NEOCORTICAL LONG-TERM DEPRESSION AND DEPOTENTIATION IN THE ADULT, FREELY MOVING RAT

By

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ABSTRACT

Information is believed to be stored in the brain by constructing new neural circuits, and these circuits are shaped by changes in the strength of the synaptic connections between the neurons making up the circuit. According to most theories of memory, new circuits can be formed by either increasing or decreasing the strength of synaptic connections. Bidirectional modifications in synaptic efficacy are also central components in recent computer simulations of learning and memory. While long-term potentiation (LTP) has been the focus of extensive research into the mechanisms underlying information storage in the mammalian brain, long-term depression (LTD) and depotentiation, its depressive counterparts, have not. Furthermore, most of the LTD research has involved the use of anaesthetized animals and in vitro slice preparations, making it more difficult to determine the role of this synaptic phenomenon in learning and memory in the intact behaving animal.

This thesis provides the first detailed examination of: 1) the induction and decay of both LTD and depotentiation in the neocortex of the awake, freely moving animal; 2) the effects of N-methyl D-Aspartate receptor (NMDAR) blockade on the induction of LTD, LTP, and depotentiation (NMDA receptor activation is known to play a major role in most forms of LTP); and 3) the interactions between these synaptic phenomena.

LTD was expressed as a significant reduction in the amplitude of both short- and long-latency field potential components. Depotentiation was expressed as a long

lasting decrease in the amplitude of a previously enhanced late component. LTD was found to be greater in magnitude and longer lasting when the conditioning stimulation was repeated. However, unlike LTP induction, the conditioning stimulation was equally effective whether spaced over hours or days.

NMDA receptor antagonism blocked LTP induction and instead produced a depression effect similar to LTD. Unlike LTP, LTD and depotentiation were found to be NMDAR-independent in the neocortex of the freely moving rat.

LTP and LTD are both reversible phenomena and LTD-inducing stimulation can modulate the effects of LTP-inducing stimulation. LTD-inducing stimulation, when delivered following to LTP-inducing stimulation, attenuates the induction rate for potentiation. LTD and depotentiation may play important roles in the ongoing experience-induced modification of neuronal connectivity. Furthermore, these results are consistent with the hypothesis that potentiation and depression reflect the physiological instantiation of a bidirectional learning rule.

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CHAPTER ONE

GENERAL INTRODUCTION

One of the most important features of the mammalian central nervous system is its capacity for processing and storing information. While learning involves the acquisition of new information, memory involves the storage of that information for later use. The neural basis of memory is usually studied indirectly by monitoring the effects of brain damage on subsequent cognitive abilities or by measuring neural activity in terms of the hemodynamic, magnetic, or electrical field changes. Learning and memory involve ongoing adaptations of brain circuitry throughout the lifetime in response to the environment and are generally thought to result from alterations in synaptic connectivity within the central nervous system (Iriki, Palvides, Keller & Asanuma, 1989; Hebb, 1949). The synaptic connectivity changes create new networks or circuits that are believed to represent newly acquired memories. The connectivity changes are also believed to rely on use-dependent enhancements of synaptic strength, which occur under situations of correlated pre- and post-synaptic activity (Hebb, 1949). This thesis describes some of the functional characteristics of, and the putative mechanisms involved in, synaptic strength modulation in the mammalian brain.

1.1 Memory Systems

The physical representation of a memory is referred to as the *engram* or *memory trace*. Although there is considerable information regarding the properties of memory formation and decay, studying the physical manifestation of memory remains difficult, beginning with a determination of where do memories reside. In the 1920s, Karl Lashley set out to determine the effect of various brain lesions on learning in rats. At the time, Lashley framed his work on the generally accepted belief that the engram could be located in specific areas of the neocortex based on the parcellations delineated in Broadman's cytoarchitectural maps. Lashley trained a rat to go through a maze to get a food reward. With repeated training, the rat reduced the time necessary to obtain the reward. Providing they were sufficiently large, brain lesions performed prior to training slowed the rat's progress, and brain lesions after training returned rats to the pre-training levels of performance. However, the memory deficits were not localised to specific brain regions, suggesting that the memory traces were distributed throughout the cortex.

In subsequent research, psychologists have distinguished several types of memory and have determined that there is considerable localization of function that was missed in Lashley's work. We now know that different types of information require the engagement of different neural systems. Two major subdivisions of memory are declarative (explicit) and nondeclarative (implicit). Declarative memory, memory for facts and events, is associated with awareness and intention to recall. It is generally rapidly acquired, flexible, and prone to distortion (Cohen and Squire,

1980; Squire, 1993). Nondeclarative memory includes priming, motor skill and emotional memory. It is nonconscious, slowly acquired (except for priming), and inflexible (Squire, 1992).

Declarative memory is associated with the awareness that one is remembering and is usually tested using recognition or recall tests. These memories rely on structures in the medial temporal lobe, including the hippocampus and the entorhinal, parahippocampal, and perirhinal cortices (Squire, 1992). Lesions to these structures produce deficits in declarative memory tasks (Scoville and Milner, 1957; Zola-Morgan et al., 1986; Squire et al., 1989; Squire and Alvarez, 1995) and functional imaging studies show activation in these areas during the encoding and recall of declarative memories (see Schacter and Wagner, 1999 for review). Declarative memory can be further subdivided into episodic memory, involving recollections associated with a time and place, and semantic memory, which is the recollection of facts without the environmental and temporal context.

Patients with bilateral medial temporal lobe lesions show both anterograde and retrograde amnesias (Scoville and Milner, 1957; Squire, 1992). They cannot acquire new episodic memories nor retrieve episodic memories stored shortly prior to the time of lesion. They can, however, retrieve declarative memories learned in the more distant past, suggesting that the storage of such information may depend, at least temporarily, on intact and functional medial temporal lobes.

Another type of memory associated with awareness involves the short-term retention of a perceptual representation and is termed working memory. Working and

declarative memory are dissociable because amnesic patients can show profound deficits in one form while retaining normal function in the other. Amnesic patients experience severe explicit memory deficits but normal working memory, and patients with parietal or frontal lobe lesions show poor working memory but normal explicit memory (Warrington et al., 1971). Among the implicit memories, lesion studies show that priming is particularly dependent on the neocortex. Priming involves pretraining such that the subject is better able to identify words that they have seen previously compared to novel words. Patients with localized lesions of the neocortex can show modality-specific impaired performance on priming tasks, while declarative and working memory remain normal (Tulving & Schacter, 1990). Motor skill learning refers to the progressive increase in speed and accuracy associated with given movements that occur with practice, and this process is distributed among the striatum, supplementary motor area, premotor cortex and primary motor cortex (Grafton et al., 1992).

Classical conditioning has been a major focus of memory research since the pioneering work of Pavlov (1927). By repeatedly pairing a neutral perceptual cue such as a tone, with a stimulus such as a piece of food that leads to a predictable or unconditioned response such as salivation, Pavlov showed that the subjects learn the relationship between the neutral cue and the appropriate response. The neutral cue has thus become the conditioned stimulus. Prior to conditioning, the neutral cue does not elicit salivation. After training, the neutral, now conditioned, cue reliably elicits salivation. The neural mechanisms of classical conditioning reside in a number of

CNS structures, including the cerebellum.

Emotional conditioning involves the pairing of a neutral stimulus such as a light, with a stimulus such as electric shock that evokes an emotional response (e.g., fear). The initially neutral stimulus comes to elicit a conditioned fear response (freezing, increased heart rate, urination, defecation, etc.). Fear conditioning experiments with rats have shown that the amygdala is important for this type of learning (LeDoux, 1995; Maren & Fanselow, 1996).

A significant role for the hippocampus in declarative memory was identified following neuropsychological research involving human patients that had undergone bilateral lesions of both the hippocampus and surrounding cortical structures (Scoville & Milner, 1957; Penfield & Milner, 1958). Lesion studies involving higher primates, including humans, have shown the importance of the hippocampus for the initial storage of information and the transfer of this information to long-term storage (Squire & Zola-Morgan, 1991). Clinical evidence also indicates that remote memories are spared following hippocampal lesions suggesting that other regions of the brain, including the neocortex may subserve the long-term storage of memory (Squire & Zola-Morgan, 1991).

While the hippocampus has the capacity to acquire new information rapidly, the neocortex is widely considered to be the site for long-term storage (Squire & Zola-Morgan, 1991; McClelland, McNaughton & O'Reilly, 1995; Murray & Bussey, 2001). Numerous studies show that the neocortex, like the hippocampus, displays plasticity. The monocular deprivation studies of Hubel & Weisel (1963, 1970)

illustrate one form of neural plasticity that is induced when the developing visual system is partially deprived of input. This work inspired an extensive body of research that focused on use-dependent neural plasticity in the visual cortex (Kirkwood & Bear, 1994a, 1994b; Aroniadou & Teyler, 1992; Singer & Artola, 1991; Berry, Teyler, & Taizhen, 1989). In particular, synaptic efficacy can be enhanced in neocortical neurons in slice preparations following exposure to the appropriate conditioning stimulation.

Iriki et al. (1989) found similar forms of synaptic plasticity in the motor cortex following electrical stimulation of the somatosensory cortex. They suggested that input to the motor cortex from the somatosensory cortex may be involved in the acquisition and/or retention of motor skills, and that the mechanisms involved in synaptic enhancement may subserve this learning and memory. Furthermore, coactivation of thalamocortical and corticocortical afferents has been shown to enhance the synaptic strength of thalamic input to the motor cortex (Iriki et. al., 1991).

The hippocampus is widely considered to be critical for the initial storage of declarative memories. It receives extensive input from neocortical systems and feeds information back to those same systems (McClelland et al., 1995). It has been suggested that the hippocampus provides a compressed trace for the temporary linking of component neocortical traces that must be activated together to read out the memory in its entirety. The hippocampus is required for reactivating the full neocortical representation (McClelland et al., 1995). The existence of temporally

graded amnesia further suggests that the conversion of short-term to long-term memory requires some period of consolidation. After multiple reinstatements, there may be sufficient links established between the component neocortical traces that they can now stand alone. This is a type of consolidation process.

Although the number of memory systems is the subject of ongoing debate, this topic is only briefly discussed here as it pertains to understanding the difficulty involved in studying the molecular and cellular mechanisms subserving a biological process that often seems almost intractable in its complexity. Memory is clearly distributed, but there is also a division of labors among the various memory systems. In this thesis, the focus is on the synaptic mechanisms that may be common to some or all of these systems. The target of these experiments is the neocortex, because it is believed to play a major role in long-term memory.

To understand the experimental approaches undertaken to study synaptic plasticity in the neocortex, it is important to consider its macroanatomical arrangement and cellular composition. The following section describes the overall arrangement and cellular constituents of the neocortex.

1.2 Cortical circuitry

Although all neocortical areas show a similar laminar organization, generally consisting of six discrete layers, different neocortical regions vary in both subcortical (Uylings, van Pelt, Parnavelas, & Ruiz-Marcos, 1994), and corticocortical connectivity (White, 1991) as well as in their specific cellular constituents (Campbell,

1905; see Kemper & Galaburda, 1984 for review). Functionally dissociable regions of the neocortex may be defined based on electrophysiological (Welker, 1976; Woolsey and Van der Loos, 1970; Mountcastle, 1957; Powell & Mountcastle, 1959), cytoarchetectonic (Caviness Jr, 1975), anatomical (White, 1991) and connectivity (Woosley 1958, 1964; see Rosenquist, 1985 for review) studies. The rat neocortex shows an organization and physiology that is similar to that seen in other mammalian species, and is well suited for neurophysiological and neuroanatomical investigations. The pyramidal cell is the dominant neuron and the primary output neuron in the neocortex, but there are also a variety of non-pyramidal neurons.

Extensive analysis and categorization of non-pyramidal cell types has been provided by various staining methodologies including Golgi impregnation (Fairen & Valverde, 1979), Golgi-electron microscopy (Fairen et al., 1981) and Nissl (Peters & Yilmaz, 1993) staining. Non-pyramidal neocortical neurons such as axon tuft, marinotti (White, 1991), chandelier (Marin-Padilla, 1987) and stellate (Lund, 1973; Lorente de No, 1938) cells exhibit wide variations in morphology including patterns of dendritic arborisation (Lund, 1973; Jones, 1975), axonal arborisation (LeVay, 1973), and synaptic connectivity (Gray, 1959).

Although the pyramidal cell is the primary output and association neuron in the cortex, the diversity that exists within the pyramidal cell group is only beginning to be explored in detail. Modern neuroanatomy has provided several possible techniques for the identification and categorization of pyramidal cell sub-types. Pyramidal neurons may be classified based on the projection site and laminar location

of the cell body by retrograde labelling techniques (Peters & Jones, 1984). This technique has been extended through combination with intracellular labelling methods, thereby permitting analysis of pyramidal cell morphology (Katz, 1987) and the distinction of cell subclasses. Pyramidal cells have also been further characterized into subclasses based on their electrophysiological behavior. As reported by White (1991), regular-spiking cells are so named because they exhibit relatively long action potentials, prominent spike-frequency adaptation, and brief excitation followed by prolonged inhibition in response to local stimuli. These regular-spiking neurons were all identified as pyramidal cells occurring in layers II through VI of the cortex. Bursting cells are pyramidal cells that generate bursts of three to five action potentials and are located in cortical layers IV or in upper layer V (White, 1991).

Although typically characterized by a uniformly laminar arrangement of cells throughout all cortical areas (White, 1991, Peters and Jones, 1984), neocortical compartmentalization is evident through both the vertical and horizontal planes (White, 1991). It was Lorente de No (1938) that first postulated a vertical columnar organization of intrinsic neurons within the cortex. The vertical orientation of pyramidal cells across cortical layers is an important aspect of neocortical cytoarchitecture.

The pyramidal cell is so named for its basically triangular profile, the apex of which points toward the cortical surface with the base pointing toward the white matter. Pyramidal neurons typically possess a thick dendrite emanating from the

apex and extending toward the cortical surface, while its basal dendrites extend from the cell body in an oblique, downward direction forming profuse branching patterns in all directions shortly after leaving the cell body (White, 1991).

The apical dendrite of a pyramidal cell is composed of a terminal apical tuft, which forms extensive arborisation, and a dendritic trunk that runs perpendicular to the pial surface, and transverses several cortical laminae with limited branching between the cell body and the apical tuft. Dendritic arborisations originating from large layer V pyramidal cells usually terminate in molecular layer I whereas those of small layer V pyramidal cells terminate in layer III (Uylings, van Pelt, Parnavelas and Ruiz-Marcos, 1994). Similarly, the axons of layer V pyramidal neurons are differentiable by size in that large pyramidal neurons tend to project subcortically while small pyramidal neurons provide callosal and ipsilateral intracortical projections (Uylings et al., 1994).

Pyramidal cell bodies are found from layer II through to layer VI. Synapses within layer I are a major source of input to many pyramidal cells, and these contacts are made with the most distal branches of the pyramidal cell's apical dendrites (layer 1 of the neocortex is a dense neuropil about 0.1-0.15 mm thick in rats, consisting largely of axons and synapses from many sources and dendrites arising from neuron somata in other layers). The apical dendrite of layer V pyramidal neurons generally ascends as a thick trunk through layers IV and III with relatively few oblique branches, but branches profusely in layers II and I to form the apical tuft. Layer V

pyramidal neurons, therefore, receive a substantial portion of their synaptic contacts more than 1.0 mm from the cell body.

The dendritic tuft comprises approximately 25% of the dendritic membrane in deep-layer pyramidal neurons and receives a correspondingly large number of inputs. Layer I receives widespread thalamic inputs as well as various cortico-cortical connections. For example, the projections from visual areas V2 and V3 to primary visual cortex V1 ascend directly to layer I synapses and travel up to 4.0 mm horizontally, making widespread synaptic contacts (Cauller and Conners, 1994). In addition to the primary efferent projection axon, cerebral cortical pyramidal cell axons show extensive collateral branching with many synapses forming immediately proximal to the neuron. There are also numerous horizontal connections that can extend substantially in primary motor cortex (M1) (Huntley & Jones, 1991; Hess & Donoghue, 1994). Field potential recordings in slices of M1 have revealed extensive and functionally strong horizontal pathways spanning layers II, III and V (Aroniadou & Keller, 1993; Donoghue et al., 1996). Pharmacological studies have shown these connections to be glutamatergic (Keller, 1993; Hess and Donoghue, 1994) and capable of expressing activity-dependent synaptic plasticity (Baranyi & Feher, 1978; Baranyi et al., 1991).

