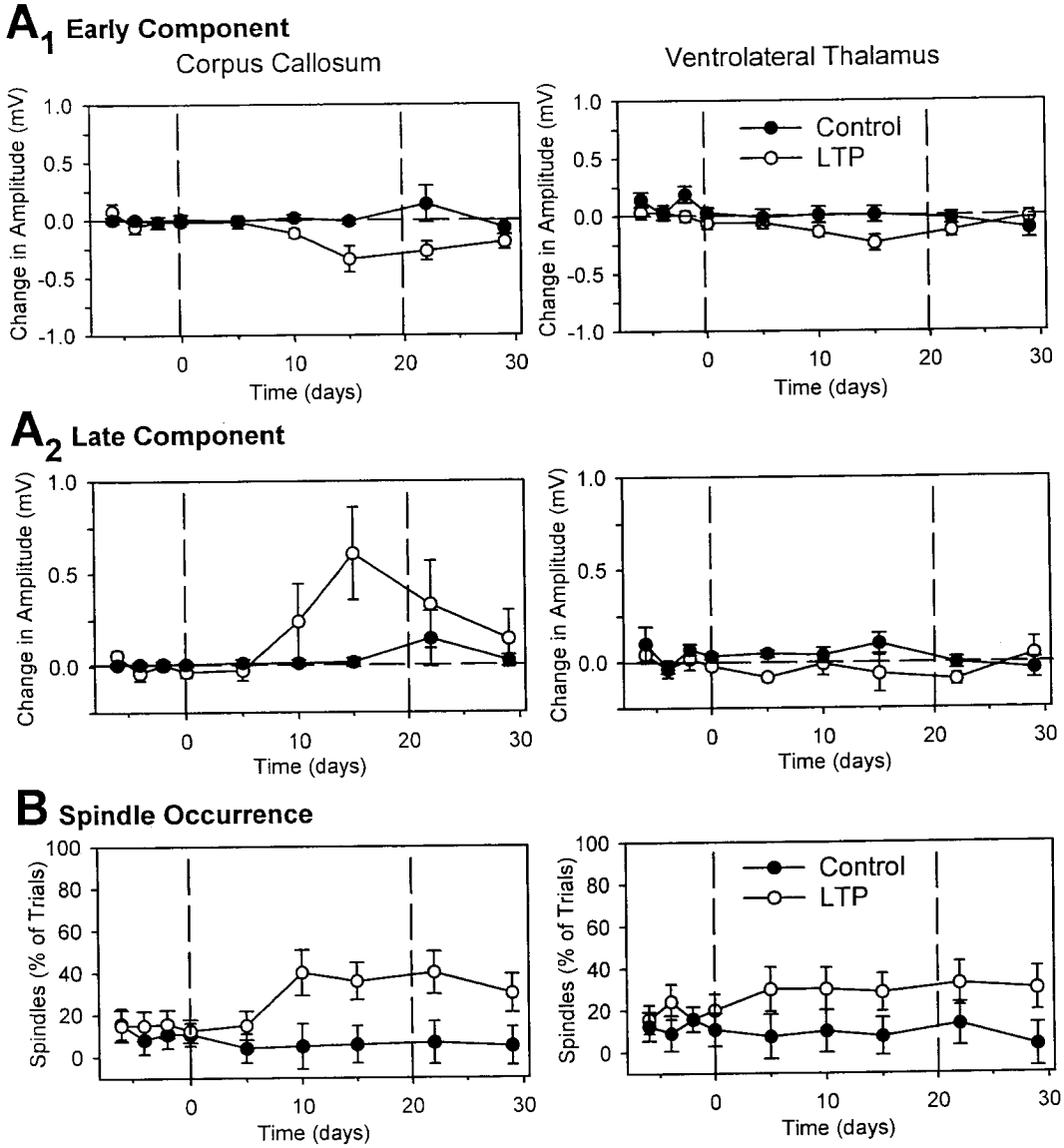

A1 Early Component
Control (CC)
Control (Thalamus)
LTP (CC)
LTP (Thalamus)
Amplitude (mV)
Pre LTP
Post LTP
Stimulation intensity (μA)
A2 Late Component
B Evoked Spindles
Spindles (% of Trials)

Figure 11. Changes in synaptic responses and increases in the reliable induction of spindles occurred with a similar time-course over the 15-day LTP induction period. Daily tetanization of the corpus callosum led to changes in synaptic responses evoked by corpus callosum stimulation, but had no significant effect on responses evoked by thalamic stimulation (A). Similarly, increases in the reliable induction of spindles were observed for the corpus callosum, but not for the thalamus (B). Vertical dashed lines indicate the duration of the LTP induction period, and horizontal lines indicate no change from baseline. Pulse intensity was 100 μA for the corpus callosum and 200 μA for the ventrolateral thalamus.


A1 Early Component
Corpus Callosum
Ventrolateral Thalamus
Change in Amplitude (mV)
1.0
0.5
0.0
-0.5
-1.0
0
10
20
30
Time (days)
Control
LTP
A2 Late Component
B Spindle Occurrence
Spindles (% of Trials)
100
80
60
40
20
0

CHAPTER FOUR

EFFECTS OF LTP ON RESPONSES EVOKED BY DIRECT STIMULATION OF LAYER V HORIZONTAL PROJECTIONS IN THE SENSORIMOTOR CORTEX

C.M. Werk, and C.A. Chapman

Stimulation patterned after the theta rhythm enhances the induction of both LTP (Werk & Chapman, 2003) and LTD (Werk, Klein, Nesbitt, & Chapman, in press) in the sensorimotor cortex. In addition, the previous chapter has shown that LTP of this region enhances the ability of single stimulation pulses delivered to the corpus callosum to evoke spindle waves in the sensorimotor cortex. Spindle waves occur at theta-frequency, reflect mass synchronization of neurons, and are thought to reflect the activation of large areas of the neocortex. This strong 10 Hz activity pattern may contribute to mechanisms of memory consolidation by enhancing re-activation of neuronal pathways, and by promoting the long-term strengthening of these same pathways. Through experiments indicating that theta-patterned stimulation induces LTD effectively, this thesis has contributed to a literature (Werk & Chapman, 2003) showing that stimulation at the frequency of the theta rhythm facilitates lasting changes in synaptic strength in the neocortex.

One way that theta-frequency activation may be particularly effective at inducing LTP is through enhanced activation of horizontal layer V projections. The strength of synaptic responses mediated by horizontal projections was recorded directly before and after induction of LTP in the next experiment. This was done to determine if enhancements of the polysynaptic component of field potential responses is due to a potentiation of polysynaptically activated connections within layer V or simply due to enhanced synaptic strength in monosynaptic inputs to the cortex. Direct test-pulse stimulation of horizontal projections was predicted to yield larger field potentials within layer V following LTP induction as compared to baseline recordings.

Abstract

Repeated, daily tetanization of the corpus callosum in awake rats has been shown to lead to long-term potentiation (LTP) of early monosynaptic and late polysynaptic components of field potential responses in the sensorimotor cortex. Both the early and late responses are generated by excitatory synaptic currents in layer V. The enhanced polysynaptic component could result from LTP of monosynaptic inputs to layer V neurons that, in turn, drive activation in horizontal collaterals. In addition, the enhanced late component could result in part from potentiation of synapses within horizontal projections. To determine if synapses within horizontal projections undergo LTP, recordings in the sensorimotor cortex were obtained from an array of 5 electrodes arranged in a rostro-caudal array from +4 to 0 mm anterior to bregma. Synaptic responses were evoked by stimulation of the corpus callosum at 2.0 mm anterior to bregma, and by direct stimulation of the deep layers to activate horizontal collaterals. Recordings were obtained before and after 15 days of daily tetanization to induce LTP. Tetanization induced significant LTP of differentially recorded responses evoked by corpus callosum stimulation. However, there was no significant LTP of the superficial-negative component of responses recorded at superficial sites at any distal location within the electrode array. In addition, no LTP was observed in superficial negative responses evoked by direct cortical stimulation via the central electrode in the array. These findings suggest that LTP of polysynaptic responses evoked by corpus callosum stimulation is due mainly to a potentiation of monosynaptic inputs to the sensorimotor cortex.

Key words: Potentiation, motor cortex, rat, polysynaptic, layer V

Synaptic plasticity in the neocortex is thought to be the primary mechanism through which long-term memories become consolidated. Long-term potentiation (LTP) is a model for memory consolidation and, for successful induction of LTP in the sensorimotor cortex *in vivo*, high-frequency stimulation of the corpus callosum must be spaced and repeated over multiple days (Trepel & Racine, 1998; Chapman et al., 1998). This slow induction of LTP is consistent with the idea that LTP reflects mechanisms of memory consolidation, which is thought to be a gradual process.

Responses recorded from the sensorimotor cortex consist of two main components; an early component reflecting largely monosynaptic activation and a late component reflecting both monosyaptic and polysynaptic activation. Induction of LTP results in a decrease in the early component which reflects an increase in repetitive spiking of layer V neurons (Chapman et al., 1998). Potentiation of the late polysynaptic component of field responses is believed to occur at least partly due to strengthening of horizontal layer V collaterals (Hess and Donoghue, 1994, 1996; Urban, Kossut, & Hess, 2002; Werk & Chapman, 2003), but this idea has not yet been directly tested.

The theta (7-14 Hz) EEG rhythm may play a role in strengthening synapses within horizontal layer V connections during memory consolidation. Naturally occurring activity at theta-frequency is observed in the neocortex during slow-wave sleep and awake immobility in the form of large amplitude (~10 Hz) spindle waves. This rhythm reflects mass synchronization of neuronal activity across the neocortex, and this synchronization could promote synaptic plasticity. Theta-patterned stimulation has been shown to be effective at inducing both LTP (Werk & Chapman, 2003) and long-term depression (LTD; Werk, Klein, Nesbitt, & Chapman, in press) in the sensorimotor cortex.

Theta-patterned stimulation may be effective at inducing LTP and LTD of the late component in the sensorimotor cortex by enhancing the spread of activation across the cortex. Similarly, normally occurring theta frequency activities may promote memory consolidation through enhancing the strength of horizontal projections.

The idea that induction of LTP leads to a strengthening of horizontal projections is supported by motor mapping studies. Teskey and colleagues (2002) studied the functional consequences of lasting potentiation in the caudal forelimb area induced by seizure activity evoked by kindling of the corpus callosum, and used microstimulation to measure changes in the functional output of the motor cortex. They found that seizure-induced increases in synaptic strength are correlated with expansion of the motor map in the caudal forelimb area. These changes in motor maps following kindling may have occurred through increases in synaptic strength of horizontal connections. That is, increasing the strength of horizontal connections may link a larger area of cortex to motor output neurons activated by microstimulation.