To the extent that pyramidal cell organization and physiology define the functional units within the neocortex, investigation of pyramidal cell distribution and function may provide insights into how regions of the cortex are processing information.

The second main type of neocortical neuron is the stellate cell. In primary sensory areas there is a heavy concentration of stellate neurons in layer IV (Peters and Jones, 1984), which are termed granule cells for their small size relative to pyramidal cells. Stellate cells are typically subdivided into two subclasses differentiated by the presence (spiny) or relative absence (non-spiny) of dendritic spines (Peters & Jones, 1984). These neurons are multipolar with dendrites extending from the somata in all directions and are distinguished from pyramidal neurons by the absence of an apical dendrite. While the axons of the spiny stellate cell typically extend from the cell body towards the white matter and branch profusely in the vicinity of the cell body, the axons of the non-spiny stellate cell branch out only within the cortex. Spiny stellate neurons have been identified in the primary visual (Lund, 1973), auditory (Winer, 1984a, 1984b) and somatosensory (Jones, 1974; Killacky, 1983) cortices.

The vertical organization of information flow in the rat neocortex is seen most clearly in the barrel fields, with each barrel representing single specialized sensory hairs from the periphery (Woosley & Van der Loos, 1970). Each barrel in the posteromedial barrel field represents one mystacial vibrissa, and stimulation of a specific vibrissum activates the population of neurons within the associated barrel (Welker, 1976), reflecting one component of an isomorphic representation of the body surface within the primary somatosensory cortex (Olavarria et al., 1984). The importance of the vibrissal component is evidenced by the extensive amount of cortical area it occupies (Welker, 1971).

The vertical columnar organization of the barrel fields is clearly seen in the

increased cytochrome oxidase activity that is found in the barrel hollows (Land and Simons, 1985; Wong-Riley and Welt, 1980), the distribution of layer 4 thalamic inputs that are localized in the barrel hollows, elevated 2-deoxyglucose uptake in vertical columns after stimulation in layers III through V and VI (Durham and Woosley, 1978; Melzer et al., 1985), and in the organization of pyramidal cell modules where apical dendrites of layer V pyramidal cells arc towards the barrel wall as they ascend from layer V into layer IV (White and Peters, 1993).

Cell bodies in the neocortex are organized in six layers parallel to the pial surface. The characterization of these discrete laminations between the pial surface and the underlying white matter is based on variations in cell packing density and the shapes and sizes of the neurons. Conversely, the motor cortex is thought to have only 5 layers and to be missing a layer IV (Donoghue and Wise, 1982; but see Skoglund et al., 1997).

In primary motor cortex (M1) there is a somatotopic representation such that specific cortical areas control movement in specific parts of the body, such as the arm, face, or leg. This somatotopy holds only for major body parts. Each of these major divisions is composed of separate, distributed networks of neurons for which somatotopy, in the customary sense, does not apply (Sanes & Donoghue, 2000). These networks are composed of large populations of neurons supporting widely distributed functions within the subregion (Sanes & Donoghue, 1997). Extensive functional mapping of the arm region of M1, for example, has been realized with intracortical electrical stimulation, which has provided a link between the stimulated

brain area and the body part in which movement is induced. When a comprehensive map of this underlying pattern of organization is assembled, however, multiple, widely distributed and overlapping locations for a given body part were observed (Donoghue et al., 1992; Nudo et al., 1992). A similar distributed internal representation has also been shown for the face (Huang et al., 1989) and the leg areas (Gould et al., 1986). Moreover, while global effects on arm movements are produced by focal inactivation of M1 in the monkey, the actions of individual parts are not blocked (Schieber & Poliakov, 1998).

It is clear that the neocortex is composed of a variety of neural processing systems, as evidenced by its complex anatomical arrangement. Additionally, the neocortex possesses mechanisms capable of altering synaptic strength within and between these systems as an adaptive response to environmental stimuli.

1.3 Models of Memory Mechanisms

How do we localize, let alone determine the mechanisms supporting a single memory *trace* or *engram*? There are approximately one million pyramidal cells in the hippocampus alone and an overwhelming number of synapses, and current experimental techniques only permit the simultaneous recording of about 100 pyramidal cells (Wilson & McNaughton, 1993).

1.3.1 Neural Network Models (Hebb rule and its variants)

Computer models of neural networks provide a means for trying to understand

how information is stored as patterns of synaptic strengths, or weights, in neural assemblies of various architectures. In this way, we might be able to understand information flow, encoding, and storage in at least simulated networks. Modeling can provide a test area for theories of information processing and learning algorithms.

Inspired by evidence for recurrent connections between neighboring cells in the cortex, Hebb postulated that cortical circuits support self-sustained activity that reverberates in what he termed "cell assemblies". There was already evidence that reverberating activity could last up to 500 ms. Hebb further suggested that one activated cortical circuit could, through converging projections, activate other cortical areas, leading to a chain of activations he called a "phase sequence". Although these ideas were quite speculative, there is a growing literature placing spike timing and spike synchrony experiments at the forefront of theoretical research on cortical neural processing (Abbott and Sejnowski, 1999). Hebb further postulated that by adjusting the connection strengths between neurons, using an activity-dependent mechanism for synaptic plasticity, new circuits could be constructed. In this way, the "memory trace" is encoded and the reinstatement of the reverberatory activity is facilitated. Many neural network models incorporate Hebbian theories of synaptic modification (Muller and Stead, 1996; Zador, Koch and Brown, 1990; Brown et al., 1990).

According to Hebb, synaptic strength increases when the pre- and postsynaptic cells fire together in time, but it is also essential that the synaptic strength not become too great (Hasselmo and Bower, 1993; Hasselmo and Schnell, 1994; Muller et al., 1996) and that the network does not saturate, with all connections

maximally enhanced. In many types of neural network models, learning is based on the *bidirectional* modification of synaptic weights. This alteration of synaptic efficacy employs simple activity-dependent rules that resemble the Hebb rule (Hebb, 1949) with the further provision that un-correlated pre- and post-synaptic activity leads to a decrease in synaptic strength. Networks that follow such rules can store multiple memories (Hopfield, 1982), organize topographic maps (Pearson, Finkel, & Edelman, 1987), develop input selectivity (Bienenstock, Cooper, & Munro, 1982), and find optimum solutions (Hopfield & Tank, 1986). These modelling data suggest that there must also be some physiological mechanism for long-term decreases of synaptic strength, in order to limit the saturation of network connectivity. Even after 50 years, Hebb's theory continues to be useful as a general framework for relating behavior to synaptic organization through the dynamics of neural networks.

1.3.2 Electrophysiological Models of Memory Mechanisms

The distributed nature of the memory trace makes studying the physiology of memory difficult. Researchers rarely know exactly where to look. Model phenomena that may utilize the same underlying mechanisms provide an alternative approach. Currently, the most heavily utilized models focus on activation-induced changes in synaptic function of known monosynaptic pathways. The most popular of these models is long-term potentiation (LTP).

1.3.2.1 Long-Term Potentiation

LTP is an experimental model of synaptic plasticity typically expressed as a long-lasting enhancement of the efficacy of synaptic transmission within activated pathways. This increased efficacy is reflected as an increase in the amplitude of an evoked post-synaptic response following intense, repetitive synaptic activation, and it can be induced by application of high frequency electrical stimulation (Bliss and Lomo, 1973; Bliss and Collingridge, 1993; Tsumoto, 1992; Tsumoto, 1990). LTP was first described in the rabbit hippocampal formation (Bliss and Lomo, 1973; Lomo, 1971) and was subsequently demonstrated in several excitatory synapses in the central nervous system of vertebrates (Iriki et. al., 1991, 1989; Artola and Singer, 1987; Racine, Milgram, and Hafner, 1983). Numerous studies have documented the induction of LTP in a variety of CNS pathways in both chronic and *in vitro* preparations (Malenka, 1994; Tsumoto, 1990,1992; Bliss and Collingridge, 1993; Kirkwood and Bear, 1994a; Racine et al., 1983; Beiko and Cain, 1998; Trepel and Racine, 1998).

LTP effects are specific to the activated pathway, but co-active pathways interact to produce "associative" LTP. Combined inputs can result in more LTP, particularly for weak inputs, than when each input is stimulated alone (Brown, Kairiss, and Keenan, 1990). While increases in synaptic strength are well documented, decreases in synaptic efficacy have received less attention.

1.3.2.2 Long-term Depression

Neural networks endowed with the ability to bidirectionally modify synaptic strength have considerably more flexibility and power than those incorporating only unidirectional changes. Theoretically then, there should exist a form of synaptic plasticity allowing for decreases in synaptic strength. There are various examples of use-dependent decreases in synaptic strength, which have been collected under the blanket term long-term depression (LTD). LTD is a long lasting decrease in the efficacy of synaptic transmission and can be measured as a decrease in the amplitude of evoked responses following patterned electrical stimulation of the afferents. Like LTP, LTD is not simply a single phenomenon and must be considered a generic term used to describe a variety of long lasting decreases in synaptic efficacy. LTD and LTP have been considered by many to reflect the instantiation of a bidirectional memory rule. However, while LTP has proven to be quite reliable, LTD has often proven difficult to demonstrate.

As a consequence of inducing LTP in one set of synapses, a heterosynaptic form of LTD can be induced in neighbouring synapses converging onto the same population of postsynaptic neurons (Lynch et al., 1977). This depression was first demonstrated as a decrease in the field response of commissural-CA1 synapses following the induction of LTP in the Schaffer collateral inputs to the same cells. The term "heterosynaptic depression" is applied because the depression depends on a burst of activity generated at synapses other than those being modified. This form of LTD occurs when converging afferents drive strong postsynaptic activity in the

absence of pre-synaptic activity in the inactive afferents to the same cells. Heterosynaptic depression appears to be triggered by strong postsynaptic depolarization (Pockett et al., 1990), is promoted by NMDAR activation (Abraham & Wickens, 1991; Desmond et al., 1991; but see Bradler and Barrionuevo, 1990), and may be mediated by calcium entry via voltage-dependent calcium channels (VDCCs; Wickens and Abraham, 1991). There are two hypotheses regarding heterosynaptic LTD mechanisms. According to the first hypothesis, when Ca^{2+} influx via NMDARs at active synapses reaches a high enough concentration, the Ca^{2+} ions passively diffuse to neighboring inactive synapses. This moderate elevation of Ca^{2+} at the inactive synapses may trigger a biochemical cascade resulting in heterosynaptic depression. Heterosynaptic LTD, however, is not always NMDAR-dependent and calcium influx does not rely solely on NMDARs. The second hypothesis recognizes that voltage-dependent Ca^{2+} channels (VDCCs) also allow calcium influx across the postsynaptic membrane and that activation of metabotropic glutamate receptors (mGluRs) can trigger a release of calcium from internal stores. However, if the critical mechanisms require activation of distant VDCCs, current source density analyses indicate that the affected synapses must be close enough in proximity to the source of the depolarization for the spread to be effective (White et al., 1988, 1990).

Using slice preparations, researchers have also found that prolonged presynaptic activity correlated with moderate postsynaptic depolarization can elicit a homosynaptic LTD in the rat hippocampus (Dudek & Bear, 1992; Mulkey & Malenka, 1992; Artola, Brocher & Singer, 1990; Dunwiddie & Lynch, 1978), the

visual cortex (Kirkwood & Bear, 1994b), the somatosensory and motor cortices (Castro-Alamancos et al., 1995; Hess & Donoghue, 1996) and in the cat sensorimotor cortex (Kitagawa et al., 1997). Homosynaptic LTD is confined to the synapses that were active during the conditioning stimulation and is typically induced by administration of 900 pulses at 1 Hz; an example is LTD in the hippocampus (Bramham & Srebro, 1987; Dudek & Bear, 1992; Mulkey & Malenka, 1992). Similar parameters have been used to induce hippocampal LTD in the acute and awake, behaving preparations (Staubli & Scafadi, 1997). LTD of striatal synapses, on the other hand, is typically induced using a higher frequency of stimulation (HFS; 50-100 Hz) applied for 1-5 s (Calabresi et al., 1992; Lovinger et al., 1993; Walsh, 1993). The striatal LTD also requires pharmacological or electrophysiological interventions to limit the level of postsynaptic activity during the HFS.

Hippocampal LTD is blocked by NMDAR antagonists (Dudek & Bear, 1992; Mulkey & Malenka, 1992; Desmond et al., 1991; Thiels et al., 1996; Manahan-Vaughan, 1997; but see Stanton & Sejnowski, 1989; Bolshakov & Siegelbaum, 1994) and by strong hyperpolarization, and Ca^{2+} chelation (Mulkey & Malenka, 1992), confirming that it depends on postsynaptic depolarization, NMDAR activation and increased postsynaptic Ca^{2+}. It is also saturable by repeated administration of LFS, reversible by subsequent LTP induction and can, itself, reverse previously established LTP (depotentiation).

1.3.2.3 Depotentiation

Previously established LTP in the rat hippocampus can be reversed or 'depotentiated' by sustained periods of LFS (Barrionuevo, 1980; Staubli and Lynch, 1990; Fujii et al., 1991; Larson et al., 1993; Wexler & Stanton, 1993; O'Dell and Kandel, 1994; Bashir & Collingridge, 1994; Doyle et al., 1997; Fujii & Sumikawa, 2001; Chen et al., 2001). Depotentiation has been induced in vitro (Fujii et al., 1991) and in vivo (Staubli & Lynch, 1990; Heynen et al., 1996) and has also been demonstrated in the amygdala in vitro (Aroniadou-Anderjaska et al., 2001). Depotentiation shares many induction requirements with LTD. Like LTD it is typically induced by LFS (Barrionuevo, 1980; Staubli and Lynch, 1990; Fujii et al., 1991; O'Dell & Kandel, 1994) and is blocked by antagonism of NMDARs (Fujii et al., 1991; O'Dell et al., 1994; but see Bashir & Collingridge, 1994), but it has also been shown to depend on mGluR activation (Bashir et al., 1993; Bortolotto et al., 1994). Depotentiation is restricted to a limited time window after the induction of LTP (Fujii et al., 1991; O'Dell & Kandel, 1994; Staubli & Chun, 1996) and is reversible by subsequent HFS (Dudek & Bear, 1993; Mulkey & Malenka, 1992; Heynen et al., 1996). In hippocampal area CA1 Xu et al. (1998) showed that HFS-induced LTP could be rapidly and completely reversed by exposure to a new, non-stressful environment without causing any change in baseline transmission in a control pathway. As in previous studies, this depotentiation was found to be time dependent, because LTP induction and maintenance of established LTP were not affected. An important question is whether or not LTD and depotentiation represent

the same synaptic phenomenon.

1.4 Biophysical and Pharmacological Mechanisms of Synaptic Plasticity

The pattern of afferent activity can change synaptic strength, but it is the subsequent biochemical activation of a variety of receptors and complex second messenger systems that mediates this process.

1.4.1 Glutamate and Glutamate Receptors

Excitatory amino acid receptors, including glutamate receptors, are a critical component of neural circuitry, because they are the principal mediators of excitatory synaptic activity and are involved in activity-dependent alterations in synaptic efficacy. Glutamate receptors are found throughout the mammalian brain and glutamate is the major excitatory neurotransmitter (Hollmann & Heinemann, 1994). Glutamate and aspartate are neurotransmitters of the majority of intrinsic cerebral cortical pyramidal and spiny stellate cells and of thalamic relay cells (Huntley et al., 1994).

The glutamate receptors have been organized into two groups based on their mechanism of action. The longest studied are the ionotropic glutamate receptors, which are ligand-gated channels that are permeable to cations. They have been further divided into three broad subtypes based on pharmacological and electrophysiological properties. Classified according to their strongest agonist they are: α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors, kainate

(KA) receptors, and N-methyl-D-aspartate (NMDA) receptors. More recently, Sugiyama et al (1987) identified a family of G-protein coupled receptors termed metabotropic glutamate receptors. Metabotropic glutamate receptors are coupled to a variety of second messenger systems via G proteins. Currently there are eight different mGluR subtypes that have been further subdivided into 4 groups based on amino acid sequence similarity, the second messenger system to which they are coupled via the G-protein, and agonist pharmacology. Group I mGluRs ($mgluR_1$ and $mgluR_5$) stimulate the mobilization of intracellular Ca^{2+} via phosphoinositide (PPI) hydrolysis, whereas Group II mGluRs ($mgluR_2$ and $mgluR_3$) and Group III mGluRs ($mgluR_4$ and $mgluR_{6-8}$) are negatively coupled to adenylyl cyclase activity. A fourth, as yet unassigned, group of mGluRs are coupled to phospholipase D (PLD).

Each group of ionotropic glutamate receptors is composed of a distinct set of receptor subtypes based on common electrophysiological and pharmacological properties and similar gene sequences: subunits GluR1-4 constitute the AMPA receptors; subunits GluR5-7 and KA1 and KA2 (kainate-binding proteins) constitute the kainate receptors; and subunits NMDAR1 and NMDAR2A-D compose the NMDA receptors (Huntley et al., 1994). Molecular cloning and functional expression techniques have shown that each of the ionotropic glutamate receptor subtypes (AMPA, KA, and NMDA) is composed of assemblies of subunit proteins. NMDA receptors, for instance, display low amplitude elicited currents when composed of only NMDAR1 subunits, are non-functional when composed of any NMDAR2 subunits without NMDAR1, and display large amplitude elicited currents when the

NMDAR1 subunit is combined with any of the NMDAR2 subunits. This suggests that the NMDAR1 subunit may be obligatory for all functional NMDA receptors. The integral channel of the NMDA receptor-channel complex is highly permeable to Ca^{2+}, Na^{2+} and K^{+} and the resultant increase in postsynaptic calcium concentration is thought to mediate the induction of neuronal plasticity. This receptor is distinguished from all others by the voltage-dependent channel block by Mg^{2+}.

AMPA receptors are the principal excitatory neurotransmitter receptors in the brain and are composed of four different subunits (GluR1-4) that can assemble in different combinations (Hollmann & Heinemann, 1994; Seeburg, 1993). These four receptors subunits are of similar size. The GluR1 subunit is expressed in the forebrain, hippocampus and neocortex (Hollmann & Heinemann, 1994). Zamanillo et al. (1999) have shown that LTP is absent in CA3 to CA1 synapses in mice lacking the GluR1 subunit of the AMPA receptor. Protein phosphorylation regulates the GluR1 subunit at two sites on its intracellular carboxy-terminal domain (Lee et al., 2000; Roche et al., 1996). While serine 845 is phosphorylated by PKA, protein kinase C (PKC) and CaMKII (Roche et al., 1996; Barria et al., 1997; Mammen et al., 1997) phosphorylate serine 831.

Analysis of LTP in the CA1 region of the hippocampus (Kelso, Ganong & Brown, 1986) has shown that LTP induction satisfies the Hebb rule such that synaptic weights are increased when there is concomitant presynaptic activity and postsynaptic depolarisation. Induction of LTP involves the N-methyl-D-aspartate (NMDA) class of glutamate-activated channels in the postsynaptic membrane (Collinridge, Kehl &

McLennan, 1983). These channels open only if there is both presynaptic release of glutamate and sufficient postsynaptic depolarisation (Mayer & Westbrook, 1984; Nowak et al., 1984), providing a physiological instantiation of the Hebb rule. Opening of the NMDA channel leads to the influx of Ca^{2+} (Jahr & Stevens, 1987; Teyler, Cavus & Coussens, 1995) that is critical for increasing synaptic strength (Mulkey & Malenka, 1992). The increase in synaptic efficacy is observed as an increase in the amplitude of an evoked excitatory postsynaptic potential (EPSP) or field potential, and the increased synaptic currents are carried primarily by the non-NMDA class of glutamate-activated channels (Muller, Joly & Lynch, 1988).

The responsiveness of a postsynaptic cell to presynaptically released glutamate is contingent upon the position and number of glutamate receptors in the postsynaptic membrane. Thus, the endocytotic and exocytotic mechanisms that regulate the surface expression of these receptors may be crucially important to the induction and/or expression of LTP, LTD and depotentiation. Xiao et al. (1994, 1995) have shown that LTD induced in hippocampal area CA1 is expressed as a decrease in both NMDA and AMPA currents. While it has long been assumed that NMDA receptors are static, recent research has shown that NMDAR stability is dependent on the NR2B subunit of the NMDAR (Roche et al., 2001). Roche et al. (2001) have shown that NMDARs can be internalized within the postsynaptic membrane following the deletion of the PDZ-binding domain of NR2B and that the synaptic protein PSD-95 inhibits NR2B internalization. Phosphorylation of NR2B, however, inhibits binding to PSD-95, thereby permitting receptor internalization.

AMPARs are also important for the induction of LTP, LTD and depotentiation. In cultured hippocampal neurons, enhanced miniature excitatory postsynaptic potentials following brief activation of NMDARs reflect a rapid insertion and increased clustering of AMPARs at the surface of the dendritic membrane (Lu et al., 2001). While LTP is thought to involve an increase in functional AMPAR number (Isaac et al., 1995; Liao et al., 1995; Durand et al., 1996; but see Grosshans et al., 2002), LTD and depotentiation have been linked to decreases in AMPARs. In hippocampal neuron cultures, Carroll et al. (1999) have shown that LTD induction is NMDAR-dependent, and is accompanied by a decrease in the amplitude and frequency of miniature excitatory postsynaptic currents. Using immunocytochemical analyses, they have also shown that the induction of LTD in this preparation caused a concomitant decrease in the number of AMPARs without causing any change in NMDA receptor clusters. Furthermore, both the LTD and the concomitant reduction in AMPAR number were dependent on NMDAR activation during the induction protocol.

1.4.2 Ca^{2+} Levels Related to Covariance of Pre-and Postsynaptic Activation Patterns.

In order for synaptic weights to be decreased, there must be a mechanism for converting a small net excitation to a biochemical change. Under anti-Hebb conditions of low correlation between presynaptic and postsynaptic events, low level Ca^{2+} fluxes can trigger second messenger cascades that ultimately decrease the connection strength of the affected synapses (Lisman, 1989; Kirkwood & Bear, 1994;

Mulkey & Malenka, 1992). The Ca^{2+} entry occurs mainly via non-NMDA glutamate-activated channels (MacDermott, Mayer, Westbrook, Smith & Braker, 1986), glutamate-stimulated release from internal postsynaptic stores (Murphy & Miller, 1989), and voltage-dependent Ca^{2+} channels (Llinas & Sugimori, 1980; Ross & Werman, 1987) all producing moderate increases in intracellular postsynaptic Ca^{2+} concentrations (Lisman, 1989).

When pre- and postsynaptic events are strongly correlated, the same Ca^{2+} entry mechanisms are activated, but the Mg^{2+} block at the NMDAR is released allowing large influxes of Ca^{2+} through the NMDA channel. Ca^{2+} fluxes will now exceed the threshold for triggering LTP.

As Ca^{2+} is a requirement for both LTP and LTD induction, Kirkwood and Bear (1994a) proposed a dual threshold for the induction of LTP and LTD. If postsynaptic increases in Ca^{2+} reach the lower threshold, LTD induction occurs, but if the higher threshold is reached, LTP is induced. Using repetitive low frequency stimulation, LTD at hippocampal synapses has been shown to last for more than 1 hour (Dudek and Bear, 1992, 1993), and this form of LTD is dependent upon increased post-synaptic Ca^{2+} during stimulation (Tsumoto, 1990; Mulkey and Malenka, 1992). Ultimately, then, the level of Ca^{2+} activity may determine whether LTP or LTD will be induced by neural activation. Several researchers have shown that LTP induction requires substantially higher increases in postsynaptic calcium concentration than does LTD (Lynch et al., 1983; Lisman, 1989; Bröcher et al., 1992; Mulkey & Malenka, 1992; Malenka and Nicoll, 1993; Kirkwood and Bear, 1994a;

Cummings et al., 1996; Hansel et al., 1997; Zhang & Poo, 2001).

LTP, LTD and depotentiation in the CA1 region of the hippocampus are all blocked by D-AP5, while only LTP is blocked by (3-((+/-)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid) CPP, suggesting that LTP and LTD are induced by pharmacologically distinct NMDA receptors (Hrabetova and Sacktor, 1997). While the requirement for postsynaptic calcium is well documented, its primary sources are less clear. In addition to a possible contribution of the NMDA receptor, voltage-dependent calcium channels (VDCCs) permit the entry of calcium into the postsynaptic neuron, depending on the membrane potential. Metabotropic glutamate receptors (mGluRs) can trigger the release of calcium from internal stores. Either of these routes may be sufficient to cause the small rise in the postsynaptic calcium concentration required for LTD. The dependence of LTD on mGluRs involves the activation of the 1,4,5-inositol triphosphate-linked subtypes of the mGluR, which is responsible for the increase in postsynaptic calcium concentration required for LTD induction (Kato, 1993). Metabotropic glutamate receptor-dependent mechanisms have also been shown to contribute to depotentiation effects (Kato, 1993; Haruta et al., 1994; O'Mara et al., 1995).

1.4.3 Phosphatases and Kinases

This differential involvement of Ca^{2+} in the induction of LTP and LTD suggests that the Ca^{2+} signal itself could determine whether synaptic weights increase or decrease. The synaptic weight of each synapse could, for example, depend on the

Ca^{2+}/calmodulin kinase II (CaM-kinase II) molecules stored within the postsynaptic density (Kennedy, Bennett, & Erondu, 1983). The postsynaptic density is a cytoplasmic structure immediately proximal to the postsynaptic receptors within each dendritic spine (Siekevitz, 1985). Ca^{2+} stimulates CaM kinase II to phosphorylate other enzymes and itself (autophosphorylation) (Saitoh & Schwartz, 1985; Miller & Kennedy, 1986). After Ca^{2+} has stimulated the autophosphorylation of the CaM kinase II at 2 or 3 of the 30 phosphorylation sites on this enzyme, the enzyme effectively switches to an "on" state in which it will phosphorylate itself and other substrates even after Ca^{2+} is removed (Lisman, 1989), providing an enduring record of changes in intracellular postsynaptic Ca^{2+} concentration lasting from minutes to hours. In this "on" state, the Ca^{2+}-independent autophosphorylation enables the CaM kinase II molecules to resist the resetting effects of phosphatase molecules such that these molecules can retain information with the stability required for long-term memory. Furthermore, the number of CaM kinase II molecules switched to the "on" state provides a measure of the increased intracellular postsynaptic Ca^{2+} concentration, effectively converting and storing the synaptic activity as a chemical gradient. The large influx of postsynaptic Ca^{2+} during LTP induction increases the synaptic weight by increasing the number of CaM kinase II molecules that are switched to the "on" state.

Moderate levels of Ca^{2+} influx, on the other hand, lead to dephosphorylation of the CaM kinase II. Dephosphorylation occurs via a phosphatase, specifically phosphatase 1 (Lisman, 1989) which is localized to the postsynaptic density (Sheilds,

Ingebritsen, & Kelly, 1985). More moderate increases of postsynaptic calcium during LFS bind to calmodulin and initiate activation of the phosphatases calcineurin (PP2B) and protein phosphatase 1 (PP1) (Mulkey et al., 1994). Under conditions of moderate Ca^{2+} influx, such as LTD induction, Ca^{2+}/Calmodulin activated conversion of ATP to cAMP via adenylate cyclase is increased, whereas conditions of high Ca^{2+} influx, inhibit this process (Lisman, 1989). Phosphatase 1 is active when bound to phosphorylated inhibitor 1 and inactive when inhibitor 1 is dephosphorylated (Nairn, Hemmings, Walaas & Greengard, 1988). Because inhibitor 1 is phosphorylated by a cyclic AMP-dependent kinase and dephosphorylated by the Ca^{2+}/ CaM-dependent phosphatase 2b (calcineurin), the level of Ca^{2+} influx can indirectly modulate the number of phosphorylated CaM kinase II molecules by altering the balance between kinase-dependent phosphorylation for increases in synaptic efficacy (LTP) and phosphatase-dependent dephosphorylation for decreases in synaptic efficacy (LTD). Thus Ca^{2+} acts as a biochemical postsynaptic second messenger storing a long-lasting measure of gradations of synaptic strength through gradations in the number of Ca^{2+} / CaM kinase II molecules switched "on".

It is also important to understand the biological machinery that mediates the long-term maintenance of synaptic plasticity. LTP that decays relatively rapidly, for instance, involves the post-translational modification of proteins (Lovinger et al., 1987; Malinow et al., 1988). A more persistent form of LTP, however, requires gene transcription and the synthesis of new proteins (Krug et al., 1984; Nguyen et al., 1994; Frey et al., 1996). The same dependencies likely apply to LTD.

Although most kinases are only briefly activated by second messengers, an autonomously active kinase could persistently potentiate synapses to maintain LTP over more intermediate durations (Lisman, 1989; Malinow, Madison and Tsien, 1988; Schwartz and Greenberg, 1987). Although persistently active forms such as CaM kinase II, PKA and PKC do exist, and they are important in induction, not all are required for maintenance. While inhibition of CaM kinaseII (Otmakhov, Griffith and Lisman, 1997), PKA (Malinow et al., 1988) or most forms of PKC (Denny, Polan-Curtain, Rodriguez, Wayner and Armstrong, 1990) during or immediately following the conditioning stimulus prevents LTP induction, it does not reverse previously established LTP (Malinow et al., 1988; Otmakhov et al., 1997; Denny et al., 1990). However, one autonomously active, independent catalytic domain of an atypical PKC isozyme does increase with (Sacktor, Osten, Valsamis, Jiang, Naik and Sublette, 1993) and is required for LTP maintenance for at least several hours (Ling, Benardo, Serrano, Blace, Kelly, Crary and Sacktor, 2002).

1.5 Synaptic Plasticity in the Neocortex

The hippocampus clearly possesses the capacity for synaptic weight change required under Hebb's rule for information storage in a network of neurons. It is important, then, to determine whether or not the neocortex, the putative site for long-term memory, also possesses this capacity.

1.5.1 Neocortical Long-Term Potentiation

LTP has been demonstrated in a number of neocortical sites in acute preparations (Racine, Wilson, Teskey & Milgram, 1994; Iriki et. al., 1989) and slice preparations (Kirkwood & Bear, 1994a; Brocher et. al., 1992; Sutor & Hablitz, 1989), but it has only recently been demonstrated in awake freely moving rats following non-epileptogenic stimulation (Racine et al., 1995). Whereas LTP in most subcortical structures can be rapidly induced, reaching near asymptotic levels with only one stimulation session, the neocortex requires that the stimulation be spaced and repeated. It is not yet clear what mechanistic differences underlie these differences in LTP induction rules, but there is a greater complexity of neural connectivity in the neocortex compared with the hippocampus (Thomson & Deuchars, 1994; Mumford, 1991, 1992; Tsumoto, 1990).

The maintenance of neocortical LTP can only be monitored for minutes or hours in the anaesthetized (acute) and slice preparations (Arondiadou, & Keller, 1995; Kimura, Caria, Melis, & Asanuma, 1994; Wilson & Racine, 1983; Castro-Alamancos et al., 1995). Also, neocortical LTP in the slice is often dependent on the reduction of inhibiton by application of GABAergic blockers such as bicucculine (Kirkwood & Bear, 1994; Bear & Kirkwood, 1993; Hess, Aizenman & Donoghue, 1996; Hess et al., 1996).