Activation of inputs to the motor cortex may promote plasticity in horizontal connections. Hess and colleagues (1996) used theta-burst stimulation *in vitro* to assess LTP of horizontal motor pathways in layer II/III. They found that bicuculline (a $GABA_A$ antagonist) was required to induce LTP of the layer II/III motor pathway and that co-activation of converging vertical inputs enhanced LTP in the inputs to layers II/III. Widespread potentiation of layer II/III responses have also been observed following stimulation of layer VI in slices of somatosensory cortex (Lee, Weisskopf, & Ebner, 1991). This research shows that responses in layer II/III are resistant to potentiation but that LTP can be successfully induced in these horizontal projections under some

conditions in the *in vitro* preparation.

If horizontal connections are potentiated by LTP induction, then structural changes in the cytoarchitecture of neurons involved could be expected. Ivanco, Racine and Kolb (2000) induced LTP in the sensorimotor cortex *in vivo* and then used Golgi staining to examine morphological changes in layer III pyramidal neurons. They found increases in dendritic morphology and increased spine density in the sensorimotor cortex following LTP. These morphological changes following LTP in sensorimotor cortex pyramidal cells *in vivo* support the idea that synaptic plasticity may take place partly through strengthening of horizontal projections in the sensorimotor cortex *in vivo*.

In the present study, we wanted to determine if theta-patterned stimulation would promote LTP within layer V projections in the sensorimotor cortex, or if LTP in this pathway was due mainly to enhanced monosynaptic projections that, in turn, enhanced polysynaptic responses. If horizontal connections mediate the enhanced polysynaptic responses following LTP induction, then synaptic responses evoked by direct stimulation of layer V collaterals should be enhanced at nearby recording sites. Therefore, to determine if synapses within horizontal projections undergo LTP, test-pulses were delivered to the corpus callosum and to the center of a five-electrode array used to activate horizontal projections directly. Paired-pulse (PP) facilitation was also used to measure the ability of horizontal projections to respond preferentially to activation at the 100 ms period of the theta rhythm.

Method

Surgery

Male Long-Evans rats (300-400 g) were anesthetized with ketamine and xylazine (90 and 8 mg/kg, respectively) and placed in a stereotaxic frame. Bipolar, Teflon-coated, stainless steel twisted-wire electrodes (125 μm exposed tips) were implanted in the right corpus callosum (anterior, 2.0; lateral, 2.0; ventral, 2.6 mm from pia) and sensorimotor cortex (anterior, 2.0; lateral, 4.0; ventral, 1.8 mm from bregma). Tip separation was 0.5 mm for the corpus callosum electrode and 1.2 mm for the recording electrode in sensorimotor cortex. Monopolar recording electrodes were also placed 2, and 1 mm posterior and 1, and 2 mm anterior to the bipolar recording electrode (see Figure 12). Vertical placements were adjusted to maximize the early monosynaptic component of field potentials and to minimize current thresholds. Maximizing the amplitude of the early monosynaptic component is expected to also maximize the degree of polysynaptic activation, and to therefore enhance the amplitude of the late component of responses in the awake animal. A stainless-steel jeweler's screw in the left posterior parietal bone served as the ground electrode. Electrode leads were connected to gold-plated pins, mounted in a connector, and the assembly was stabilized using dental cement anchored to the skull with screws. Animals were housed individually on a 12 h light-dark cycle and handled every 2-3 days during a 2 week recovery period. All experimental testing was conducted during the lights-on period.

Stimulation and Recording

Animals were habituated to a 30 x 40 x 30 cm Plexiglas chamber, and recordings were collected while animals were in a quiet resting state. Biphasic, square-wave

constant current stimulation pulses (0.1 ms duration) were delivered to the corpus callosum using a linear stimulus isolation unit (A-M Systems, Model 2200) driven by a computer DAC channel (50 kHz). Evoked field potentials were filtered (0.1 Hz to 5 kHz passband), amplified (A-M Systems, Model 1700), and digitized at 20 kHz (12 bit) for storage on computer hard disk (Datawave Tech., Experimenter's Workbench).

Paired-Pulse Facilitation. Pairs of single pulses separated by 50ms, 100ms and 1000ms were delivered either to the corpus callosum or to directly to layer V in order to assess the facilitation of responses to the second pulse in both of these pathways. Pulse amplitude was set to evoke field potentials that were 60% of their response evoked by maximal stimulation. Even though the facilitation observed during delivery of pairs of pulses is very short-lasting and the stimulation intensity used was relatively low, animals were still given a full week after delivery of the paired-pulse tests before they received any other testing. This was done to ensure that there were no lasting effects on measures of LTP.

LTP Induction. Input/output tests were used to monitor field potential responses evoked by a range of stimulus intensities (0, 50, 100, 200, 300, 400, 500, 600, 800, and 1000 μA) and to assess changes in evoked responses induced by LTP induction. Eight evoked potentials were recorded and averaged at each of the test pulse stimulation intensities. The inter-pulse interval was 10 s and pulses were delivered in ascending order with respect to intensity. Input/output tests were conducted every 2 days during a 1 week baseline period and further tests were conducted every week for 2 weeks following LTP induction following stimulation of both corpus callosum inputs and direct layer V inputs. Input/output tests were also conducted every 5 days during the induction of LTP.

Evoked field potentials were recorded in all recording sites in response to test pulses delivered to the corpus callosum, and responses in distal sites were recorded following direct stimulation of the sensorimotor cortex. To induce LTP, animals received 20 pairs of trains that were patterned after the theta rhythm (pairs of 4-pulse, 300 Hz trains with a 100 ms inter-train interval; n = 12), which were delivered daily to the corpus callosum for 15 days.

Data Analysis. Amplitudes of field potential components were measured at the peaks of the antidromic spike (2 ms), the early monosynaptic (6-8 ms), and the late polysynaptic (17-18 ms) components of differentially recorded field potentials. Early and late field potential amplitudes were also measured from the superficial monopolar response of the other four recording sites. Latency from the stimulation pulse to the early and the late components across the array were measured to determine the onset of responses. PP facilitation was measured by subtracting the field potential amplitudes of the first pulse from the second pulse. Mean input/output curves were obtained after normalizing amplitudes of field potentials to the average response to 1000 μA pulses recorded in the last baseline tests. Mixed-design ANOVA's were used to analyze changes in peak amplitudes of the spike, early and late components of evoked responses across the electrode array as well as changes in paired-pulse facilitation and latency of responses.

Results

Standard histological analysis showed that bipolar electrodes were located with the superficial tip in superficial layers II/III and the deeper tip in layers V-VI. Monopolar electrodes were usually located in layers II/III at a level lateral to the superficial tip of the

bipolar electrode. See Figure 12 for a schematic illustration of the array. Analysis of differentially recorded responses in the sensorimotor cortex evoked by stimulation of the corpus callosum showed a strong potentiation of the initial antidromic spike and the polysynaptic late component of responses, but no significant change in the early monosynaptic component following 15 days of tetanization. In comparing the final baseline test with the first post LTP test, the amplitude of the spike increased significantly to 140.3 ± 22.5% of baseline values at the 1000 μA stimulus intensity [$F_{1,10}$ = 5.54, $p < 0.05$]. Similar to previous findings (Werk and Chapman, 2003), the amplitude of the early component became more variable between animals and there was no significant change at 1000 μA [$F_{8,88} = 0.87, p > 0.05$; 99.1 ± 30.1%]. The late component was significantly enhanced to 210.7 ± 49.5% of baseline values [$F_{8,88} = 2.64$, $p < 0.01$; Figure 13]. Therefore, tetanization of the corpus callosum resulted in successful LTP of bipolar responses.

Superficial negative responses evoked by stimulation of the corpus callosum were measured across the entire array to assess the spatial spread of the evoked response. In animals in which the depth of the electrodes varied, the amplitude and/or polarity of responses also varied with electrode depth. The majority of animals showed negative field potential responses, but 3 of the 12 animals had one or two positive-going responses among the 5 recording sites in the array. An example of superficial negative responses recorded across the entire array in one animal, and the group averages of amplitudes of the early and late components of the responses are illustrated in Figure 13. It could be expected that responses immediately lateral to the stimulation electrode would be the largest, but there was no systematic difference in the amplitude of the early [$F_{32,424}$ =

0.39, $p > 0.05$] or late components [$F_{32,424} = 0.24, p > 0.05$]. There was, however, a slightly shorter peak latency of responses for the electrode immediately lateral to the stimulation electrode [early, 5.3 ± 0.3 ms; $F_{4,72} = 3.29, p < 0.05$: late, 10.0 ± 0.4 ms; $F_{4,72} = 2.92, p > 0.05$], with distal recording sites showing slightly longer latencies (early, 5.9 ± 0.3 ms; late, 11.1 ± 0.4; Figure 14). Tukey's post hoc analysis revealed that the recording from the electrode directly lateral to the stimulating electrode had a significantly shorter latency than the most posterior electrode recording ($p < 0.05$), and there was a trend for the middle posterior electrode recording to have a longer latency ($p = 0.07$). The recording of the late component in the directly lateral recording site was found to be significantly shorter than the most anterior recording ($p < 0.05$), and showed a trend towards being faster than the middle anterior electrode ($p = 0.07$) and posterior ($p = 0.08$) recordings. Therefore, the latency of the onset of distal responses was generally longer than responses recorded directly lateral to the stimulation.

Paired-pulse stimulation of the corpus callosum, and direct paired-pulse stimulation of the cortex, was also used to assess the spatial spread of facilitated evoked responses across the array, and to determine if horizontal collaterals may support paired-pulse (PP) facilitation at the 100 ms interval (Figure 15). Superficially recorded responses to corpus callosum stimulation showed PP facilitation of the late component across the entire array at the 100 ms interval [P1: 0.29 ± 0.11 mV; P2: 0.99 ± 0.21 mV, $F_{2,8} = 16.63, p < 0.01$], but no changes were observed in the early component [P1: 0.88 ± 0.15 mV; P2: 0.69 ± 0.17 mV, $F_{2,6} = 0.63, p > 0.05$] at any paired-pulse interval. Similarly, there was also a significant PP facilitation of the late component [P1: -0.57 ± 0.18 mV; P2: -1.00 ± 0.29 mV, $F_{2,4} = 21.65, p < 0.01$], and no significant change in the

early component [P1: -1.48 ± 0.36 mV; P2: -0.96 ± 0.29 mV, $F_{2,6} = 4.71, p > 0.05$] following direct cortical stimulation. Tukey's post hoc analyses revealed that significant PP facilitation occurred only at the 100 ms paired pulse interval and not at paired-pulse intervals of 50 or 1000 ms for both stimulation sites (all p values < 0.05). The amount of PP facilitation was not significantly greater for posterior than anterior sites following stimulation of either the corpus callosum or by direct cortical stimulation (p values $>$ 0.05). This suggests that horizontal collaterals activated by direct cortical stimulation support PP facilitation, and are highly responsive to stimulation at this interval.

To assess the spatial spread of potentiated responses, superficial-negative responses recorded across the electrode array were compared before and after induction of LTP. Bipolar responses had shown potentiation (Figure 13), and the superficial recording from the bipolar electrode also showed potentiation of the late [$F_{1,9} = 6.55, p <$ .05] but not the early component [$F_{1,9} = 0.74, p > 0.05$]. However, there were no significant changes in the amplitudes of the early superficial negative components of responses at any of the distal electrodes in the array (all p values > 0.05). The late superficial component was also not affected significantly by LTP induction but the late component was also more variable and much smaller in baseline amplitude (all p values $>$ 0.05; Figure 16).

To determine if horizontal layer V projections may undergo LTP following tetanization of the corpus callosum, superficial negative responses evoked by direct cortical stimulation via the central electrode of the array were compared before and after LTP induction. Similar to results for corpus callosum stimulation, there was no significant change in the amplitude of the superficial negative component in any location

in the electrode array following LTP induction (all p values > 0.05; Figure 17). In addition, similar to responses evoked by corpus callosum stimulation, the late component of responses was variable and low in amplitude during baseline recordings, and also showed no change following LTP induction (all p values > 0.05).

Discussion

The importance of monosynaptic inputs for the potentiation of polysynaptic responses in the sensorimotor cortex has been demonstrated here. We found LTP of polysynaptic connections, evidenced by potentiation of the bipolar late response, which likely resulted from the strengthening of monosynaptic inputs. The potentiation of the late component could also have resulted from increased repetitive firing of monosynaptically activated neurons because this repetitive firing could in turn drive synaptic responses in horizontal connections, and this idea is supported by the enhanced repetitive spiking that was observed (Figure 13). A clear facilitation of the late component across the whole electrode array was observed during paired-pulse stimulation of the corpus callosum at the100 ms interval. In addition, we found PP facilitation following direct stimulation of the *cortex* using the 100 ms interval, suggesting that synapses within horizontal projections are capable of showing short-term facilitation, and suggesting that these synapses are sensitive to the effects of 10 Hz stimulation. This suggests that short-term facilitation within these projections may contribute to the thalamocortical augmenting response (Castro-Alamancos & Connors 1996a, b). Long-term potentiation probably does not require potentiation of synapses within these horizontal connections however, because we found that there was no potentiation of the early or late components of responses in distal recording sites evoked

by direct stimulation. Monosynaptic inputs from the corpus callosum may therefore be primarily responsible for LTP of the sensorimotor cortex.

Although previous researchers have suggested that horizontal projections are strengthened following LTP induction (Hess, et al., 1996; Ivanco, et al., 2000; Werk & Chapman, 2003), the present findings argue against this conclusion. There are a variety of factors that suggest that the experimental preparation used here has provided a good test of whether horizontal projections are strengthened during LTP induction. The array preparation provided reliable results for corpus callosum stimulation in that we observed superficial negative responses across the array in most animals. This indicates that the electrodes recorded the superficial negative component of responses generated from layer V stimulation (Chapman et al., 1998). In addition, the latencies of distal recordings were longer than the latencies for the central recording site which suggests that responses across the array were due, in part, to the spatial and temporal spread of responses via layer V horizontal projections. We also observed PP facilitation following direct stimulation of the horizontal fibres, and this indicates that responses recorded in this way have the potential to display strong facilitation effects. If these projections can support short-term facilitation, then LTP of these pathways should also have been apparent if potentiation of the horizontal projections had occurred. Finally, similar to previous findings, all animals showed successful induction of LTP of the bipolar responses, so that the preparation could test if there are corresponding changes in the strength of horizontal projections during LTP.

Long-term potentiation of the neocortex is believed to be dependent on NMDA receptor activation (Kitagawa, Inoue, Nishida, Nishimura, Yamamoto, & Nishimura,

2004; Malenka & Nicoll, 1995; Trepel & Racine, 1998; Werk & Chapman, 2003). For example, Trepel and Racine (1998) were able to successfully block the induction of LTP induced *in vivo* in the motor cortex by administering daily injections of an NMDAR antagonist. Hess, Aizenman, and Donoghue (1996) also found that an NMDA antagonist (APV) was effective at blocking theta-burst stimulation-induced potentiation of horizontal projections in layer II/III of the motor cortex *in vitro*. Therefore the strengthening of polysynaptic responses within horizontal projections in the motor cortex appear to be dependent of NMDAR-mediated LTP of direct cortical inputs.

Polysynaptic responses have been shown to be generally resistant to potentiation. Inhibition of GABA-mediated IPSPs by bicucullin has been used to effectively potentiate horizontal projections in the sensorimotor (Chagnac-Amita & Connors, 1989) and motor (Hess & Donoghue, 1994) cortices *in vitro*. However, when converging inputs in the motor cortex were co-activated with theta burst stimulation, GABA antagonists were not required (Hess et al., 1996). In this case, even theta-burst stimulation, which can be effective at inducing cortical LTP in monosynaptic input pathways (Kirkwood, Rioult, & Bear, 1996; Singer, 1993; Werk & Chapman, 2003), was not sufficient to induce LTP of horizontal projections unless converging inputs were stimulated simultaneously (Hess et al., 1996). Layer V horizontal connections were also resistant to potentiation in the present study, and it is possible that our preparation did not show potentiation because of a lack of sufficient activation of the horizontal projections. However we stimulated the corpus callosum in the intact animal, which has massive inputs to the sensorimotor cortex, and the evoked responses were very large. In addition, LTP of bipolar responses was observed similar to previous studies, so potentiation clearly occurred. Therefore,

either horizontal projections in the sensorimotor and motor cortices require different mechanisms for potentiation of the *in vivo* preparation may simply provide a more naturalistic representation of plasticity and there may be no enhanced synaptic strength of horizontal connections in the sensorimotor cortex following LTP induction.

The finding that PP facilitation can be observed following both corpus callosum and direct cortical stimulation provides the strongest evidence that LTP in the sensorimotor cortex does not require potentiation of layer V horizontal projections. This is the first time that horizontal projections have been directly investigated electrophysiologically *in vivo* and the PP facilitation effects demonstrate the responsiveness of these projections in this preparation. The lack of potentiation of horizontal projections in the presence of short-term PP facilitation of responses at the same recording sites emphasizes the importance of monosynaptic inputs for the LTP of polysynaptic inputs from the corpus callosum.

Figure 12.

Bipolar electrodes were placed in the corpus callosum and sensorimotor cortex. Distal electrodes were placed 0, +1, +2, +3, and +4 mm relative to bregma (A). The schematic diagram (B) of a sagital section showing the layers of the sensorimotor cortex illustrates that the deep tip of the bipolar recording electrode was located in deep layer V and that the superficial tip and the monopolar distal electrodes were located in superficial layer III/IV.

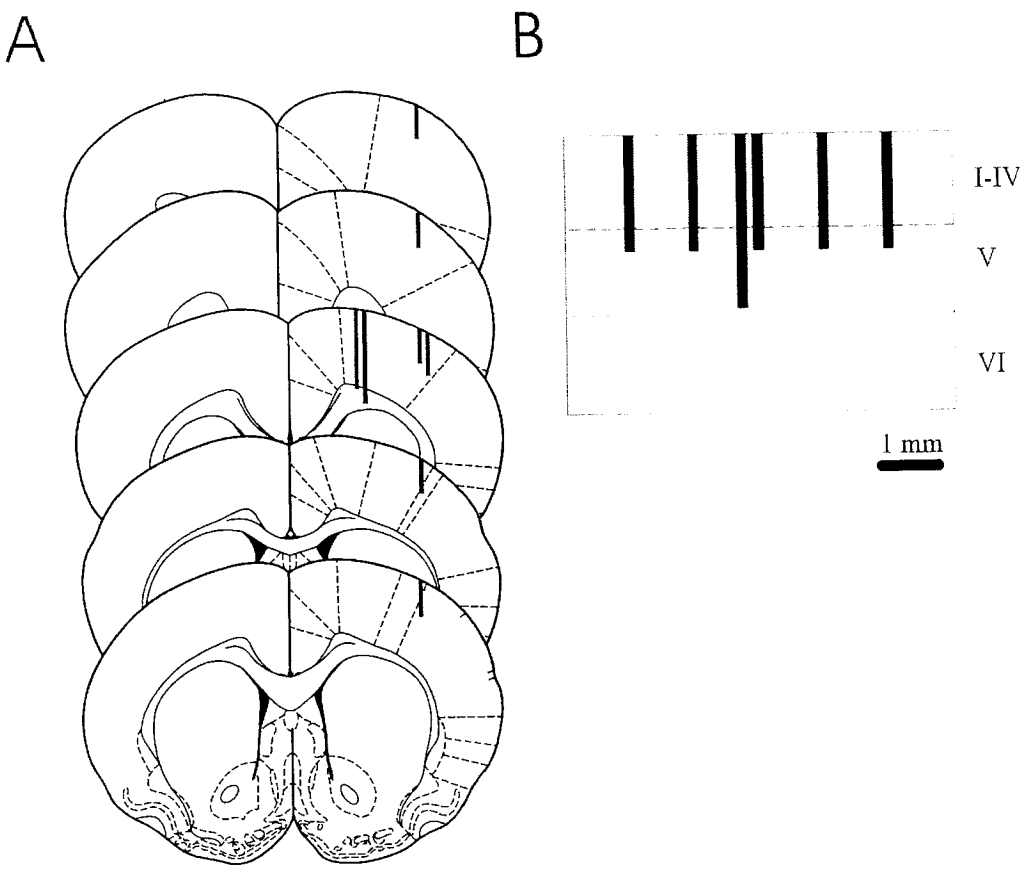

A
B
I-IV
V
VI
1 mm

Figure 13.

Representative traces are shown for differentially recorded field potentials before (A_1) and after (A_2) LTP induction (calibration 10 ms, 1.0 mV). Traces are also superimposed for comparison (A_{1+2}). Symbols show the latencies of the spike (♦), early (●) and late (■) components. Averaged, normalized results of input/output tests (B) show an increase in the initial spike (B_1), a reduction in the early component (B_2), and an increase in the late component (B_3) following the induction of LTP.