Racine et al (1995) found that neocortical LTP in chronic preparations was reliably induced, without reducing inhibition and was long lasting. However, the LTP induction required repeated daily sessions of electrical stimulation applied to the

white matter. The LTP was expressed most clearly as an increase in the population spike and late component amplitudes (Racine et al., 1995; Trepel et al., 1998). The monosynaptic EPSP component was also enhanced, but it is normally obscured by the distributed population spike components (Chapman et al., 1998). Chapman et al. (1998) showed that the amplitude of the early component is only clearly observable when the cell firing is attenuated by either anaesthetic or during frequency of following tests (80 hertz).

1.5.2 Neocortical Long-term Depression and Depotentiation

LTD has been induced at neocortical synapses using both LFS (Berry et al., 1989; Krikwood et al., 1993) and HFS (Artola et al., 1990; Hirsch & Crepel, 1990; Kimura et al., 1990; Kato, 1993). In the in vitro slice preparation homosynaptic LTD of visual cortical responses recorded in layer III can be induced by LFS applied to layer IV and this LTD is both reversible and NMDAR dependent (Kirkwood et al., 1993; Kirkwood et al., 1994). LTD has also been shown to be NMDAR dependent in vitro in the somatosensory and motor cortices (Castro-Alamancos et al., 1995) and thalamocortical synapses in the barrel cortex (Feldman et al., 1998). However, LTD at corticostriatal synapses (Calabresi et al., 1992; Lovinger et al., 1993; Walsh 1993) and some visual cortical synapses (Artola et al., 1990; Kato, 1993) have been observed despite the application of NMDAR antagonists. Also, HFS delivered during NMDAR blockade has resulted in the induction of LTD in visual (Teyler et al., 1990) and prefrontal (Hirsch & Crepel, 1991) cortices. Visual cortical LTD has been

blocked by mGluR antagonism but not NMDAR blockade (Kato, 1993; Haruta et al., 1994). There is considerable controversy in the literature regarding the mechanisms as well as the functions of LTD in the neocortex. Even the importance of calcium remains unclear. Calcium chelators, injected into the postsynaptic neuron, have been shown to block the induction of LTD by HFS (Brocher et al., 1992; Hirsch & Crepel, 1992) or block the induction of LTP without blocking LTD (Kimura, 1990; Yoshimura et al., 1991).

Behavioral LTD has been observed in the kitten visual cortex. By comparing eyelid suture- and tetrodotoxin-induced monocular deprivation, Rittenhouse et al. (1999) were able to show that monocular deprivation-induced LTD was greater in pathways originating from the sutured eye. While tetrodotoxin eliminates synaptic activity, suturing of the eyelid limits vision to residual retinal activity and the responses to nonpatterned diffuse illumination changes. This suggests that homosynaptic LTD underlies monocular deprivation.

The vast majority of demonstrations of LTD and depotentiation in the neocortex are based on in vitro slice and in vivo anesthetized preparations. Also, most of this work has focused on LTD; research on depotentiation has been sparse.

It remains to be seen whether or not the artificially induced synaptic phenomena, LTP, LTD and depotentiation, represent memory mechanisms. If they do, it is still not clear how the events recorded in the slice and acute preparations, which can only be monitored for minutes or hours, relate to long-term memory. Furthermore, in in vitro preparations, differences in the chemical solutions,

temperature, stimulus protocol and developmental age of the specimen can produce plasticity with different forms and longevity. It is not always clear how these effects relate to those in intact, awake preparations. It is of critical importance that these phenomena also be studied in the awake behaving animal, without the extreme deafferentation of the slice preparation or the anesthesia of the acute preparation. Although LTD is generally accepted as a valid form of activity-dependent synaptic plasticity, the mechanisms of LTD and its relation to LTP and depotentiation remain unclear. Furthermore, it is unknown whether the various forms of LTD and depotentiation occur in the intact neocortex. The following chapters outline the experimental investigation of the induction and decay properties of LTD and depotentiation. They also address the pharmacological properties of these phenomena, and the interactions between LTD and potentiation in the neocortex of the awake, behaving rat.

CHAPTER TWO

Long-Term Depression and Depotentiation in the Sensorimotor Cortex of the Freely Moving Rat

Various neural network models incorporate rules for the bidirectional modification of synaptic weights that are based on variations of the "Hebb synapse" (Hebb, 1949). These bidirectional learning rules specify that connection weights will be *increased or decreased* depending on whether the pre- and post-synaptic activity is *correlated or uncorrelated*, respectively (Brown et al., 1990). Such rules prevent the saturation of enhanced connection weights and maintain the capacity of the circuit to store new information.

The implementation of bidirectional learning rules in real neuronal circuits requires mechanisms that support both increases and reductions in synaptic strength. Experimentally, increases in synaptic strength can be induced by brief, high frequency stimulation of afferent pathways, while prolonged low-frequency stimulation leads to reductions in synaptic drive. These phenomena are referred to as long-term potentiation and long-term depression (or LTD). Depression of potentiated responses is referred to as depotentiation.

Long-term synaptic potentiation (LTP) is currently the most widely investigated model of the synaptic mechanisms underlying memory formation in the mammalian brain (Bliss and Lomo, 1973; Tsumoto, 1992; Bliss and Collingridge, 1993). It can be reliably induced in several forebrain structures, including the

hippocampus and neocortex (e.g., Racine et al., 1983; Beiko and Cain, 1998; Trepel and Racine, 1998).

The literature is not entirely consistent, however, regarding the induction of LTD and depotentiation, at least in the hippocampus. Stimulation protocols that successfully induced LTD and depotentiation in the dentate gyrus *in vitro* were ineffective *in vivo* (Errington et al., 1995; Abraham et al., 1996). In the freely moving rat, prolonged low-frequency stimulation of hippocampal area CA1 has been reported to produce a number of outcomes, including reliable LTD (Manahan-Vaughan, 1997), depotentiation but no LTD (Staubli and Lynch, 1990; Doyle et al., 1997), or neither depotentiation nor LTD (Errington et al., 1995). Both LTD (Thiels et al., 1994; Heynen et al., 1996) and depotentiation (Heynen et al., 1996) have been successfully induced in area CA1 in the anaesthetized *in vivo* preparation. Staubli and Scafadi (1997), on the other hand, were unable to induce LTD in this preparation using similar patterns of stimulation, and Barrionuevo et al. (1980) could only induce depotentiation.

The few demonstrations of LTD or depotentiation in the neocortex have been conducted in slice (Kirkwood and Bear, 1994b; Castro-Alamancos et al., 1995) or anesthetized (Tsumoto and Suda, 1979; Burette et al., 1997) preparations. In this paper, we demonstrate reliable long-term depression and depotentiation of field potentials recorded from the sensorimotor cortex of the chronically prepared rat. The use of this preparation also allowed us to confirm that the LTD and depotentiation effects were long lasting, and to examine some of the stimulation parameters that

affect the longevity of these effects. Because LTP induction in this preparation requires repeated daily sessions of stimulation (Racine et al., 1995; Trepel and Racine, 1998), we also tested for differences in LTD induced by single or multiple stimulation sessions.

MATERIALS AND METHODS

Animals and Surgery

Fifty-four male Long-Evans rats (300-400 g) from the McMaster University Breeding Colonies were used in these experiments. Rats were anaesthetized with Somnotol (sodium pentobarbital, 65 mg/kg i.p.) and received atropine (1.2 mg/kg) to prevent respiratory distress. Twisted wire bipolar electrodes were constructed from Teflon-coated stainless steel wire (120 μm diameter), and the exposed tips were separated by 1.0 mm for cortical placements and 0.5 mm for the callosal stimulating electrodes. Electrodes were implanted into either the sensorimotor cortex (M1 bordering on S1) or the white matter in the same coronal plane. Sensorimotor cortex electrodes were placed 2.0 mm anterior to bregma and 3.5 or 4.0 mm lateral to the midline at a depth of 2.0 mm from pia (Paxinos and Watson, 1997). White matter electrodes were placed 2.0 mm anterior to bregma, 2.0 mm lateral to the midline, at a depth of 3.0 mm from pia. Electrode depths were adjusted during surgery to maximize field response amplitudes. The electrodes were connected to gold-plated pins and inserted into a connector plug that was anchored to the skull surface with dental cement and 4 stainless steel screws. One screw in the right occipital bone served as a ground electrode.

In this preparation, the electrode resistance is always around 20 KΩ. To verify that the low-frequency stimulation parameters used in the following experiments did not change the resistance, we monitored resistance in both stimulating and recording electrodes in 3 animals before and after multiple sessions of

stimulation. The resistances were 19.32, 19.05 and 20.64 KΩ for the stimulating electrodes and 20.91, 21.09 and 23.44 KΩ for the recording electrodes (measured at 250 μA). These resistances did not change after three sessions of low-frequency stimulation (which reliably produced a long-term depression effect).

Rats were housed individually on a 12 h/12 h light/dark cycle and tested during the light cycle. A two-week recovery period preceded experimental testing.

Stimulation and Recording

Input/Output Tests. During input/output (I/O) tests, stimulation pulses were delivered at varying intensities to the appropriate stimulation site (see below), and evoked field potentials were monitored in the cortex. Single 0.1 msec biphasic square wave pulses were delivered through constant current isolation units at a frequency of 0.1Hz. The I/O test included 8 responses evoked at each of 10 logarithmically spaced intensities (16, 32, 64, 100, 160, 250, 500, 795, 1000, and 1260 μA). The responses were filtered (0.3 Hz to 3 kHz), amplified, digitized at 10 kHz, and stored on a computer hard drive. Two or three baseline I/O tests, separated by 48 hours, were used to confirm the stability of the evoked responses.

For each experiment, animals were divided into experimental and control groups that were matched for response morphology, amplitude and threshold. Control groups did not receive train stimulation, but otherwise followed the same regimen as experimental groups.

Long-Term Potentiation. In experiments requiring LTP induction, 30 high-frequency trains were delivered once per day for ten days. Each 24 ms train consisted of 8-pulses at 300 Hz, and the trains were delivered once every 10 sec. Pulse intensity was 1260 μA, and the pulse duration was 0.1 ms. Paper records of EEG activity were monitored to confirm that epileptiform discharges were not evoked. Input/output tests were recorded 1 and 7 days after the LTP induction procedure to confirm that the potentiation effects were long lasting.

Long-Term Depression and Depotentiation. To induce long-term depression, low-frequency stimulation (1 Hz, 900 pulses, 1260 μA) was delivered immediately following the last baseline I/O test. To examine depotentiation, the low-frequency stimulation was delivered eight days following LTP induction. Input/output tests were recorded immediately, 1 day, 2 days, and 7 days after low-frequency trains to determine the longevity of the effects.

Design

There were three experiments. The first dealt with both depotentiation and LTD effects produced by stimulation of the contralateral homologous site. The second experiment compared depotentiation effects produced by ipsilateral and contralateral stimulation. The third experiment dealt with LTD induced by ipsilateral white matter stimulation.

Depotentiation and LTD of the Interhemispheric Response. The first experiment examined whether LTP induced in the right sensorimotor cortex by tetanization of the left

motor cortex could be reversed or depotentiated by low frequency stimulation. In addition, low frequency stimulation applied to unpotentiated animals provided a test for LTD.

Twenty-four hours after the second baseline I/O test, LTP was induced in 9 animals by 10 days of high-frequency stimulation. The high-frequency stimulation was applied directly to the left sensorimotor cortex. An additional eight animals served as unpotentiated controls. Responses were recorded in the homologous site in the right hemisphere. I/O tests were recorded 1 and 7 days after LTP induction to confirm that the potentiation effects were truly long lasting. Five of the potentiated animals were then tested for depotentiation, while 4 of the animals remained unstimulated to confirm that the potentiation was maintained in the absence of low-frequency stimulation. Similarly, 4 of the unpotentiated control animals received low-frequency stimulation to test for LTD effects in the contralateral field potential. The remaining 4 control animals served to ensure that the baseline responses remained stable. I/O tests were recorded immediately and 1 day, 2 days and 7 days after delivery of low-frequency trains to determine the longevity of the depotentiation and LTD effects.

Depotentiation of Ipsilateral versus Contralateral Responses. The transhemispheric depotentiation effects in the previous experiment were found to be reliable, but short lived. In this experiment, the longevity of depotentiation induced by contralateral stimulation was compared to depotentiation induced by ipsilateral stimulation. Recording electrodes were implanted into the right sensorimotor cortex in all animals. Stimulating electrodes were implanted into either the contralateral

homologous site (n = 7) or into the ipsilateral white matter (n = 6). Following 3 baseline I/O tests, high frequency trains were used to induce LTP in all animals. Seven days following LTP induction, low-frequency trains were delivered and the extent of depotentiation induced in the contralateral and ipsilateral stimulation groups was compared. To monitor the early phase of the decay of depotentiation, I/O tests were recorded immediately following the low-frequency stimulation, hourly for six hours, and after 8, 10 and 12 hours. Follow-up I/Os were also collected 24 and 48 hours post-treatment.

Long-term Depression. In the final experiment, LTD of field responses evoked by stimulation of the ipsilateral white matter was examined, and LTD effects induced by either single or multiple low-frequency trains were compared. Further, the effect of spacing the trains over time was also assessed. Stimulating electrodes were implanted into the white matter of the right hemisphere and recording electrodes were placed ipsilaterally into sensorimotor cortex. Following 3 baseline I/O tests, the animals were divided into three experimental groups (single-train, n=6; massed-trains, n=6; spaced-trains, n=6; and one control group, n=6). Animals in the single train group received one low-frequency train, while animals in the multiple train groups received 10 trains. The massed-trains group received 10 trains on a single day at hourly intervals. The spaced-trains group received one train per day for 10 days.

In the single-train group, I/O recordings were taken immediately, 1, 2, 4, 8, and 12 hours following the low-frequency train to monitor the early phase of LTD decay. In the massed-trains group, I/O tests were recorded after each train, and in the spaced-trains

group, I/O tests were recorded before and after each train. In all groups, I/O recordings were taken every day for 7 days, and weekly for two weeks, following the last low-frequency train.

Data Analysis

Changes in the field potentials over LTD (or LTP) and decay sessions were measured by subtracting the final baseline responses from all other baseline and depressed (or potentiated) responses. All data points were thus standardized to the final baseline response. For depotentiation experiments, changes were assessed relative to the last post-LTP I/O test. Measures (mV change from baseline) were taken at two latencies representing the early, monosynaptic, and late, polysynaptic, components (Chapman, et al., 1998). Changes in response amplitudes (in mV) were analysed using mixed design ANOVAs and the Tukey HSD post-hoc test. The changes are expressed as percent of baseline amplitude (group means) evoked at low to midrange intensities (see below).

Histology

Rats were deeply anaesthetized with urethane (2.0 g/kg) and perfused through the heart with formol-saline. Frozen brain sections were cut at 40 μm and stained with Cresyl violet to verify electrode placements.

RESULTS

Histological examination confirmed that the electrode tips were in their intended targets for all animals. The M1 field potentials were as characterized by Chapman et al. (1998). Both contralateral and ipsilateral responses showed overlapping monosynaptic EPSP and population spike components. The population spikes tended to be small, began at about 3 ms and repeated over 9-33 ms. The polysynaptic component peaked at about 18.0 ms for contralateral responses and 19.5 ms for ipsilateral responses. Ipsilateral responses often contained a broader and more complex waveform, compared to the contralateral response, which may account for the slightly longer latency to peak.

Depotentiation of Interhemispheric Responses

Long-term Potentiation. Representative responses evoked in the right sensorimotor cortex by stimulation of the homologous site in the left hemisphere are shown in Figure 2-1. LTP induction was characterized by an increase in the repetitive population spike activity (which masks the enhancement of the early monosynaptic EPSP) and a potentiation of the longer-latency, polysynaptic late component (Trepel and Racine, 1998; Chapman et al., 1998). Subsequent depotentiation and LTD effects in the contralateral responses were reliably seen only in the late component, so only late component measures are reported for this experiment.

The largest potentiation effects for the late component were observed at intermediate test pulse intensities. At an intensity of 795 μA, the late component increased by 1.35 ± 0.26 mV from baseline amplitude, while control animals showed a change of only 0.12 ± 0.06 mV ($F(1,15)=16.69$; $p<0.001$). The potentiation decayed by

only 16.6 ± 8.0 % during the following 7 days (Fig. 2-2). Responses evoked in the control animals remained stable throughout the experiment.