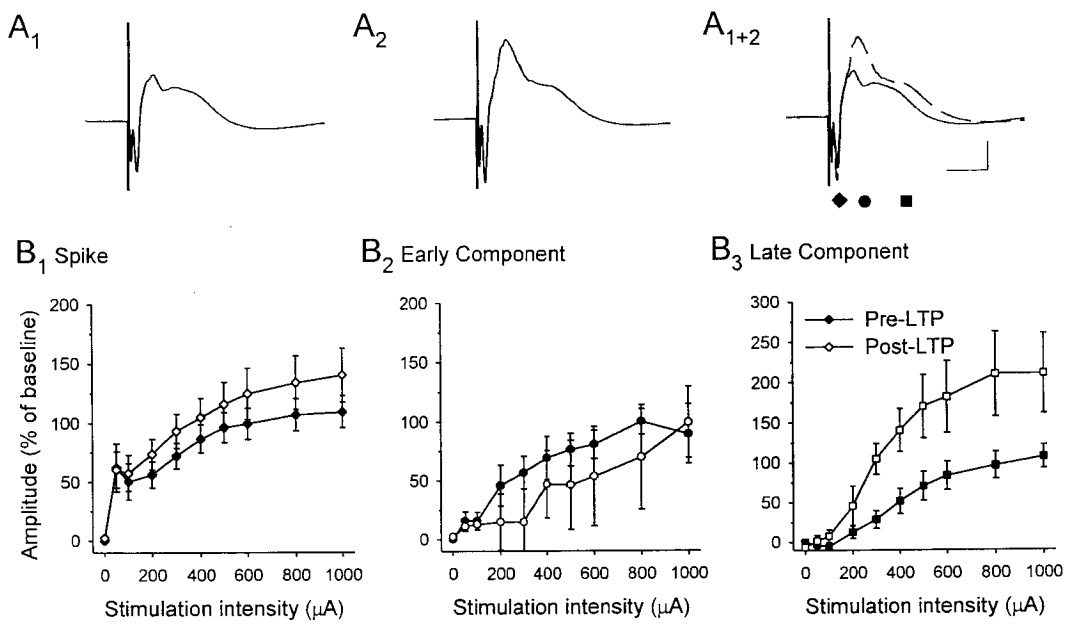

A1
A2
A1+2
B1 Spike
B2 Early Component
B3 Late Component
Amplitude (% of baseline)
Stimulation intensity (μA)
Stimulation intensity (μA)
Stimulation intensity (μA)
Pre-LTP
Post-LTP

Figure 14.

Representative monopolar field potentials evoked by corpus callosum stimulation are shown at the indicated rostro-caudal locations relative to the stimulating electrode. A dashed line indicates the latency of the early component of the direct recordings (A; calibration 10 ms, 1.0 mV). Line graphs show the average baseline amplitudes of the early (B_1) and late (B_2) components of responses for corpus callosum stimulation evoked by 1000 μA pulses. The peak latencies of the early and late components from the pulse (C) are shown as a function of stimulation site. The recordings directly lateral from the stimulation pulse had shorter latencies than distal recordings.

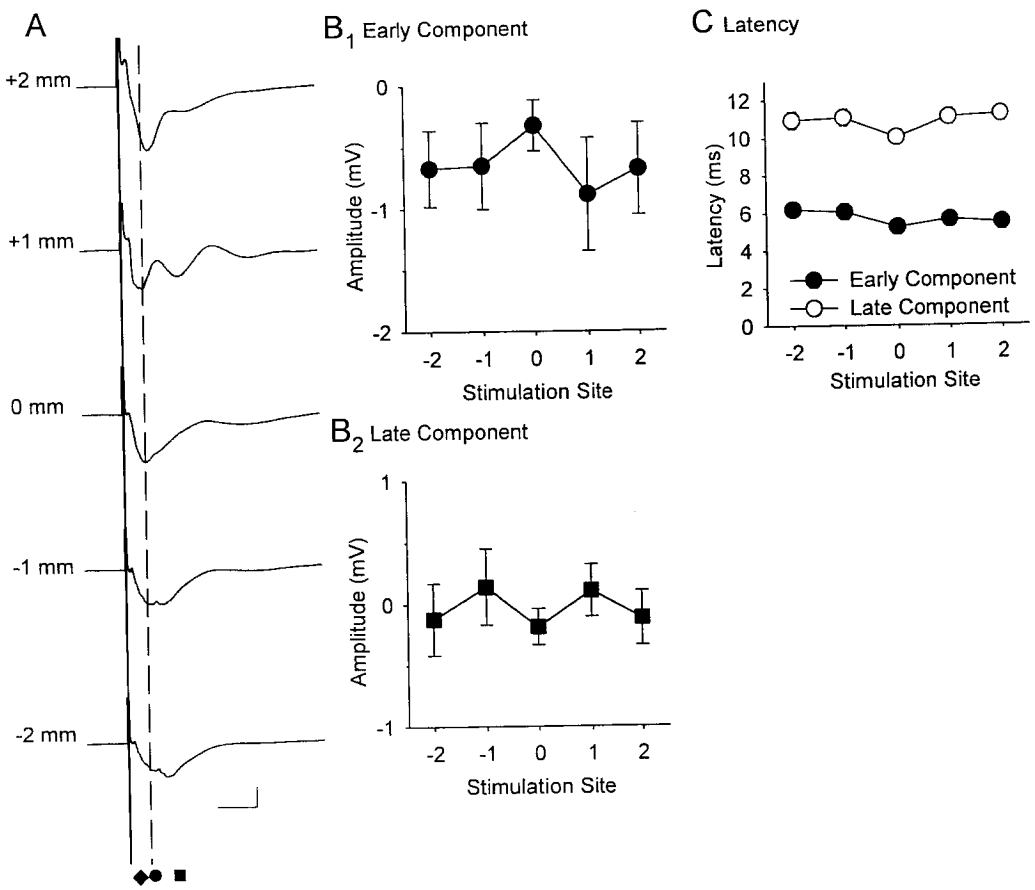

A
+2 mm
+1 mm
0 mm
-1 mm
-2 mm
B1 Early Component
Amplitude (mV)
0
-1
-2
-2 -1 0 1 2
Stimulation Site
B2 Late Component
Amplitude (mV)
1
0
-1
-2 -1 0 1 2
Stimulation Site
C Latency
Latency (ms)
12
10
8
6
4
2
0
Early Component
Late Component
-2 -1 0 1 2
Stimulation Site

Figure 15.

Representative traces and averaged change in amplitude of field responses as a function of stimulation site (B) are shown for the indicated paired-pulse intervals following corpus callosum stimulation (A_1) and direct stimulation (A_2; calibration 10 ms, 1.0 mV). The late component was facilitated at all recording positions in the array following corpus callosum (B_1) and direct stimulation (B_2) at the 100 ms interval, but not at either the 50 or 1000 ms intervals.

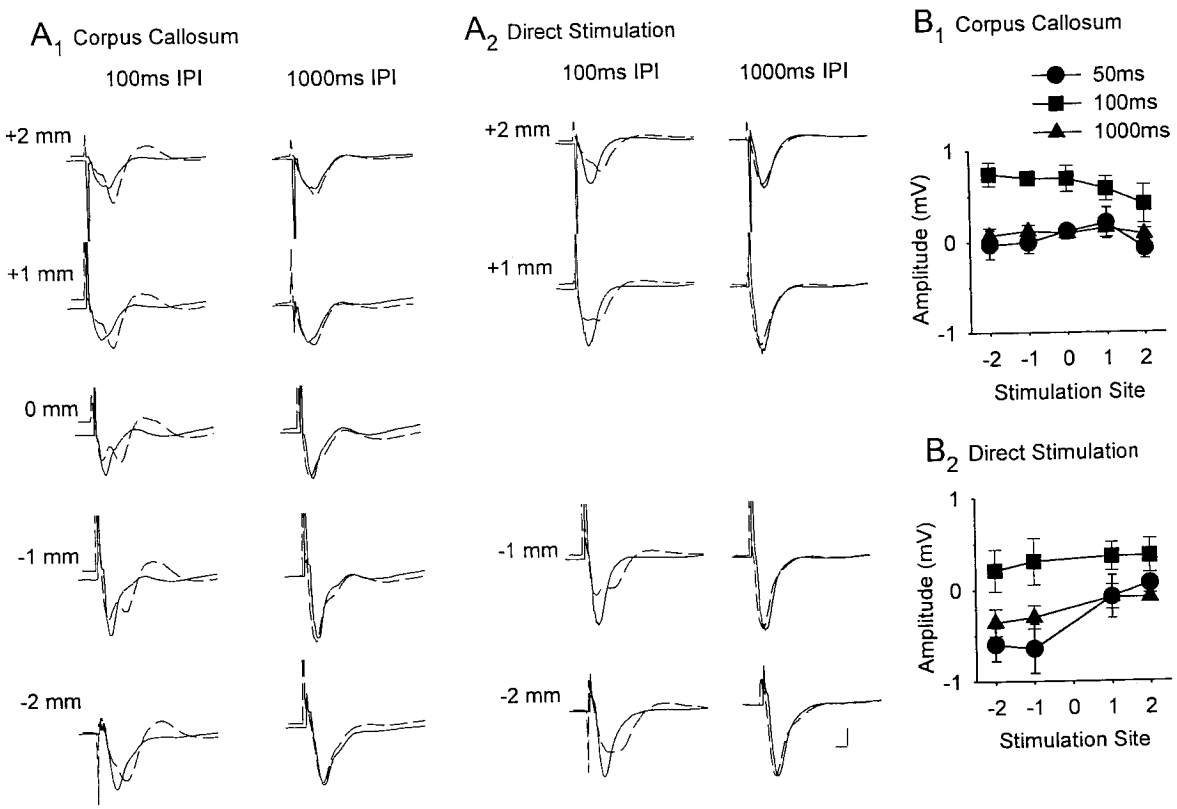

A1 Corpus Callosum
100ms IPI
1000ms IPI
+2 mm
+1 mm
0 mm
-1 mm
-2 mm
A2 Direct Stimulation
100ms IPI
1000ms IPI
+2 mm
+1 mm
-1 mm
-2 mm
B1 Corpus Callosum
50ms
100ms
1000ms
Amplitude (mV)
1
0
-1
-2 -1 0 1 2
Stimulation Site
B2 Direct Stimulation
Amplitude (mV)
1
0
-1
-2 -1 0 1 2
Stimulation Site

Figure 16.

Averaged input-output curves are shown for monopolar superficial negative responses at the indicated recording sites (+2, +1, 0, -1, -2) relative to the stimulating electrode evoked by corpus callosum stimulation before and after LTP induction. Potentiation effects were only found for the late component (A_2) of responses in the direct recording sites, and no significant changes were observed in the early component (A_1) from any recording. Representative traces illustrate the variability of responses across recording sites (B: calibration 10 ms, 1.0 mV).

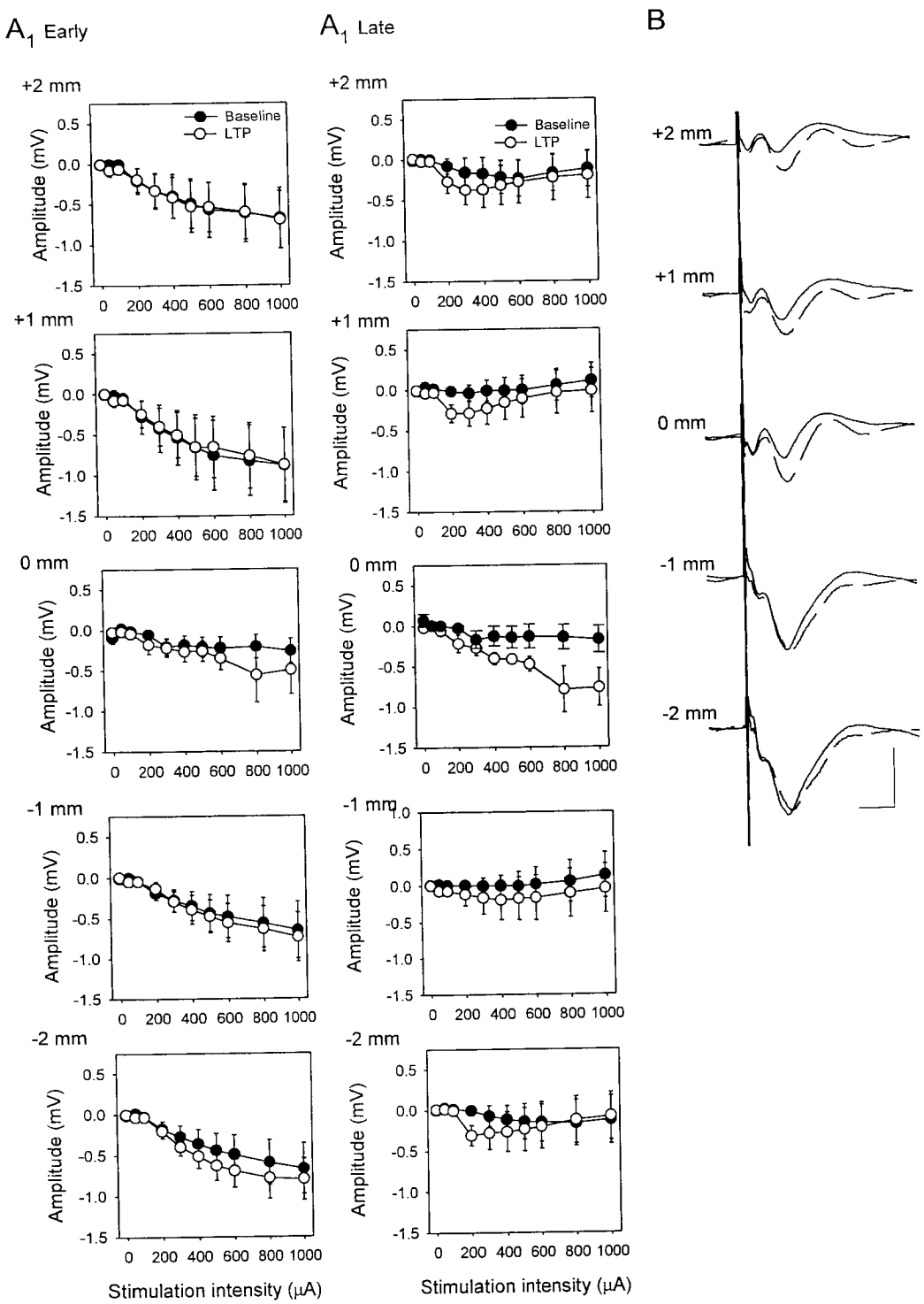
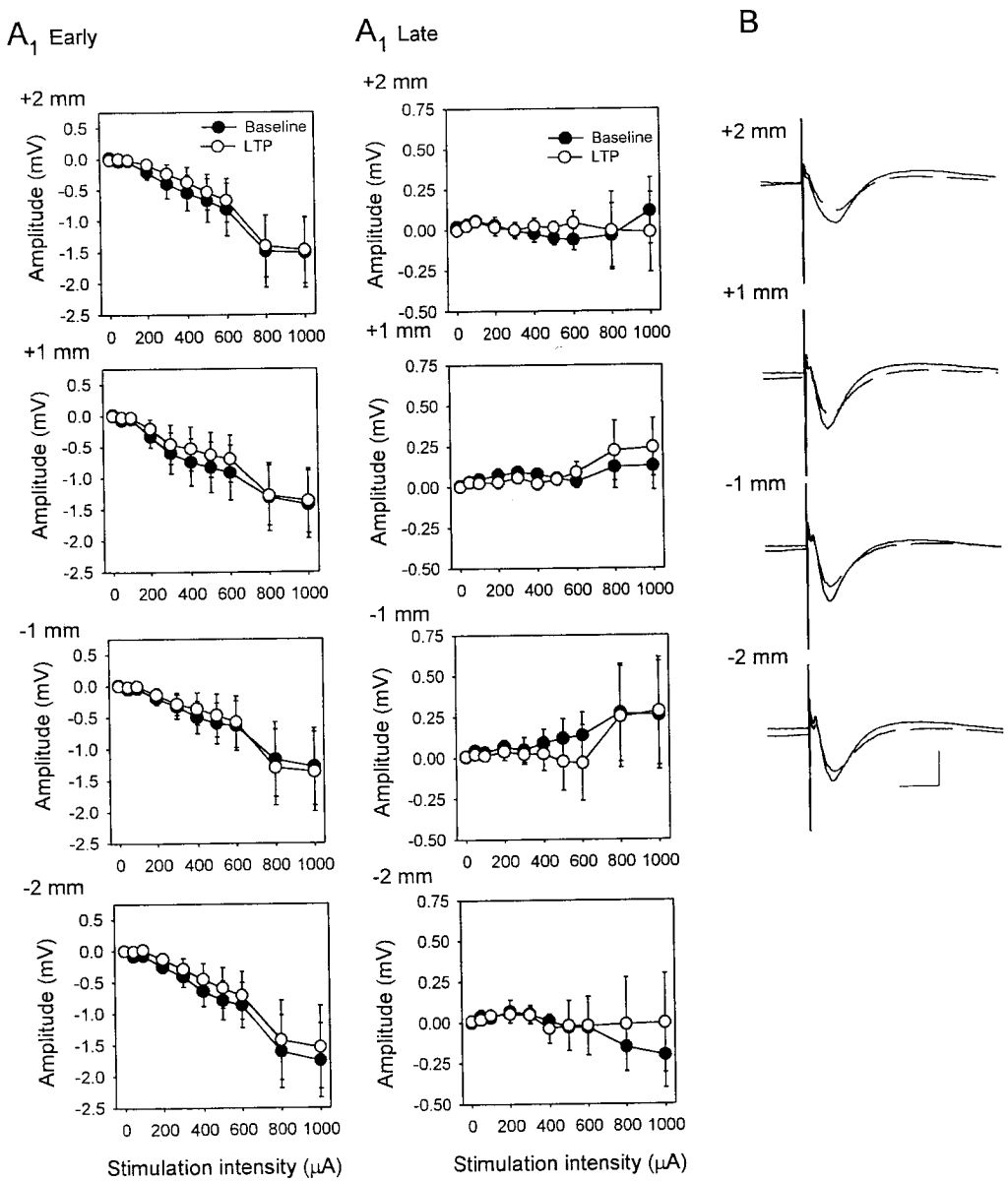

A1 Early
A1 Late
B
+2 mm
+1 mm
0 mm
-1 mm
-2 mm
Baseline
LTP
Amplitude (mV)
0.5
0.0
-0.5
-1.0
-1.5
0 200 400 600 800 1000
Stimulation intensity (μA)

Figure 17.

Representative traces (B: calibration 10 ms, 1.0 mV) and averaged input-output curves are shown for monopolar field potentials evoked by deep layer stimulation before and after LTP induction. Averaged, normalized input-output curves show no consistent changes in the amplitude of early (A_1) and late (A_2) components of responses following LTP induction.


A1 Early
A1 Late
B
+2 mm
+1 mm
-1 mm
-2 mm
Baseline
LTP
Amplitude (mV)
Stimulation intensity (μA)

CHAPTER FIVE

GENERAL DISCUSSION

This thesis has dealt with the neural mechanisms that contribute to the consolidation of motor memory in the sensorimotor cortex, and alterations in synaptic strength are thought to play a major role in this consolidation. Increases in the strength of synaptic responses have been observed previously in the sensorimotor cortex following training on a skilled reaching task, and this has provided strong evidence that changes in synaptic strength in this area do contribute to motor learning (Monfils & Teskey, 2004b; Rioult-Pedotti et al., 2000). The functional relevance of changes in the strength of horizontal layer V connections has also been demonstrated by changes in motor maps (Monfils et al., 2004; Teskey, et al., 2002), and potentiation of synaptic strength has been shown to be dependent on NMDAR activation (Trepel & Racine, 1998; Werk & Chapman, 2003). Strong spindle activity occurs during SWS, and this could play a major role in initiating cellular mechanisms that normally promote lasting synaptic plasticity and the consolidation of motor memory (Sejnowski & Destexhe, 2000). Here I have used electrophysiological stimulation and recording methods to investigate the relationship between low-frequency stimulation patterns, the induction of short-term synaptic facilitation effects, and the induction of long-term changes in synaptic strength within layer V of the sensorimotor cortex.