Depotentiation and LTD. Low frequency stimulation delivered 8 days after LTP induction resulted in a reliable depotentiation of the late component compared to controls, but the earlier components were not markedly affected (Fig. 2-1B). The potentiated animals showed a mean late component depotentiation of 42.0% immediately after low-frequency stimulation. This depotentiation effect was short-lived, however, and the responses increased back to potentiated levels in less than 24 hours (Fig. 2-2). Responses in potentiated animals that did not receive low-frequency stimulation were only slightly reduced in amplitude from the previous day's level. A repeated measures ANOVA performed on the data following low frequency stimulation showed that the difference between the groups was significant ($F(1,7)=202.7$; $p<0.001$). Two of the four animals that were not potentiated showed a transient depression in the late component following low-frequency stimulation, (Fig. 2-1C&2-2), but this effect was not statistically significant.

Depotentiation of ipsilateral vs. contralateral responses

In the previous experiment, when stimulation was applied to the homologous site in the contralateral hemisphere, the depotentiation effects were significant, but short-lived. The effects were also restricted to the polysynaptic components. The second experiment was designed to determine if depotentiation might be more robust and long-lasting in response to ipsilateral white matter stimulation.

Long-Term Potentiation. A 400 μA pulse was added to the I/O test to increase resolution at the midrange intensities. Maximum LTP effects occurred at low to midrange test pulse intensities following both contralateral (125 μA; n=2 of 7, 250 μA; n=2 of 7 and 400 μA; n=3 of 7) and ipsilateral (125 μA n=1 of 6, 250 μA n=4 of 6 and 400 μA n=1 of 6) tetanization. Both groups displayed an enhancement in the population spike (Fig. 2-3B) and late component (Fig. 2-3C) amplitudes reflected by significant main effects of session ($F(1,11=90.2$; $p<0.001$ and $F(1,11)=26.25$; $p<0.001$, respectively). These LTP effects were slightly larger in the contralateral responses than in the ipsilateral responses, but these differences were not statistically significant ($F(1,11)=1.85$; $p>0.2$ and $F(1,11)=3.55$; $p>0.08$, respectively).

Depotentiation. Although a few animals showed what appeared to be a stimulation-dependent depotentiation of the early component in the ipsilateral response, this effect was not significant (Fig. 2-3A&2-3B). There was also no significant difference between ipsilateral and contralateral effects in the early component measures. The decreases in late component response amplitudes following low-frequency stimulation in both groups are reflected in a main effect of tetanization ($F(1,11)=19.33$; $p<0.002$). As in the first experiment, the low-frequency stimulation produced only a weak and short-lived change in the response amplitude of the contralateral response. The depotentiation was nearly complete, however, in the late component of the ipsilateral response, with amplitudes returning to the baseline levels recorded before LTP was induced (Fig. 2-3C). The depotentiation effect was still strong 24 hours later. The

contralateral response, on the other hand, returned to potentiated levels after 24 hours, and analysis of the pre- and post-low-frequency stimulation measures, between ipsilateral and contralateral responses, showed a nearly significant interaction effect ($F(1,11)=4.16$; $p<0.06$). This experiment confirmed that potentiated responses could be depressed by low-frequency stimulation, and showed that these effects were still evident at 24 h.

Long-Term Depression

The long-term depression of interhemispherically-evoked baseline responses observed in the first experiment was quite weak and short-lived. This experiment was designed to test LTD effects produced by low-frequency stimulation of ipsilateral white matter, and to determine if LTD could be more effectively induced by massed or spaced stimulation trains than by delivery of a single low-frequency train. Results showed that the amount of LTD induced depended on whether the animals received single or multiple trains of stimulation (Fig. 2-4), and this was reflected in a significant main effect of group for both the early ($F(3,20)=7.45$; $p<0.002$) and late ($F(3,20)=6.41$; $p<0.004$) component measures. Subsequent comparisons will be pair-wise between groups.

LTD Induced by a Single Low-Frequency Train. A single LFS train produced a small, nonsignificant depression of the early component (Fig 2-4; Tukey HSD; $p>0.42$) and a statistically reliable depression of the late component (Fig. 2-4; Tukey HSD; $p<0.001$) compared to controls. There was a mean decrease of 54.0% in late component field response amplitudes immediately following low-frequency stimulation. The maximum LTD effect occurred at midrange intensities (160 μA, n=3 of 6 and 250 μA

n=3 of 6). Although the mean response amplitude was still depressed by 29.0% 24 hours later, the difference was no longer statistically significant (Tukey HSD; $p>0.13$). After 1 and 2 weeks, the decrease in response amplitude was further reduced to 13.0% and 13.7% respectively.

All six animals exhibited an increase in evoked potential thresholds, such that the stimulus intensity required to evoke a response was greater after LTD induction than during baseline recording. To assess whether or not the LTD effect was associated with neural damage, high-frequency trains were subsequently applied to the white matter to determine if LTP could still be induced. Responses were enhanced by the third day of high-frequency stimulation, and reached asymptotic levels after 8 or 9 days of stimulation (data not shown). There was a 10-fold increase in the amplitude of the late component at low to midrange intensities (160-250 μA) following LTP induction, indicating that the affected synapses are capable of supporting further plastic changes following LTD induction.

LTD Induced by Massed, Multiple Stimulation Trains. Multiple low-frequency stimulation trains delivered on one day produced significant LTD effects, and both early and late components were affected (Fig. 2-4 A&B). Moreover, the LTD effect was significantly larger at the end of the 10 stimulation trains than it was after the first train for both the early ($F(9,45)=8.84$; $p<0.001$) and late ($F(9,45)=4.40$; $p<0.001$) components (Fig. 2-5B&C). Maximal depression was observed at low to midrange intensities (160-250 μA). Immediately following the LFS, the mean amplitude of the early component was depressed by 39.9% from baseline, which was significantly different from controls

(Tukey HSD; $p<0.001$). During the first 24 hours, and at 7 and 14 days post conditioning, the decrease in response amplitude decayed to 28.6%, 20.4% and 11.7%, respectively. Seven days following the LFS (Fig 2-4), the LTD effect was not quite significantly different from controls (Tukey HSD; $p>0.08$) or single session animals (Tukey HSD; $p>0.06$).

The late component response amplitudes were decreased by a mean of 76.7% immediately after the trains (Tukey HSD; $p<0.001$). After 24 hours, response amplitudes were at 47.8%. Responses showed further recovery 1 and 2 weeks post-stimulation to 35.5% and 27.0%, respectively. The late component LTD effect was still significantly different from controls after two weeks (Tukey HSD; $p<0.04$).

LTD Induced by Spaced, Multiple Stimulation Trains. Multiple low-frequency stimulation trains delivered once per day for 10 days produced a large LTD effect (Fig. 2-4; Tukey HSD; $p<0.001$). The immediate depression showed somewhat steeper decay rates during each of the first five, compared to the second five days (Fig. 2-5C). Again, the depression was maximal at low to midrange intensities (160 μA). Response amplitudes were decreased by 5.6% and 45.0% immediately following the *first* low-frequency train and by 33.5% and 70.2% immediately following the *tenth* train for the early and late components, respectively. Both the early ($F(9,45)=6.10$; $p<0.001$) and late ($F(9,45)=9.14$; $p<0.001$) component measures showed a significant main effect of session over the LTD induction phase. While the early component response was still significantly depressed, compared to controls, 7 days following the application of the low-frequency trains (Tukey HSD; $p<0.02$), it was no longer significantly different from

the depression seen in the single session group. The late component remained significantly depressed at two weeks compared to both control (Tukey HSD; $p<0.003$) and single session groups which received only one LFS session (Tukey HSD; $p<0.04$).

DISCUSSION

Long-term potentiation is difficult to induce in the neocortex, *in vivo*, using stimulation procedures that are effective in sub-cortical sites (e.g., Racine et al., 1994; Racine et al., 1995; for review, see Tsumoto, 1990). However, tetanization of the white matter can induce LTP in the neocortex of the awake, freely moving animal as long as the stimulation trains are spaced and repeated (Racine et al., 1995; Trepel and Racine, 1998). Using a similar preparation, we show here that LTP can also be induced by stimulation of the homologous site in the contralateral neocortex. Furthermore, we have shown that low frequency stimulation can induce a depression of potentiated responses (i.e., depotentiation) similar to that reported previously in sub-cortical sites (e.g., Bashir and Collingridge, 1994) and in neocortical slice preparations (Artola and Singer, 1993; Kirkwood and Bear, 1994a). We have also demonstrated that long-term depression effects can be induced in naive animals. Finally, we have found larger and longer-lasting LTD effects when multiple low-frequency trains are applied to ipsilateral white matter. These results indicate that neocortical LTD is reliably induced, long lasting and dependent upon the number of stimulation trains.

The evoked potentials observed in these experiments are similar to those reported previously (e.g., Vanderwolf et al., 1987, and Chapman et al., 1998). The early component of the field response evoked by ipsilateral white matter stimulation was shown by Chapman et al. (1998) to consist of both monosynaptic EPSPs and population spikes, while the late component is driven polysynaptically. The morphology and plasticity of contralaterally-evoked responses were similar to responses evoked

ipsilaterally, but it remains to be determined if they are generated by the same layer V substrates known to underlie the salient components of ipsilaterally-evoked responses (Chapman et al., 1998).

Depotentiation effects were larger and longer-lasting when low-frequency stimulation was delivered to the ipsilateral white matter than when it was delivered to the contralateral cortical site. Responses remained depressed for at least 24 hours following ipsilateral stimulation, but decayed back to baseline within 24 hours following contralateral stimulation. The difference might be due to a stronger activation of afferents by ipsilateral stimulation, but the long-term potentiation effects were similar for groups receiving ipsilateral and contralateral stimulation (Fig. 2-3). Another possible explanation for the stronger depression effects with ipsilateral stimulation is a greater activation of thalamic and/or neuromodulatory systems that could then feed back onto the sensorimotor cortex. Some of these systems may facilitate induction of LTD or depotentiation.

The LTD effect induced by a single low-frequency train decays rapidly, over hours, while LTD induced by multiple stimulation sessions lasts for at least two weeks. Multiple stimulation sessions may therefore recruit distinct mechanisms that mediate a longer-lasting form of LTD. LTD, like LTP, may consist of multiple components with different decay time-constants (see Racine et al., 1983; Krug et al., 1984; Frey et al., 1988; Matthies et al., 1989; Jeffery et al., 1990; Abraham et al., 1993 for discussion of multiple components for LTP). Both short- and long-term depression effects are also observed in *in vitro* slice preparations, where a large, initial effect of low-frequency

stimulation decays within several minutes, unmasking a more durable but less substantial effect (Dudek and Bear, 1992; Artola and Singer, 1993; Kirkwood and Bear, 1994a, 1994b). *In vitro* responses recorded in the motor and somatosensory cortices (Castro-Alamancos et al., 1995) hippocampus (Mulkey and Malenka, 1992; Dudek and Bear, 1993) and visual cortex (Bröcher et al., 1992) express both long- and short-term depression effects, which are dissociable based on their NMDA receptor dependence and independence, respectively.

Both the massed and spaced multiple train protocols produced comparable amounts of LTD immediately following stimulation and also showed similar rates of decay during the daily and weekly follow-up I/O measures. The spacing of stimulus trains is therefore not as critical for LTD induction as it is for LTP induction (Trepel and Racine, 1998).

Kirkwood and Bear (1994a) proposed a dual threshold for LTP and LTD induction that is dependent upon the degree of NMDA receptor activation and Ca^{2+} entry. Repetitive 1-3 Hz stimulation of the Schaffer collaterals induced a depression of synaptic efficacy that lasted for more than 1 hour (Dudek and Bear, 1992, 1993), and this form of LTD requires an increase of post-synaptic Ca^{2+} during stimulation (Tsumoto, 1990; Mulkey and Malenka, 1992). Ultimately, then, the level of Ca^{2+} activity may determine whether LTP or LTD will be induced by neural activation (Lisman, 1989; Bröcher et al., 1992; Kirkwood and Bear, 1994b). Furthermore, LTP, LTD and depotentiation in the CA1 region of the hippocampus are all blocked by D-AP5, while only LTP is blocked by CPP, suggesting that LTP and LTD are induced by pharmacologically distinct NMDA

receptors (Hrabetova and Sacktor, 1997). Metabotropic glutamate receptor-dependent mechanisms have also been shown to contribute to LTD and depotentiation effects (Kato, 1993; Haruta et al., 1994; O'Mara et al., 1995).

Although both early and late components showed reliable depression effects, they were somewhat more robust for the late components, particularly for depotentiation. This late component depression could be mediated by a decreased volley from monosynaptically-driven sites, or by direct changes within the polysynaptic pathways themselves. If the responses are passively driven by changes in the monosynaptic sites, the failure to find robust monosynaptic depotentiation may simply indicate that our procedures were not sufficiently sensitive to detect those effects. Other manipulations, such as the application of barbiturate anaesthetics, have also shown that the depression of polysynaptic responses can be detected more easily than the depression of monosynaptic responses (e.g., Chapman et al., 1998). Alternatively, the difference in reliability of the early and late component depression effects may indicate that they are partially independent phenomena. Some portion of the late component LTD and depotentiation effects, for example, might be expressed within the horizontal pathways.

The results of these experiments are consistent with theoretical models of learning and memory that require bidirectional modifications in synaptic efficacy. Most neural network models, however, use symmetrical learning rules, with equivalent rates of increment and decrement of synaptic weights. Our results raise the interesting question of why the nature of neocortical LTP and LTD effects, in chronic preparations, are so dissimilar. In contrast to LTP, LTD reached asymptotic levels rapidly, decayed relatively

quickly, and could be induced with a single session of stimulation. Moreover, the LTD effects were most readily induced in the polysynaptic components. Although the *in vivo* neocortex can support bidirectional synaptic plasticity, the rules governing this plasticity may be biased towards the weakening of horizontal connections that mediate polysynaptic responses.

FIGURE CAPTIONS

Figure 2-1. Changes in field potential amplitude evoked in the right sensorimotor cortex by high- and low-frequency stimulation of the homologous contralateral site. Representative field responses are shown for both a control and a potentiated animal (A_1 and A_2), a potentiated and depotentiated animal (B_1 and B_2), and a control and LTD animal (C_1 and C_2). The solid lines represent pre-stimulation responses and the dashed lines represent the response after conditioning stimulation had been delivered to the experimental animals. **A:** High-frequency stimulation caused an enhancement in the repetitive population spike activity (") associated with an apparent reduction in the amplitude of the early monosynaptic component (%) and the enhancement of a longer latency polysynaptic component (!). **B&C:** Depotentiation and LTD of field responses evoked in the sensorimotor cortex. While potentiated (B_1) and control (C_1) responses remain stable in the absence of low-frequency stimulation, depotentiation (B_2) and LTD (C_2) of the late component are induced by the delivery of a single low-frequency (1 Hz, 15 min) train. Stimulation intensity was 795 μA. Horizontal calibration: 25 ms; vertical calibration: 1mV.

Figure 2-2. The mean change from baseline amplitude (±SEMs) of the late component in the sensorimotor field potential is shown over days. Stimulation was delivered to the cortex and the response was recorded in the contralateral homologous site. Following two days of baseline tests, the LTP animals received 10 days of high-frequency stimulation trains (break in x-axis). Values indicate the change in the late

component amplitude relative to the last baseline I/O test. Following the induction of LTP, 5 of the potentiated animals (HFS + LFS), and 4 of the control animals (LFS) received low frequency trains (1 Hz, 15 min). I/O measures were collected daily for two days and again 1 week later to monitor the longevity of these effects. The LTD effect was not significant and the depotentiation effect decayed within 24 hours.

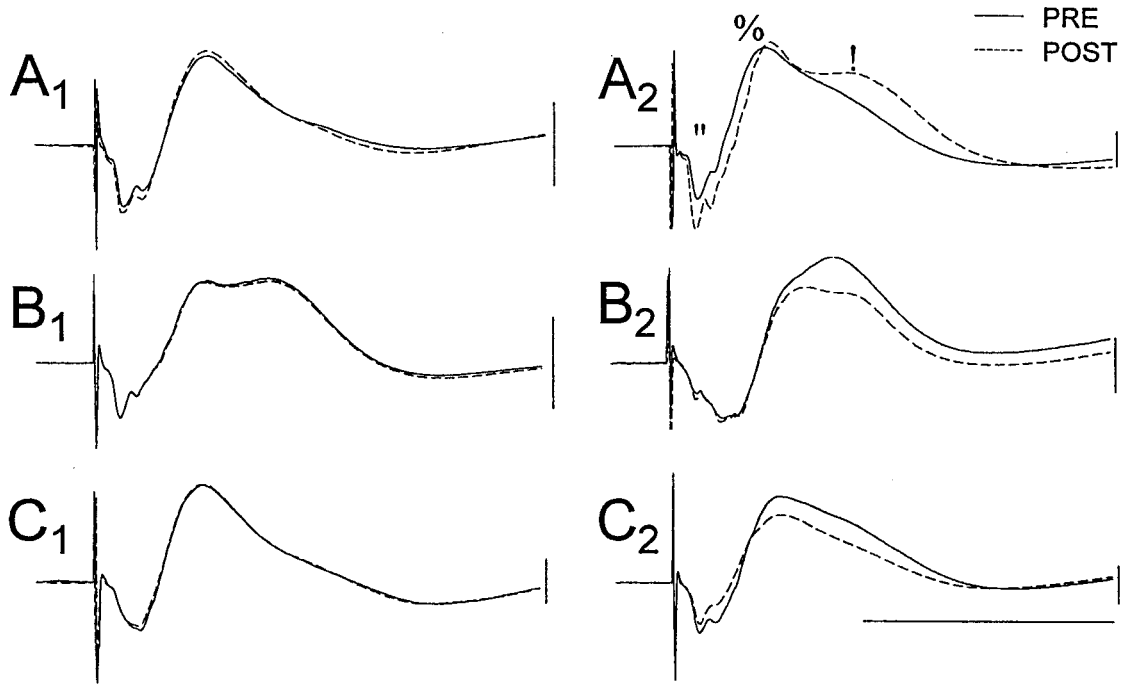
Figure 2-3. A comparison of depotentiation effects induced in sensorimotor responses by low-frequency stimulation of either the contralateral homologous site or the ipsilateral white matter. **A:** Representative sweeps taken from one animal in the ipsilateral group comparing baseline to potentiated and depotentiated responses. LFS caused a decrease in population spike amplitude and number, as well as a decrease in the amplitude of the late component. Horizontal calibration: 25 ms; vertical calibration: 1mV. **B&C:** The mean change from baseline amplitudes (± SEMs) of the early monosynaptic (B) and late polysynaptic (C) components in sensorimotor cortex field potentials are shown here for the contralateral (open squares) and ipsilateral (filled circles) stimulation groups. Values indicate the change in the late component amplitude relative to the last baseline I/O test. Both population spikes and late components were clearly enhanced following 10 days of high-frequency stimulation, and these changes persisted with little decay for the next 7 days. Depotentiation was induced by the delivery of a single low frequency train (1 Hz, 15 sec), and is reflected as a decrease in amplitude relative to the pre-LFS measures. These effects were only significant for the late component. While depotentiated

responses were still evident after 24 hours in the ipsilateral group, they had recovered back to the potentiated levels in the contralateral group. Stimulation intensity was 250 μA.

Figure 2-4. Multiple low-frequency trains induced a larger and longer lasting LTD effect in ipsilaterally-evoked sensorimotor cortex responses than did a single train. **A&B:** Changes in the amplitude of the early (A) and the late (B) field potential components in a control group and in three groups receiving different patterns of low-frequency stimulation. Following 3 baseline test sessions, experimental groups received either 1 train (SINGLE TRAIN), or multiple trains. Multiple train groups received either 10 trains over 10 hours (MASSED-TRAINS) or 10 trains over 10 days (SPACED-TRAINS). Control animals did not receive low-frequency stimulation. Values indicate the change in the late component amplitude relative to the last baseline I/O test. A substantial LTD effect was found in all experimental groups, and the multiple train stimulation produced the largest and longest lasting effects. The stimulation intensity was 250 μA. **C&D:** Representative sweeps from animals in the single train (C) and spaced-trains (D) LTD groups. The baseline responses are compared to the responses evoked two weeks after the induction of LTD. Horizontal calibration: 25 ms; vertical calibration: 1mV.

Figure 2-5. Multiple sessions augment the LTD effect observed after the first session of low-frequency (1 Hz for 15 minutes) stimulation. **A:** Representative sweeps from

an animal in the spaced-trains group comparing the first session and tenth session LTD effect to the last baseline response. Horizontal calibration: 25 ms; vertical calibration: 1mV. **B&C:** Changes in response amplitudes for the early monosynaptic (B) and longer latency polysynaptic (C) components monitored during LTD induction in the multiple train groups. Trains were delivered either hourly (MASSED TRAINS) or daily (SPACED TRAINS). I/O tests were recorded both before and after each train in the spaced trains groups, so that the decay of the LTD effect could be observed for each 24 hrs post-stimulation period. The final point in the graph shows response amplitudes 24 hours after the last low-frequency train. Similar amounts of LTD were observed for both spaced and massed groups.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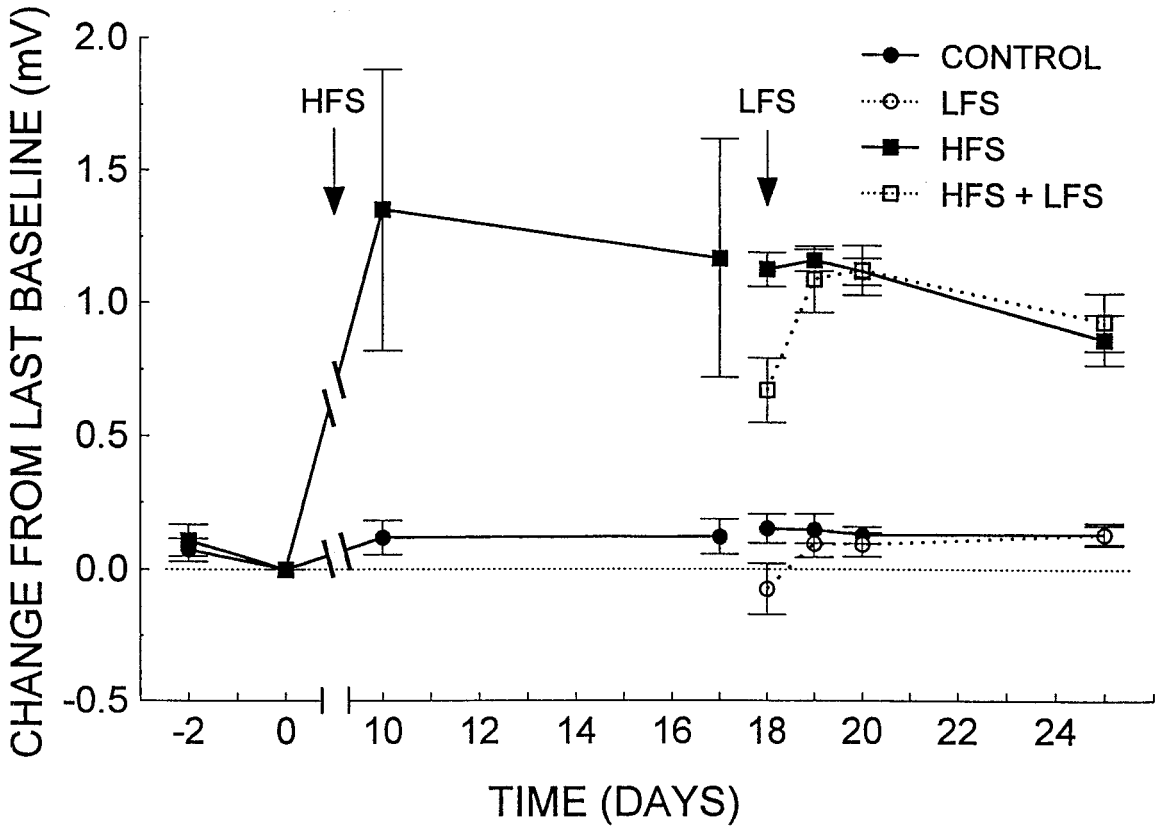
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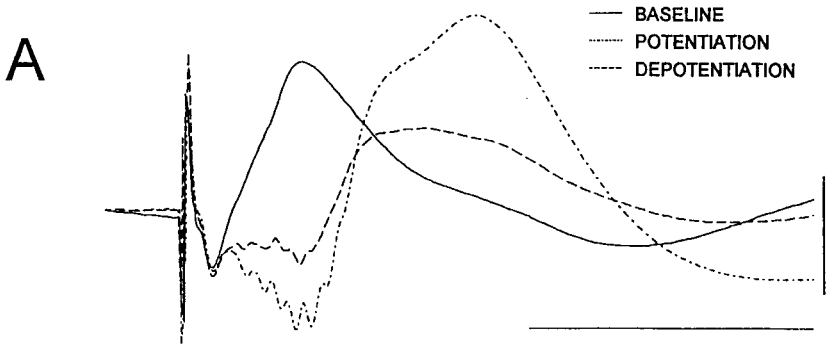
TIME (DAYS)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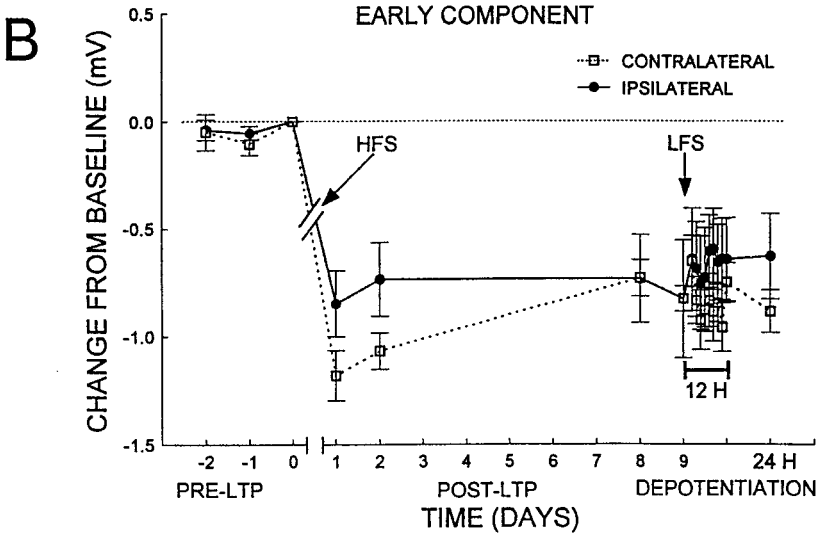
B
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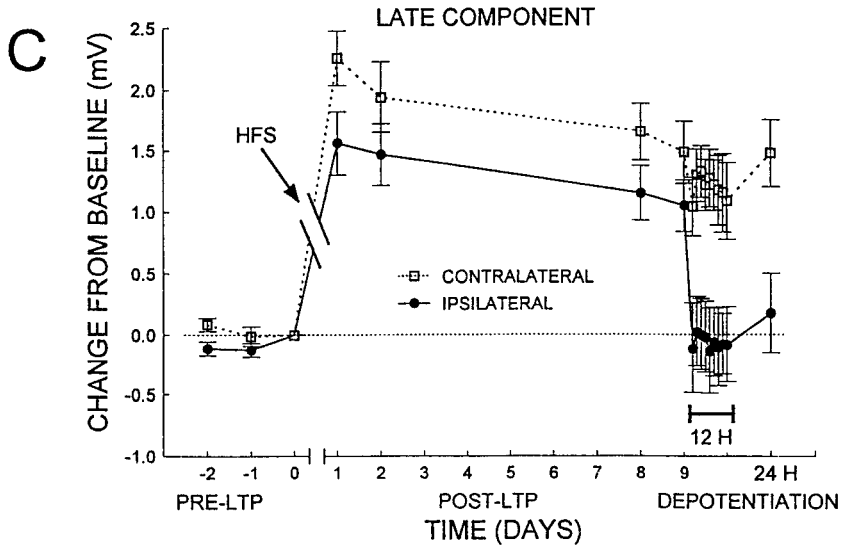
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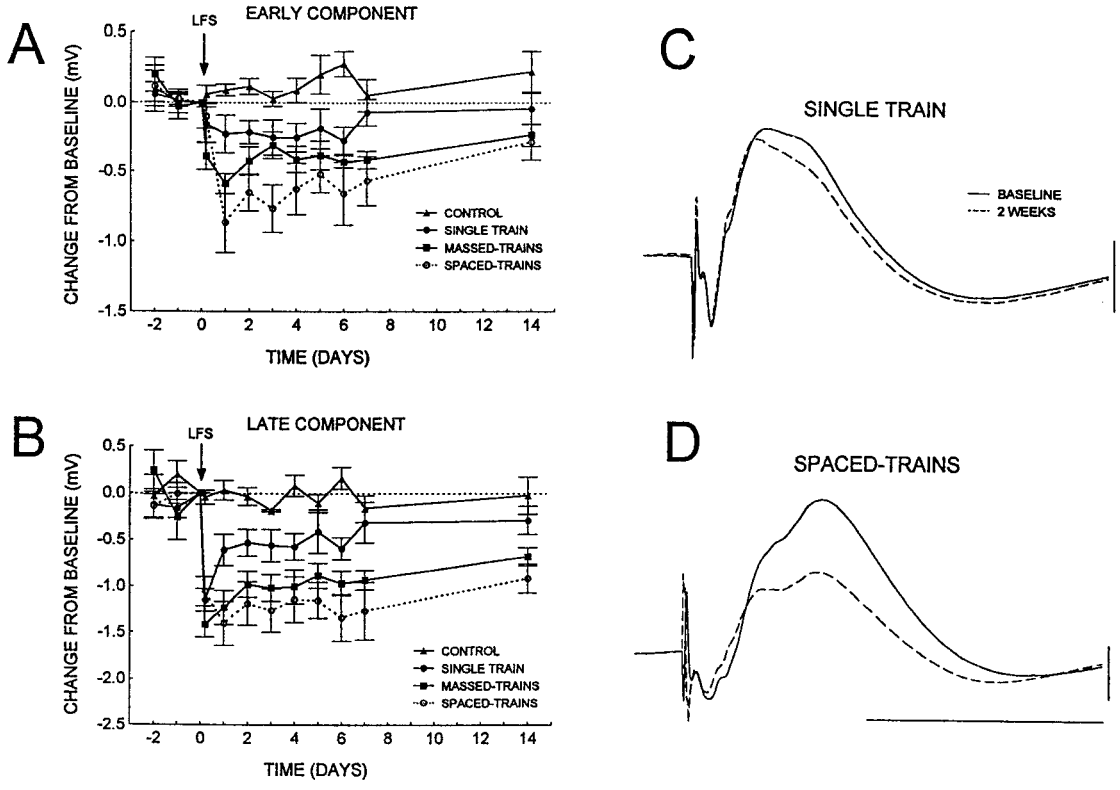
PRE-LTP
POST-LTP
DEPOTENTIATION
TIME (DAYS)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TIME (DAYS)
CONTROL
SINGLE TRAIN
MASSED-TRAINS
SPACED-TRAINS
B
LATE COMPONENT
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CHANGE FROM BASELINE (mV)
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CONTROL
SINGLE TRAIN
MASSED-TRAINS
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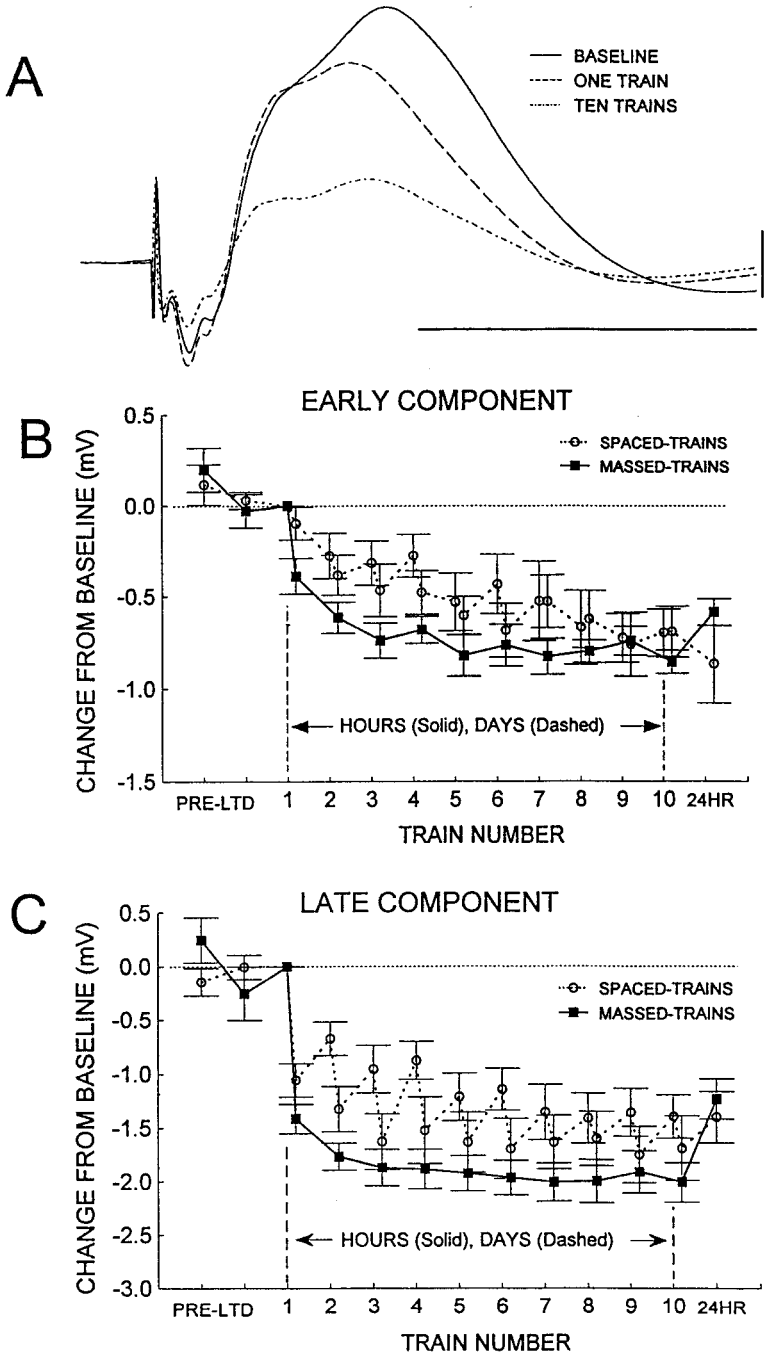
SPACED-TRAINS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HOURS (Solid), DAYS (Dashed)
PRE-LTD 1 2 3 4 5 6 7 8 9 10 24HR
TRAIN NUMBER
C
LATE COMPONENT
SPACED-TRAINS
MASSED-TRAINS
CHANGE FROM BASELINE (mV)
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0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
HOURS (Solid), DAYS (Dashed)
PRE-LTD 1 2 3 4 5 6 7 8 9 10 24HR
TRAIN NUMBER