In Chapter two, stimulation patterned after the theta rhythm, at a frequency similar to spindle activity, was shown to be more effective than standard low-frequency stimulation at inducing LTD in the sensorimotor cortex (Figures 5, 6). The greater LTD observed following the 10 Hz trains was likely due to the short-term synaptic facilitation observed during the prolonged theta-patterned stimulation. The occurrence of spindle activity during sleep may be a way for similar short-term facilitation effects to occur in

the neocortex during motor learning. Consistent with this idea, an interaction was shown here between LTP and the induction of spindles in Chapter three, where the ability to evoke spindle activity in the sensorimotor cortex was increased following LTP induction (Figures 10, 11). In Chapter 4, synaptic responses mediated by horizontal projections were measured directly before and after LTP of the sensorimotor cortex, and no change in these responses were observed. This finding suggests that LTP is largely due to enhanced monosynaptic inputs (Figures 16, 17) rather than a strengthening of the polysynaptically activated synapses within horizontal layer V collaterals. Therefore, the induction of bidirectional plasticity in the neocortex is responsive to the theta-frequency rhythm and is likely not dependent on strengthening of polysynaptic horizontal projections, but appears to be largely dependent on long-lasting strengthening of monosynaptic inputs to the neocortex.

Short-Term Facilitation.

Short-term facilitation at the frequency of the theta rhythm likely plays major role in enhancing the activation of cellular mechanisms that cause long-term changes in synaptic strength. A number of short-term facilitation effects related to the 10 Hz rhythm were evoked by stimulation patterns used in Chapters two, three and four. Short-term facilitation was observed in Chapter two during two types of theta-patterned stimulation: pairs of pulses separated by 100 ms (which is the period of 10 Hz; PP-LFS), and prolonged 10 Hz trains (Figure 7). Facilitation during these two types of trains could have contributed to significant long-term depression in the sensorimotor cortex due to prolonged moderate activation of NMDAR's during the 10 Hz trains. The PP-LFS trains induced short-term facilitation in the response to the second pulse of each pair, but these

trains did not induce significantly greater LTD than 1 Hz trains in which no short-term facilitation occurred. As discussed in Chapter one, LTD induction typically requires prolonged moderate activation, so perhaps the short-term facilitation evoked by the pairs of pulses was not sufficient to induce significantly greater postsynaptic Ca^{2+} influx than 1 Hz trains.

Given the nature of bi-directional plasticity, in which moderate Ca^{2+} influx can induce LTD and strong Ca^{2+} influx can induce LTP (Stanton, 1996), there was a chance that the prolonged 10 Hz trains would be intense enough to induce LTP rather than LTD. However, the prolonged 10 Hz trains appear to activate a sufficient level of Ca^{2+} influx to induce lasting reductions in synaptic strength without inducing LTP. This finding was not too surprising because 10 Hz is still a fairly low frequency compared to the 300 Hz trains typically used to induce LTP in this area (Trepel & Racine, 1998; Chapman et al., 1998; Werk & Chapman, 2003). In addition, the 10 Hz trains were delivered for 20 seconds every 2 minutes over a prolonged 15-minute induction period. Therefore, the prolonged short-term facilitation of synaptic responses during the 10 Hz train is consistent with the criteria for induction of LTD.

High-frequency stimulation delivered in a pattern related to the theta rhythm can be used to induce larger LTP in the sensorimotor cortex than high-frequency trains alone (Werk & Chapman, 2003). To induce LTP in Chapters three and four, two high frequency trains of 4-pulses were delivered separated by a 100 ms interval (Figure 3). Short-term facilitation, which occurred during the response to the second train, is likely responsible for the greater ability of these trains to induce LTP as compared to single high-frequency trains that do not induce this form of short-term potentiation (Werk &

Chapman, 2003). Therefore, stimuli that employ even a single 100 ms interval are sufficient to enhance long-lasting changes in synaptic plasticity, which suggests that mechanisms of plasticity in the sensorimotor cortex are particularly sensitive to activation at the frequency of the theta rhythm.

Naturally occurring spindle activity during SWS may enhance changes in synaptic plasticity during learning due to increased activation of horizontal layer V projections. Paired-pulse facilitation was observed in Chapter four across the entire rostro-caudal extent of the electrode array at the paired-pulse interval of 100 ms, but not at 50 ms or 1000 ms intervals. This emphasizes that theta-patterned stimulation is effective at inducing short-term facilitation in horizontal neocortical pathways. Strengthening of synaptic pathways has also been shown here to enhance theta-frequency activity in the form of spindle activity; as seen in Chapter three, the ability to evoke spindles was increased following LTP induction (Figure 10). Taken together, stimulation patterns related to the theta rhythm were clearly shown to be effective at inducing short-term facilitation in the sensorimotor cortex.

The increase in spindle activity reported in Chapter three may have been due to strengthening of monosynaptic inputs to the cortex and/or to strengthening of synapses within horizontal pathways during LTP induction. Spindle waves occur at essentially the same frequency as stimulation used to induce the augmenting response, and likely invoke similar short-term facilitation effects. Castro-Alamancos and Connors (1996a) proposed that the augmenting response may lead to cooperativity among horizontal fibers due to the short-term recruitment of more polysynaptically activated neurons. In Chapter four, however, lasting potentiation of horizontal projections was not observed following the

induction of LTP. Paired-pulse facilitation was observed in recording sites across the entire array (Figure 15) suggesting that there was a potential for increases in synaptic strength in horizontal pathways, and that the lack of LTP of these projections was not due to a ceiling effect on the amplitude of the responses. This strongly suggests that strengthening of synapses within horizontal projections is not required for LTP to occur. So, augmenting that occurs during theta frequency stimulation may recruit more horizontal projections in the context of short-term facilitation effects, but appears to not necessarily strengthen these projections over the long-term. Therefore, short-term facilitation during theta-patterned trains is likely to have also significantly enhanced activation of monosynaptically activated neurons, and this may have lead to increased long-term potentation of monosynaptic inputs to the cortex.

Mechanisms of Long-term Changes in Synaptic Strength.

The research presented in Chapters two and four has shown that repeated induction of short-term synaptic facilitation effects can promote long lasting changes in synaptic strength in the sensorimotor cortex. There are a number of mechanisms which may be responsible for the facilitation of responses during the theta-patterned stimulation used here to induce LTP and LTD. Short-term facilitation effects occur primarily through a mechanism in which repetitive stimulation leads to increases in residual pre-synaptic Ca^{2+}. This increased Ca^{2+} enhances the release of neurotransmitter vesicles (Zucker, 1989). Short-term facilitation can lead to an enhancement of long-term changes in synaptic plasticity because of increased synaptic transmission which leads to larger postsynaptic depolarization and greater *post*synaptic Ca^{2+} influx (Castro-Alamancos & Calcagnotto, 1999). The greater postsynaptic $Ca^{2+}$is important because it can strengthen

synapses through activation of calcium-dependent protein kinases (calmodulin, and CaMKII), and these kinases can quickly lead to increases in AMPA-mediated synaptic responses through phosphorylation of AMPA receptors (Madison, Malenka, & Nicoll, 1991; for review see Malinow & Malenka, 2002) and can also lead to more permanent changes in synaptic function dependent on changes in gene expression activated by cAMP response element binding protein (Bailey, Bartsch, & Kandel, 1996). Short-term facilitation effects that enhance postsynaptic calcium influx promote cellular changes that enhance synaptic strength in the hippocampus (Lynch, 1989; Kullmann, Perkel, Manabe, & Nicoll, 1992; Ogura, Nakazawa, & Kudo, 1992), and in the neocortex (Larkum, Watanabe, Nakamura, Lasser-Ross, & Ross, 2003; Yuste & Katz, 1991).

Theta-frequency stimulation is particularly effective at facilitating cortical responses that recruit NMDAR activation, and NMDAR activation is probably enhanced by short-term facilitation during LTP induction (Castro-Alamancos, Donoghue, & Connors, 1995; Trepel & Racine, 1998; Werk & Chapman, 2003). Although NMDAR's are not required for LTD induced by 1 Hz stimulation in the sensorimotor cortex (Froc & Racine, 2005), there may be some contribution of enhanced NMDAR activation in the greater amount of LTD that was observed using the prolonged 10 Hz trains in Chapter two. NMDAR activation during theta-patterned tetanization has been demonstrated to be important for LTD in other preparations (Bouras & Chapman, 2003; Kourrich & Chapman, 2003), so perhaps the short-term facilitation induced by prolonged 10 Hz trains led to greater NMDAR activation and enhanced LTD.

The stimulation patterns used in Chapters two and four may have evoked a response similar to the augmenting response which is initiated largely by long-duration

inhibitory postsynaptic potentials that last for approximately 300 ms following stimulation. The increased hyperpolarization activates an inward cationic current and strongly enhances the excitability of layer V, and this facilitates synaptic responses (Castro-Alamancos & Connors, 1996a; Castro-Alamancos, 1997). Werk and Chapman (2003) also demonstrated an NMDAR-dependent facilitation of responses recorded in the sensorimotor cortex during 10 Hz trains of single pulses identical to those used in the LTD experiment in Chapter two. Thus, increased postsynaptic depolarization associated with the inward cationic current may have enhanced LTP induced by paired trains and LTD induced by prolonged 10 Hz trains of single pulses.

Inhibitory mechanisms, such as transient reductions in GABA transmission may also be important for the enhanced synaptic activation during paired trains. Theta-patterned stimulation is effective at inducing LTP in the CA1 of the hippocampus partly because activation of presynaptic $GABA_B$ autoreceptors during the first several hundred milliseconds following the first train reduces inhibitory synaptic transmission during the second train, and this reduction in GABA transmission results in increased excitatory post-synaptic responses (Mott & Lewis, 1991; Davies & Collingridge, 1996). Therefore, it is possible that transient reductions in GABA transmission are also important for short-term changes in synaptic strength in the neocortex.

Long-term changes in synaptic strength are likely due partly to changes in the morphology of the cells in the activated pathway. For example, increases in dendritic length and branching in layer III were observed following LTP in the sensorimotor cortex (Ivanco, Racine & Kolb, 2000). In addition, Monfils and Teskey (2004a) found that LTD in the sensorimotor cortex lead to a decrease in dendritic length and spine density in

layers II and V *in vivo*. Therefore, the bidirectional changes in synaptic strength observed in this thesis may have been dependent on either increases or decreases in dendritic morphology in the sensorimotor cortex.

Memory Consolidation during Sleep.

A major mechanism thought to be involved in consolidation of long-term memories is changes in synaptic strength during sleep (Gais et al., 2002; Sejnowski, and Destexhe, 2000; Smith, 1996). Strong electrical stimulation at the frequency of the hippocampal theta rhythm, which is similar in frequency to the naturally occurring spindle waves, was shown to produce large changes in synaptic responses in this thesis. Although spindle activity occurs both during SWS and awake immobility, spindles likely play a role in memory consolidation largely during SWS. Spindle waves are highly effective at activating Ca^{2+} currents in neocortical pyramidal neurons (Contreras, Destexhe & Steriade, 1997). Massive influx of Ca^{2+} occurs during SWS, which could lead to enhanced calcium-dependent gene expression (Gu & Spitzer, 1995). Therefore, spindle activity during SWS could effectively contribute to consolidation of memories through enhanced calcium-dependent gene expression leading to long-term changes in synaptic strength.