CHAPTER THREE

N-Methyl-D-Aspartate Receptor-Independent Long-Term Depression and Depotentiation in the Sensorimotor Cortex of the Freely Moving Rat

While LTP has been shown to be N-methyl-D-aspartate (NMDA) receptor-dependent in most areas of the brain studied to date (Malenka and Nicholl, 1999; Kirkwood and Bear, 1994; Collingridge and Bliss, 1987; but see Johnston et al., 1992; Komatsu et al., 1991; Grover and Teyler, 1990), there is considerable controversy regarding the importance of NMDARs in the induction of LTD in the hippocampus and neocortex both *in vitro* and *in vivo*. In the hippocampus, for example, the induction of LTD has been shown to be dependent on NMDA receptor activation using both *in vitro* (Kirkwood et al., 1993; Dudek and Bear, 1992; Mulkey and Malenka, 1992) and *in vivo* (Thiels et. al., 1996; Desmond et al., 1991; Manahan-Vaughan, 1997) methodologies. Depotentiation has also been shown to be NMDA-dependent *in vitro* (Fujii et al., 1991; Wexler and Stanton, 1993; Wagner and Alger, 1995; Muller et al., 1995; Norris et al., 1996). In the rat somatosensory and motor cortices (Castro-Alamancos et al, 1995) and at thalamocortical synapses in the barrel cortex (Feldman et al., 1998), LTD has been shown to be NMDAR-dependent.

Conversely, other research has found *in vitro* LTD in both the visual cortex (Kemp and Bashir, 1999; Oliet et al., 1997; Kato, 1993; Kirkwood et al., 1993; Teyler et al., 1990) and hippocampus (Stanton and Sejnowski, 1989; Bolshakov and

Siegelbaum, 1994) to be independent of NMDAR activation. Akhondzadeh and Stone (1995) have shown that LTD induced by muscimol administration is also NMDAR-independent. Hippocampal depotentiation has been reported in the absence of NMDAR activation (Bashir and Collingridge 1994), and striatal LTD has been shown to be blocked by mGluR antagonists but not by NMDAR antagonists (Calabresi et al., 1992; Lovinger et al., 1993. Additionally, in the neocortex, several studies have shown that patterns of afferent stimulation usually capable of inducing LTP caused the induction of LTD when delivered in the presence of an NMDAR antagonist (Trepel and Racine, 1998; Kitagawa et al., 1997; Artola et al., 1990; Aroniadou and Teyler, 1991; Hirsch and Crepel, 1990, 1991).

Previously, we demonstrated reliable long-term depression and depotentiation of field potentials recorded from the sensorimotor cortex of the chronically prepared rat following stimulation of the white matter (Froc et al., 2000). We also found that NMDA antagonism blocks LTP and uncovers or causes an LTD effect in response to the high-frequency stimulation (HFS) (Trepel and Racine, 1998), suggesting that neocortical LTD may not be NMDAR-dependent. Consequently, the first step in the present series of experiments was to confirm these effects of NMDA antagonism on the induction of LTP. We also determined the effects of the NMDA receptor blocker MK801 on the induction of neocortical LTD and depotentiation in the chronic preparation. We found that LTD and depotentiation are NMDAR-independent.

MATERIALS AND METHODS

Animals and Surgery

Fifty-two male Long-Evans rats (300-400 g) were used in the following experiments. All animals were implanted with stimulating and recording electrodes and had baseline I/O measures taken as described in chapter 2. The exposed tips of the electrodes were separated by 1.0 mm for cortical placements and 0.5 mm for white matter placements. Electrodes were implanted into the sensorimotor cortex (M1 bordering on S1) and the white matter in the same coronal plane. Recording electrodes were placed in the sensorimotor cortex 2.0 mm anterior to bregma and 4.0 mm lateral to the midline at a depth of 2.0 mm from the pial surface (Paxinos and Watson, 1997). Stimulating electrodes were placed in the nearby white matter 2.0 mm anterior to bregma, 2.0 mm lateral to the midline at a depth of 3.0 mm from the pial surface. Electrode depths were adjusted during surgery to maximize field response amplitudes.

Stimulation and Recording

Long term Potentiation. To induce LTP, 60 high-frequency trains were delivered once per day for ten days. Each 24 msec train consisted of 8-pulses at 300 Hz, and the trains were delivered once every 10 seconds. Pulse intensity was 1260 μA. Daily input/output tests were recorded during the induction phase and for 7 days after the LTP induction procedure to confirm that the potentiation effects were long lasting.

Long-Term Depression and Depotentiation. To induce long-term depression, low-frequency stimulation (1 Hz, 900 pulses, 1260 μA) was delivered daily for ten days. Similarly, to induce depotentiation, low-frequency stimulation was delivered daily for ten days immediately following the LTP induction regimen.

Experimental Design

There were three experiments. The first examined the effects of NMDAR blockade by MK801 ((5R,10S)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine hydrogen maleate) (1.0 mg/kg i.p., Sigma-Aldrich), an NMDAR antagonist, on the induction of neocortical LTP. The second dealt with the effects of MK801 on the induction of neocortical LTD. The third experiment was designed to determine the effects of MK801 on the induction of neocortical depotentiation.

MK801 and Long-Term Potentiation. Previous work in our lab showed that HFS induced LTD-like effects in the neocortex when combined with the specific NMDAR blocker CPP (Trepel and Racine, 1998). In this experiment, the effects of ten days of HFS administered in combination with MK801 were compared to the effects of HFS delivered alone. Following three baseline I/O measures, the animals were divided into three groups. For the first two groups, the animals were injected with 1.0 mg/kg MK801 (n=6) or saline (n=4), followed 150 minutes later by HFS. Each injection was preceded by an I/O test. The control group (n=3) did not receive any conditioning stimulation but otherwise followed the same regimen. I/O measures

were collected for seven days following cessation of the conditioning stimulation to monitor the longevity of any effects.

MK801 and Long-Term Depression. The second experiment was designed to determine the effects of NMDA receptor blockade during the low-frequency conditioning stimulation on the induction of LTD. Following three baseline I/O measures the animals were injected with 1.0 mg/kg MK801 (n=5) or saline (n=5), followed 150 minutes later by low-frequency stimulation. Each injection was preceded by an I/O measure. The LFS was delivered daily for 10 days. Following this induction period, I/Os were collected daily for seven days following the conditioning. A third group (n=3) received 1.0 mg/kg MK801 but did not receive any LFS. Additionally, a control group (n=5) that did not receive any conditioning stimulation allowed us to determine the stability of neocortical responses over the course of this experiment.

MK801 and Depotentiation. The third experiment was designed to assess the effects, if any, of NMDA receptor blockade on the induction of depotentiation. Following three baseline I/O measures, the animals were divided into five groups based on response morphology and amplitude. After prior induction of LTP by ten days of HFS, the LFS+MK801 and LTP decay + MK801 groups were injected with 1.0 mg/kg MK801 followed 150 minutes later by LFS (n=4) or no stimulation (n=5), respectively. A third group received daily injections of saline (n=5) followed 150 minutes later by LFS to monitor the effects, if any, of daily injections on the induction of depotentiation. Each MK801 or saline injection was preceded by an I/O

measure. The LFS was delivered daily for 10 days. A fourth group served as an LTP decay control group (n=5) and received neither LFS nor MK801 following LTP induction. Additionally, a control group (n=4) that received neither conditioning stimulation nor drug served to determine the stability of neocortical responses over the course of this experiment. Following this induction period, I/Os were collected daily for seven days following the conditioning stimulation.

Data Analysis

Changes in evoked potential amplitude were quantified by the changes in the peak amplitudes of field potential components relative to the last baseline I/O test. This was done at the midrange intensities, which typically reflect the largest experimental effect (LTP, LTD or depotentiation), 250 and 500 μA. The early and late components were measured at fixed latencies corresponding to the peak response of the components being analysed. The depotentiation effects were measured and analysed at the same latencies used to measure the LTP effects for each animal. For the late components, the LTP and depotentiation effects were maximal at longer latencies than the LTD effects, because LTP recruits polysynaptic responses and broadens the field potential. The late component peaks and the changes in those peaks were most clear following the induction of LTP and LTD, so the control responses for these experiments were analysed using the mean latencies for each component as determined from the experimental animals. Changes in response

amplitudes (in mV) were analysed using mixed design ANOVAs and the Tukey HSD post-hoc test.

Histology

Rats were anaesthetised with urethane (2.0 g/kg) and perfused through the heart with formol-saline. Frozen brain sections were cut at 40 μm and stained with Cresyl violet to verify electrode placements.

RESULTS

Electrode Placement and Behavioral Effects of Drug.

Histological examination confirmed that the electrode tips were located in their intended targets for all animals. The field potentials were as characterized by Chapman et al. (1998) who reported that both early and late components showed peak current densities around upper layer V. The mean peak latencies of the early and late components of the field responses over all animals were 7.0 msec (range 5.7 to 14.4 msec) and 24.7 msec (range 21.0 to 31.0 msec), respectively. The population spikes tended to be small, repeated over 2.0-9.0 msec, and were superimposed on the early monosynaptic component.

Animals injected with the NMDA antagonist MK801 were relatively motionless for several hours, except when exhibiting stereotypic ambulatory behaviors. Twenty-four hours after the administration of MK801, rats exhibited normal ambulatory behaviors. All of these animals groomed and gained weight normally throughout the experiment and appeared completely healthy during and following the MK801 injection protocol.

Effects of NMDAR-Blockade on LTP

HFS Alone. Both components were affected by the HFS used to induce LTP (Figs. 3-1A2 and 3-1B). There was a clear increase in the amplitude of the polysynaptic component. As in previous studies (Froc et al., 2000; Chapman et al., 1998; Trepel and Racine, 1998), there was a decrease, and often a reversal in the

amplitude, in the early component, which appears to be due to the potentiation of population spike activity. Both the number and amplitude of these spikes were increased after LTP induction. These effects reached asymptote by the end of conditioning. The greatest potentiation effects were observed at an intensity of 250 μA. The mean latency for the early monosynaptic and late polysynaptic components was 6.3 msec (range 4.3 to 8.0 msec) and 24.0 msec (range 21.0 to 28.0 msec), respectively.

HFS and MK801. In contrast to HFS alone, HFS delivered during NMDA blockade by MK801 resulted in a clear depression of both components (Figure 3-1B). In this case, the decrease in the amplitude of the monosynaptic component was not associated with any increase in the number or magnitude of population spikes and never reversed (Figure 3-1A_1). Additionally, the polysynaptic component showed a decrease in amplitude during and following the HFS conditioning. This effect reached asymptotic levels by the fourth day of conditioning and decayed in the 7 days following cessation of HFS to baseline levels. In contrast, the early component effects showed no decay in the 7 days following the cessation of HFS. The mean latency for the early monosynaptic and late polysynaptic components was 8.2 msec (range 8.0 to 9.3 msec) and 26.5 msec (range 25.0 to 31.0 msec), respectively.

Both HFS groups showed an amplitude shift in the early component yielding a significant main effect of session ($F(18,144)=2.36$; $p<0.003$). There was a significant interaction between group and session ($F(18,144)=11.92$; $p<0.001$) owing to the attenuated rate of change evidenced in the MK801-treated animals. While the HFS

control animals showed an increase of the polysynaptic component with the administration of the HFS, the MK801 treated animals showed a decrease in this component yielding a significant interaction between session and group ($F(36,180)=7.83$; $p<0.001$).

Figure 3-1C shows that, after washout of the drug, normal LTP effects are observed in both the early and late components following the re-administration of HFS to the animals that previously received 1mg/kg MK801 during the original conditioning stimulation. Moreover, the early component showed a greater amplitude shift than observed in the previous phase of the experiment, and this shift coincided with enhanced population spike amplitude and number. Thus, the effects of the NMDAR block were reversible.

Effects of NMDAR-Blockade on LTD.

The evoked responses were relatively stable for the saline-treated and MK801-alone controls. LTD effects were as characterized in the previous chapter where LFS delivered daily for ten days was reported to produce a long-lasting decrease in the amplitude of both the early and late components of the evoked response. MK801 did not appear to have any long-term effects, since the MK801-alone responses were relatively stable throughout the course of this experiment and overlapped with the saline treated controls. The mean peak latency of the early and polysynaptic components was 12.5 (range 10.0 to 14.0 msec) and 20.1 msec (range 16.0 to 27.0 msec).

When administered during the application of the low-frequency conditioning stimulation, MK801 did not alter the time course for the induction or decay of LTD. The MK801+LFS and LFS-alone groups reached similar asymptotic levels of depression by the end of the LFS. Furthermore, the LTD effect decayed at similar rates for these two groups (Figure 3-2). Both groups showed a decrease in the late component compared to the control and MK801-alone groups (Figure 3-2C) yielding a significant interaction between group and session ($F(57,266)=2.63$; $p<0.001$).