To observe any truly long-lasting changes in synaptic responses, it is thought that changes must occur in the pattern of protein synthesis. For instance, motor skill learning has been demonstrated to require protein synthesis (Luft, Buitrago, Ringer, Dichgans, & Schulz, 2004), and this protein synthesis may occur primarily during sleep. In addition, protein synthesis was measured across sleep states in Macaca mulatta monkeys and was found to occur selectively during SWS (Nakanishi et al., 1997). Therefore, increased

spindle activity during SWS, which has been observed to occur following learning (Gais et al., 2002) may play a central role in a cascade of events leading to enhanced gene expression and protein synthesis (Sejnowski & Destexhe, 2000).

Neuronal mechanisms activated during REM sleep are also likely involved in memory consolidation. Increased Ca^{2+} has been shown to enhance gene expression during REM sleep. For instance, a marker for gene expression (zif 268) was found to be re-induced during REM sleep in both the cortex and the hippocampus following LTP induction (Ribeiro, Mello, Velho, Gardner, Jarvis, & Pavlides, 2002). In addition, the expression of the immediate-early gene c-Fos was found to be three times greater during REM than during SWS (Sastre, Buda, Lin & Jouvet, 2000). Therefore, events that occur during both REM and SWS may lead to memory consolidation during sleep.

Long-term bi-directional changes in synaptic strength associated with new motor learning may involve spindle activity during SWS. The present results have shown that stimulation patterned after the theta rhythm can promote bidirectional synaptic plasticity in the neocortex, and this raises the possibility that bidirectional plasticity may occur naturally in association with neocortical spindle waves. The short-term facilitation that occurs during the theta rhythm is proposed to enhance the consolidation of memories into long-term storage during sleep episodes, and this process may occur over many days to weeks. This research is important because it provides a better understanding of the potential role of rhythmic oscillations in changes in synaptic strength in the sensorimotor cortex during motor learning. This work is also consistent with the idea that neurophysiological activities associated with sleep may contribute to motor learning.

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APPENDIX 1

ANOVA Tables

Long-Term Depression in the Sensorimotor Cortex Induced by Repeated Delivery of 10 Hz Trains *In Vivo*

Table #1

Repeated-Measures Factorial Analysis of Variance for Bipolar Early Component Amplitude during 4 Day LTD Induction

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	39.82	7	112.69	0.35
Day (B)	12	0.32	84	0.23	1.40
Stimulation Intensity(C)	7	64.35	49	4.32	14.98**
A X B	12	0.20	84	0.23	0.89
A X C	7	2.60	49	4.32	0.60
B X C	84	0.04	588	0.02	1.55**
A X B X C	84	0.02	588	0.02	0.90

Table #2

Repeated-Measures Factorial Analysis of Variance for Bipolar Late Component Amplitude during 4 Day LTD Induction

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	44.57	6	65.57	0.67
Day (B)	12	1.00	72	0.29	3.43**
Stimulation intensity(C)	7	53.78	42	3.65	14.74**
A X B	12	0.34	72	0.29	1.16
A X C	7	4.52	42	3.65	1.24
B X C	84	0.07	504	0.04	1.61
A X B X C	84	0.03	504	0.04	0.64

* $p < 0.05$

** $p < 0.01$

Table #3

Repeated-Measures Factorial Analysis of Variance for Bipolar Early Component Amplitude during 10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	3	77.51	29	38.49	2.01
Day (B)	11	5.46	319	0.45	12.10**
Stimulation intensity(C)	7	198.66	203	1.63	121.67**
A X B	33	0.85	319	0.45	1.89**
A X C	21	2.49	203	1.63	1.52
B X C	77	0.17	2233	0.04	4.46**
A X B X C	231	0.05	2233	0.04	1.27**

* $p < 0.05$

** $p < 0.01$

Simple Effects of Control versus 1 Hz Group for Bipolar Early Component Amplitude during 10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	211.50	17	42.43	4.99*
Day(B)	11	1.36	187	0.27	5.03**
Stimulation intensity(C)	7	120.36	119	1.63	74.06**
A X B	11	0.50	187	0.27	1.86*
A X C	7	6.51	119	1.63	4.01**
B X C	77	0.05	1309	0.03	1.98**
A X B X C	77	0.03	1309	0.03	0.98

Simple Effects of Control versus PP-LFS Group for Bipolar Early Component Amplitude during10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	120.93	14	36.79	3.29
Day(B)	11	1.27	154	0.44	2.90**
Stimulation intensity(C)	7	112.80	98	1.43	79.14**
A X B	11	0.67	154	0.44	1.52
A X C	7	3.24	98	1.43	2.28*
B X C	77	0.05	1078	0.04	1.38*
A X B X C	77	0.04	1078	0.04	0.91

Simple Effects of Control versus 10 Hz Trains Group for Bipolar Early Component Amplitude during 10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	61.54	14	42.91	1.43
Day(B)	11	3.23	154	0.49	6.60**
Stimulation intensity(C)	7	127.99	98	1.85	69.31**
A X B	11	1.91	154	0.49	3.90**
A X C	7	1.60	98	1.85	0.87
B X C	77	0.12	1078	0.05	2.68**
A X B X C	77	0.09	1078	0.05	1.85**

Simple Effects of 1 Hz versus PP-LFS Group for Bipolar Early Component Amplitude during 10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	0.55	24	6.78	0.08
Day(B)	1	8.93	24	0.44	20.49**
Stimulation intensity(C)	6	13.17	144	0.17	76.36**
A X B	1	0.00	24	0.44	0.01
A X C	6	0.02	144	0.17	0.14
B X C	6	0.05	144	0.02	2.59*
A X B X C	6	0.01	144	0.02	0.62

Simple Effects of 1 Hz versus 10 Hz Trains Group for Bipolar Early Component Amplitude during 10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	0.25	23	6.94	0.04
Day(B)	1	18.20	23	0.76	24.04**
Stimulation intensity(C)	6	15.15	138	0.20	76.39**
A X B	1	1.64	23	0.76	2.17
A X C	6	0.04	138	0.20	0.22
B X C	6	0.18	138	0.03	6.93**
A X B X C	6	0.11	138	0.03	4.04**

Simple Effects of PP-LFS versus 10 Hz Trains Group for Bipolar Early Component Amplitude during 10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	1.51	23	6.21	0.24
Day(B)	1	17.71	23	0.76	23.33**
Stimulation intensity(C)	6	14.03	138	0.20	69.03**
A X B	1	1.79	23	0.76	2.36
A X C	6	0.12	138	0.20	0.61
B X C	6	0.26	138	0.03	8.80**
A X B X C	6	0.05	138	0.03	1.82

Simple Effects of Duration of LTP in 1 Hz Group for Bipolar Early Component Amplitude

Source	df Effect	MS Effect	df Error	MS Error	F
Day(A)	1	3.64	12	0.81	4.49
Stimulation intensity(B)	6	6.00	72	0.15	38.71**
A X B	6	0.09	72	0.04	2.31*

Simple Effects of Duration of LTP in PP-LFS Group for Bipolar Early Component Amplitude

Source	df Effect	MS Effect	df Error	MS Error	F
Day(A)	1	0.94	10	0.18	5.22*
Stimulation intensity(B)	6	5.48	60	0.15	37.54**
A X B	6	0.02	60	0.01	2.44*

Simple Effects of Duration of LTP in 10 Hz Trains Group for Bipolar Early Component Amplitude

Source	df Effect	MS Effect	df Error	MS Error	F
Day(A)	1	6.39	9	1.14	5.59*
Stimulation intensity(B)	6	8.61	54	0.21	40.35**
A X B	6	0.11	54	0.07	1.51

Table #4

Repeated-Measures Factorial Analysis of Variance for Bipolar Late Component Amplitude during10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	3	28.98	42	39.45	0.73
Day(B)	11	0.96	462	0.42	2.28**
Stimulation intensity(C)	7	261.10	294	2.96	88.34**
A X B	33	0.68	462	0.42	1.63*
A X C	21	5.48	294	2.96	1.85*
B X C	77	0.16	3234	0.09	1.88**
A X B X C	231	0.14	3234	0.09	1.59**

* $p < 0.05$

** $p < 0.01$

Simple Effects of Control versus 1 Hz Group for Bipolar Late Component Amplitude Pre Versus Post during 10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	23.65	25	42.28	0.56
Day(B)	11	0.26	275	0.65	0.65
Stimulation intensity(C)	7	216.53	175	81.74	81.74**
A X B	11	0.85	275	2.13	2.13*
A X C	7	1.15	175	0.43	0.43
B X C	77	0.08	1925	1.14	1.14
A X B X C	77	0.11	1925	1.56	1.56**

Simple Effects of Control versus PP-LFS Group for Bipolar Late Component Amplitude Pre Versus Post during 10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	69.18	21	43.03	1.61
Day(B)	11	0.15	231	0.44	0.34
Stimulation intensity(C)	7	113.99	147	3.43	33.20**
A X B	11	0.57	231	0.44	1.31
A X C	7	15.5	147	3.43	4.51**
B X C	77	0.13	1617	0.12	1.10
A X B X C	77	0.22	1617	0.12	1.91**

Simple Effects of Control versus 10 Hz Trains Group for Bipolar Late Component Amplitude Pre Versus Post during 10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	69.18	21	43.03	1.61
Day(B)	11	0.15	231	0.44	0.34
Stimulation intensity(C)	7	113.99	147	3.43	33.20**
A X B	11	0.57	231	0.44	1.31
A X C	7	15.50	147	3.43	4.51**
B X C	77	0.13	1617	0.12	1.10
A X B X C	77	0.22	1617	0.12	1.91**

Simple Effects of 1 Hz versus PP-LFS Group for Bipolar Late Component Amplitude during 10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	30.52	20	6.00	5.09*
Day(B)	1	3.76	20	0.30	12.65**
Stimulation intensity(C)	6	14.13	120	0.33	42.53**
A X B	1	0.13	20	0.30	0.43
A X C	6	1.44	120	0.33	4.32**
B X C	6	0.11	120	0.04	2.92*
A X B X C	6	0.05	120	0.04	1.41

Simple Effects of 1 Hz versus 10 Hz Trains Group for Bipolar Late Component Amplitude during 10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	0.25	16	6.13	0.04
Day(B)	1	8.59	16	0.54	15.78**
Stimulation intensity(C)	6	17.98	96	0.38	47.95**
A X B	1	0.82	16	0.54	1.51
A X C	6	0.04	96	0.38	0.10
B X C	6	0.46	96	0.06	7.36**
A X B X C	6	0.30	96	0.06	4.89**

Simple Effects of PP-LFS versus 10 Hz Trains Group for Bipolar Late Component Amplitude during 10 Day LTD Induction Period

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	19.08	16	5.36	3.56
Day(B)	1	6.84	16	0.44	15.39**
Stimulation intensity(C)	6	10.28	96	0.32	32.56**
A X B	1	1.49	16	0.44	3.35
A X C	6	0.96	96	0.32	3.02**
B X C	6	0.66	96	0.06	11.43**
A X B X C	6	0.15	96	0.06	2.64*

Simple Effects of Duration of LTP in 1 Hz Group for Bipolar Late Component Amplitude

Source	df Effect	MS Effect	df Error	MS Error	F
Day(A)	1	3.43	10	0.51	6.67*
Stimulation intensity(B)	6	11.05	60	0.44	24.86**
A X B	6	0.09	60	0.04	2.37*

Simple Effects of Duration of LTP in PP-LFS Group for Bipolar Late Component Amplitude

Source	df Effect	MS Effect	df Error	MS Error	F
Day(A)	1	0.92	8	0.56	1.64
Stimulation intensity(B)	6	2.46	48	0.27	8.94**
A X B	6	0.16	48	0.05	3.48**

Simple Effects of Duration of LTP in 10 Hz Trains Group for Bipolar Late Component Amplitude

Source	df Effect	MS Effect	df Error	MS Error	F
Day(A)	1	2.54	5	0.47	5.45
Stimulation intensity(B)	6	7.23	30	0.48	15.06**
A X B	6	0.40	30	0.05	7.96**

APPENDIX 2

ANOVA Tables

Induction of Long-Term Potentiation Leads to Increased Reliability of Evoked Neocortical Spindles *In Vivo*

Table #1

Repeated-Measures Factorial Analysis of Variance for Bipolar Early Component Amplitude during LTP Induction following CC Stimulation

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	13.28	16	24.44	0.54
Day(B)	8	1.95	128	0.22	8.70**
Stimulation intensity(C)	6	45.50	96	1.43	31.77**
A X B	8	0.68	128	0.22	3.03**
A X C	6	0.42	96	1.43	0.29
B X C	48	0.06	768	0.02	2.60**
A X B X C	48	0.04	768	0.02	1.46*

Table #2

Repeated-Measures Factorial Analysis of Variance for Bipolar Early Component Amplitude during LTPInduction following Thalamic Stimulation

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	13.52	11	58.73	0.23
Day(B)	8	0.84	88	0.32	2.64*
Stimulation intensity(C)	6	60.09	66	4.85	12.40**
A X B	8	0.15	88	0.32	0.48
A X C	6	0.90	66	4.85	0.19
B X C	48	0.07	528	0.05	1.41*
A X B X C	48	0.02	528	0.05	0.49

Table #3

Repeated-Measures Factorial Analysis of Variance for Bipolar Late Component Amplitude during LTP Induction following CC Stimulation

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	11.90	16	15.32	0.78
Day(B)	8	0.56	128	0.21	2.67**
Stimulation intensity(C)	6	85.32	96	1.56	54.77**
A X B	8	0.44	128	0.21	2.10*
A X C	6	1.27	96	1.56	0.81
B X C	48	0.07	768	0.04	1.67**
A X B X C	48	0.08	768	0.04	1.97**

Table #4

Repeated-Measures Factorial Analysis of Variance for Bipolar Late Component Amplitude during LTP Induction following Thalamic Stimulation

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	7.24	10	6.39	1.13
Day(B)	8	0.12	80	0.11	1.01
Stimulation intensity(C)	6	13.75	60	0.85	16.09**
A X B	8	0.04	80	0.11	0.33
A X C	6	1.49	60	0.85	1.74
B X C	48	0.04	480	0.03	1.50*
A X B X C	48	0.02	480	0.03	0.61

Table #5

Repeated-Measures Factorial Analysis of Variance for Occurrence of Evoked-Spindles during LTP Induction following CC Stimulation

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	1	17363.40	17	3920.43	4.43*
Day(B)	8	1448.20	136	375.12	3.86**
Stimulation intensity(C)	6	220899.30	102	945.04	233.74**
A X B	8	1316.80	136	375.12	3.51**
A X C	6	9679.40	102	945.04	10.24**
B X C	48	382.60	816	163.58	2.34**
A X B X C	48	441.60	816	163.58	2.70**

Simple Effects of Spindle Occurrence Pre-Post LTP Induction following CC Stimulation in the Experimental Group

Source	df Effect	MS Effect	df Error	MS Error	F
Day(A)	1	838.29	8	729.48	1.15
Stimulation intensity(B)	6	19562.25	48	263.52	74.24**
A X B	6	1408.32	48	173.20	8.13**

Simple Effects of Spindle Occurrence Pre-Post LTP Induction following CC Stimulation in the Control Group

Source	df Effect	MS Effect	df Error	MS Error	F
Day(A)	1	27.90	9	114.71	0.24
Stimulation intensity(B)	6	32671.13	54	316.22	103.32**
A X B	6	129.46	54	126.57	1.02

Simple Effects of Spindle Occurrence Pre-Post LTP Induction following Thalamic Stimulation in the Experimental Group

Source	df Effect	MS Effect	df Error	MS Error	F
Day(A)	1	4921.88	8	1007.25	4.89
Stimulation intensity(B)	6	11440.15	48	634.71	18.02**
A X B	6	237.27	48	346.09	0.69

Simple Effects of Spindle Occurrence Pre-Post LTP Induction following Thalamic Stimulation in the Control Group

Source	df Effect	MS Effect	df Error	MS Error	F
Day(A)	1	17.86	9	64.98	0.27
Stimulation intensity(B)	6	7065.10	54	845.69	8.35**
A X B	6	155.88	54	104.62	0.20

APPENDIX 3

ANOVA Tables

Effects of LTP on Responses Evoked by Direct Stimulation of Layer V Horizontal Projections in the Sensorimotor Cortex

Table #1

Repeated-Measures Factorial Analysis of Variance for Bipolar Spike Amplitude during LTP Induction

Source	df Effect	MS Effect	df Error	MS Error	F
Day(A)	1	210.10	10	37.91	5.45*
Stimulation intensity(B)	8	66.18	80	2.39	27.67**
A X B	8	0.93	80	1.62	0.58

Table #2

Repeated-Measures Factorial Analysis of Variance for Bipolar Early Component Amplitude during LTP Induction

Source	df Effect	MS Effect	df Error	MS Error	F
Day(A)	1	0.01	11	1.06	0.01
Stimulation intensity(B)	8	2.13	88	0.40	5.29**
A X B	8	0.03	88	0.03	0.87

Table #3

Repeated-Measures Factorial Analysis of Variance for Bipolar Late Component Amplitude during LTP Induction

Source	df Effect	MS Effect	df Error	MS Error	F
Day(A)	11	1.88	55	0.51	3.72**
Stimulation intensity(B)	8	24.70	40	1.78	13.86**
A X B	88	0.08	440	0.04	2.11**

Table #4

Repeated-Measures Factorial Analysis of Variance for Paired-Pulse Facilitation of the Early Component Across Stimulation Sites Following CC Stimulation

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	2	0.24	6	0.38	0.63
Stimulation intensity(B)	4	0.24	12	0.10	2.76
A X B	8	0.26	24	0.14	1.83

Table #5

Repeated-Measures Factorial Analysis of Variance for Paired-Pulse Facilitation of the Late Component Across Stimulation Sites Following CC Stimulation

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	2	2.55	8	0.15	16.63**
Stimulation intensity(B)	4	0.04	16	0.07	0.58
A X B	8	0.06	32	0.07	0.97

Table #6

Repeated-Measures Factorial Analysis of Variance for Paired-Pulse Facilitation of the Early Component Across Stimulation Sites Following Direct Layer V Stimulation

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	2	1.22	6	0.26	4.71
Stimulation intensity(B)	3	0.40	9	0.40	1.05
A X B	6	0.08	18	0.04	1.93

Table #7

Repeated-Measures Factorial Analysis of Variance for Paired-Pulse Facilitation of the Late Component Across Stimulation Sites Following Direct Layer V Stimulation

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	2	1.88	4	0.09	21.65**
Stimulation intensity(B)	3	0.22	6	024	0.90
A X B	6	0.03	12	0.04	0.79

Table #8

Repeated-Measures Factorial Analysis of Variance for Early Component Amplitude during LTP Induction following CC Stimulation for Distal Recording Sites

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	4	1.80	53	7.28	0.25
Day(B)	1	0.71	53	0.64	1.11
Stimulation intensity(C)	8	6.39	424	0.31	20.83**
A X B	4	0.18	53	0.64	0.28
A X C	32	0.09	424	0.31	0.28
B X C	8	0.03	424	0.04	0.60
A X B X C	32	0.02	424	0.04	0.52

Table #9

Repeated-Measures Factorial Analysis of Variance for Early Component Amplitude during LTP Induction following Direct Layer V Stimulation for Distal Recording Sites

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	3	0.73	39	13.87	0.05
Day(B)	1	1.89	39	0.42	4.49*
Stimulation intensity(C)	8	23.77	312	0.96	24.65**
A X B	3	0.12	39	0.42	0.29
A X C	24	0.12	312	0.96	0.12
B X C	8	0.07	312	0.03	2.24*
A X B X C	24	0.01	312	0.03	0.39

Table #10

Repeated-Measures Factorial Analysis of Variance for Late Component Amplitude during LTP Induction following CC Stimulation for Distal Recording Sites

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	4	1.30	53	4.33	0.30
Day(B)	1	6.30	53	0.46	13.79**
Stimulation intensity(C)	8	0.55	424	0.28	1.96
A X B	4	0.23	53	0.46	0.50
A X C	32	0.15	424	0.28	0.54
B X C	8	0.10	424	0.05	2.21*
A X B X C	32	0.07	424	0.05	1.61*

Table #11

Repeated-Measures Factorial Analysis of Variance for Late Component Amplitude during LTP Induction following Direct Layer V Stimulation for Distal Recording Sites

Source	df Effect	MS Effect	df Error	MS Error	F
Group(A)	3	0.53	38	1.25	0.43
Day(B)	1	0.00	38	0.07	0.05
Stimulation intensity(C)	8	0.07	304	0.23	0.31
A X B	3	0.04	38	0.07	0.63
A X C	24	0.10	304	0.23	0.44
B X C	8	0.02	304	0.02	1.07
A X B X C	24	0.02	304	0.02	1.24