LTD of the early component was also induced at similar rates in the MK801+LFS and LFS-alone groups (Figure 3-2B). Both groups showed a decrease in the early component compared to the control and MK801-alone groups yielding a significant interaction between group and session ($F(57,266)=1.82$; $p<0.001$).

Effects of NMDAR Blockade on Depotentiation

All groups that received the HFS displayed similar potentiation effects in both the early monosynaptic and late polysynaptic components (Figure 3-3). The mean peak latency of the polysynaptic component was 22.6 msec (range 21.0 to 24.0). Ten days of low frequency stimulation induced a depotentiation effect in the late component (Figure 3-3C) of animals that were previously potentiated. There was, however, no difference between the groups that did or did not receive MK801 during the depotentiation phase. The decay of LTP was also unaffected by the administration of MK801 during the decay phase. There was a significant interaction between group and session ($F(92,368)=5.2$; $p<0.001$) reflecting the reduction in late

component amplitude resulting from the LFS. There were no depotentiation effects observed in the early component (Figure 3-3B).

DISCUSSION

Our first experiment confirmed the findings of Trepel and Racine (1998) who showed that NMDAR antagonism in the chronic preparation not only blocked neocortical LTP, but also resulted in an LTD effect following HFS, indicating that the pattern of afferent stimulation is not the final determinant of the direction of synaptic plasticity. Normal LTP effects were induced by HFS following washout of MK801, so repeated exposure to the NMDA receptor antagonist did not cause any permanent alteration in the capacity of these synapses to support plasticity. In addition, we show for the first time in this preparation that neither LTD nor depotentiation is blocked by the same dose of MK801 that completely blocks LTP induction. It is unlikely that higher doses of MK801 would have been more successful at blocking LTD. Abraham and Mason (1988) reported that higher doses (>1.0 mg/kg) were lethal and lower doses had no effect on LTP induction in the hippocampus.

Calcium is a requirement for both LTP and LTD induction, but LTP requires substantial increases in postsynaptic intracellular Ca^{2+} concentrations (Lynch, et al., 1983), while smaller increases are required for the induction of LTD (Mulkey & Malenka, 1992). Thus, when postsynaptic depolarization is not sufficient to induce the increases in postsynaptic Ca^{2+} required for LTP, LTD may be induced (Lisman, 1989; Kirkwood & Bear, 1994; Mulkey & Malenka, 1992). It is possible that the NMDA receptor antagonism caused by MK801 in our experiment reduced the amount of calcium influx triggered by HFS from that required for LTP induction to a level suitable for LTD induction.

Kirkwood and Bear (1994) proposed a dual threshold for LTP and LTD induction that is dependent upon the degree of NMDA receptor activation and consequent Ca^{2+} entry. As our LTD effects were unaffected by MK801, the Ca^{2+} may have entered the cells via voltage-dependent channels (Wickens & Abraham, 1991; Ross & Werman, 1987) or via a population of NMDA receptors that are not affected by MK801 and not sufficient for LTP induction. Hrabetova and Sacktor (1997) have found that LTP, LTD and depotentiation in the CA1 region of the hippocampus were all blocked by D-AP5, while only LTP was blocked by CPP, suggesting that LTP and LTD may be induced by pharmacologically distinct NMDA receptors. Metabotropic glutamate receptor-dependent mechanisms have also been shown to contribute to both LTD and depotentiation effects (Kato, 1993; Haruta et al., 1994; O'Mara et al., 1995). Antagonism of mGluRs has been shown to block visual cortical LTD (Kato, 1993; Haruta et. al., 1994) and cerebellar LTD (Hartell, 1994). Furthermore, striatal LTD is blocked by mGluR antagonists but not by NMDAR antagonists (Calabresi et al., 1992; Lovinger et al., 1993). Nicoll et al (1998) and Oliet et al. (1997) have shown two distinct forms of LTD in hippocampal pyramidal neurons, one dependent on NMDAR activation and the other requiring activation of metabotropic glutamate receptors (mGluRs). In the case of mGluR activation, Ca^{2+} is released from internal stores (Murphy & Miller, 1989). However, the literature is inconsistent regarding the role of mGluRs in the induction of LTD and depotentiation. Selig et al., (1995) found LTD to be mGluR-independent in hippocampal area CA1.

Activation of group II mGluRs blocks the *in vivo* induction of both LTP and

depotentiation in area CA1 of the rat (Holscher et al., 1997), and inhibition of group I mGluRs has been shown to block spatial learning in rats (Balschun and Wetzel, 1998). Manahan-Vaughan (1997) has shown that group I mGluRs may play a role in both LTP and LTD, whereas group II mGluRs may only be critically involved in LTD. Furthermore, Bortolotto et al. (1994) have shown that prior mGluR activation may facilitate, in an all or none fashion, the induction of LTP. Kemp and Bashir (1999) have shown that LTD in the CA1 region of adult hippocampal slice is dependent on the activation of either AMPA/kainate or mGluR receptors but not NMDARs or VDCCs. Furthermore, LTD was only blocked by the combined antagonism of AMPA/kainate and mGlu receptors.

In the hippocampus, LTD induction requires protein phosphatase (Kemp and Bashir, 2001; Mulkey et al., 1993; Lisman, 1989) and in the visual cortical slice, Kirkwood and Bear (1994) have shown that inhibition of protein phosphatase 1 and 2a blocked LTD but not LTP. The low levels of postsynaptic Ca^{2+} associated with LTD induction may activate the phosphatases causing the dephosphorylation of AMPA receptors. The high levels of postsynaptic Ca^{2+} associated with LTP induction, on the other hand, would activate the kinases causing phosphorylation. Together, the kinases and phosphatases increase and decrease, respectively, channel activity.

In conclusion, pharmacological antagonism of NMDA receptors in vivo blocks neocortical LTP in the chronic preparation but leaves LTD and depotentiation unaffected. In future work, we plan to investigate the role of mGluRs and protein

phosphatase in neocortical LTD induction.

FIGURE CAPTIONS

Figure 3-1. The effects of HFS delivered alone or in the presence of MK801. (A) Sample sweeps of sensorimotor cortex field potentials are shown for MK801+HFS **(A_1)** and HFS alone **(A_2)** animals. The solid lines represent pre-stimulation responses and the dashed lines represent the response after conditioning stimulation had been delivered to the experimental animals (vertical calibration: 1.0 mV; horizontal calibration: 10 msec). **(B)** The mean amplitudes (± SEMs) of the early and late components. Values indicate the change in the amplitude relative to the last baseline I/O test. The early component showed a clear amplitude shift (positive-going direction from layers I-V) following the HFS in both the HFS and HFS+MK801 treated groups. The morphology differences, however, suggest that the changes in the HFS group were primarily due to population spike potentiation while those in the HFS+MK801 group were due to depression. There was little decay in this component, in either group, seven days after the cessation of the HFS conditioning. The direction of the effect was more apparent for the late component, which was clearly enhanced following treatment with HFS alone, but depressed following HFS conditioning in the MK801 treated group. While the LTP effects showed some decay seven days following the conditioning, they remained strong. The depression effect, however, returned to baseline over the seven days of follow-up I/Os. **(C)** The bottom two panels show the effects of HFS delivered alone, following the washout of MK801. The early component showed the usual long-lasting amplitude shift, and

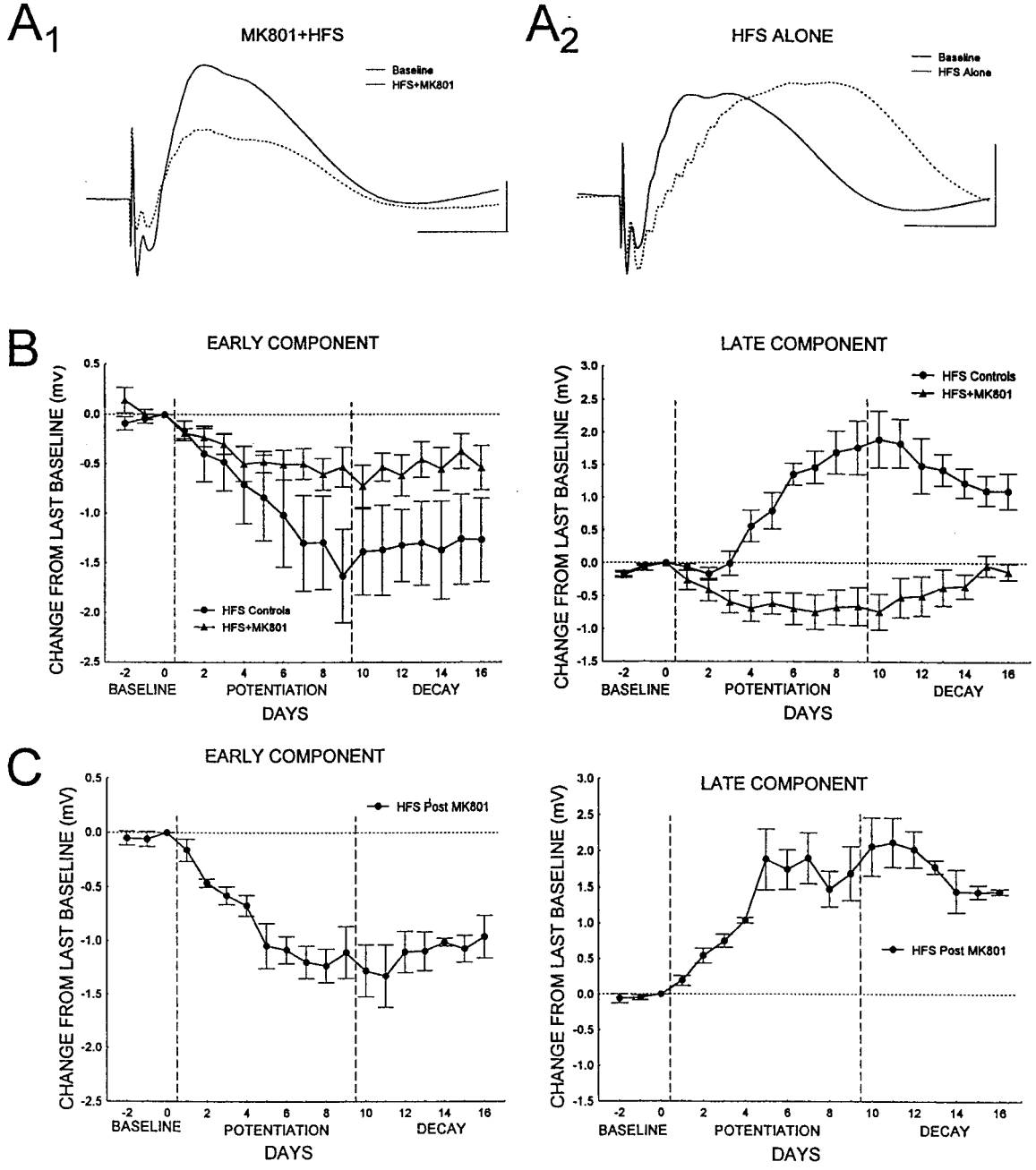
population spike potentiation. The late component amplitude was clearly increased following the HFS conditioning and remained potentiated during the decay period.

Figure 3-2. The effects of LFS delivered alone or in the presence of MK801. **(A)** Representative somatosensory cortex field responses are shown in the top panel for MK801+LFS (A_1) and LFS alone (A_2) animals. Vertical calibration: 1.0 mV; horizontal calibration: 10 msec. The mean amplitudes (± SEMs) of the **(B)** early and **(C)** late components show the clear LTD induced by LFS and the lack of an MK801 effect on the LTD. Values indicate the change in the early/late component amplitude relative to the last baseline I/O test. There was no difference between the LFS and LFS+MK801 groups in the induction rate expressed in either component, and there was little decay in the two components for either group seven days after the cessation of the LFS conditioning. MK801 alone had no effect on the evoked responses.

Figure 3-3. The effects of LFS delivered alone or in the presence of MK801 following the induction of LTP (depotentiation). **(A)** Representative somatosensory cortex field responses are shown in the top panel for MK801+depotentiation (A_1) and depotentiation alone (A_2) animals. Vertical calibration: 1.0 mV; horizontal calibration: 10 msec. The mean amplitudes (± SEMs) of the **(B)** early and **(C)** late components show little or no depotentiation effects in the early component and a clear depotentiation in the late component. MK801 had no effect on the expression of depotentiation. There was no difference between the groups in the induction rate expressed in either component and there was

little decay in the two components for either group seven days after the cessation of the LFS conditioning.

MK801+LTP


A1
MK801+HFS
Baseline
HFS+MK801
A2
HFS ALONE
Baseline
HFS Alone
B
EARLY COMPONENT
CHANGE FROM LAST BASELINE (mv)
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HFS+MK801
BASELINE
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CHAPTER FOUR

Interactions Between LTP- and LTD–inducing Stimulation in the Neocortex Of the Awake, Freely Moving Rat

Kirkwood and Bear (1994) proposed the idea of a dual threshold for synaptic plasticity such that LTD and LTP are associated with moderate and high levels of synaptic activity, respectively. While it has been shown that intense synaptic activation can produce LTP, and moderate repetitive activation can produce LTD, it has also been proposed that the threshold, direction and magnitude of synaptic change can be modulated in a homeostatic manner by prior synaptic activity. This dynamic regulation of synaptic activity has been termed 'metaplasticity' (Abraham, 1996; Abraham and Bear, 1996), and it allows the history of synaptic activation to modulate the current plasticity. Without some control over the direction and magnitude of synaptic plasticity, changes in synaptic strength could be locked into a positive feedback loop leading to runaway LTP. In a model of visual cortical plasticity, Bienenstock et al (1982) proposed an algorithm that incorporated a sliding threshold. Dudek and Bear (1992) have suggested that this sliding threshold in the Bienenstock, Cooper and Munro (BCM) model may correspond to the sliding LTP-LTD crossover point observed in frequency-response experiments (Wang and Wagner, 1999; Kirkwood et al., 1996; Christie et al., 1995). Furthermore, some neural network models have incorporated threshold adjustments that take into account the recent history of the synapse (Bear, 1996; Bear et al., 1987; Bienenstock et al., 1982).

In the hippocampal CA1 region, both LTD and depotentiation can be facilitated when the low-frequency conditioning stimulation is preceded by a high-frequency priming stimulation (Holland and Wagner, 1998). In hippocampal slices of adult (50-65 day-old) mouse, LTD can only be induced in the dentate gyrus (Wei and Xie, 1999) or CA1 region (Wexler and Stanton, 1993) when high frequency priming stimulation precedes the low-frequency conditioning stimulation. Similarly, associative LTD in the rat dentate gyrus is facilitated when a theta-frequency (5 Hz) priming stimulus precedes the conditioning stimulation (Christie and Abraham, 1992).

The prior history of the synapse affects LTP induction as well. In CA1, prior activation of synaptic inputs by LFS (Fujii et al., 1996) or weak tetani or single pulses (Huang et al., 1992) can suppress subsequent LTP induction, and these effects are NMDAR-dependent. Although this effect runs counter to that predicted by the BCM model, prior exposure to theta-frequency synaptic activity can reduce the threshold for LTP induction (Christie et al., 1995). Also, LTD has been shown to be dependent on mGluR activation (Lin et al., 2000; Fitzjohn et al., 2001; Bashir and Collingridge, 1994) and mGluR activation has been shown to prime subsequent LTP induction in the hippocampus (Cohen et al., 1998; Cohen and Abraham, 1996) and dentate gyrus (O'Leary and O'Connor, 1998).

The induction of LTP and LTD can also be modulated by prior behavioral activity. Behavioral stress, for example, can inhibit LTP induction (Diamond et al., 1994; Shors et al., 1989; Foy et al., 1987) and facilitate LTD induction in area CA1 of

rat hippocampus (Xu et al., 1997). Further, Lebel et al (2001) have shown that olfactory learning inhibits subsequent LTP induction and facilitates LTD induction. Similarly, a persistent depotentiation can be induced in the hippocampus of freely moving rats following exploration of a new, non-stressful environment (Xu et al., 1998).

Typical experimental manipulations used to induce LTD and LTP involve temporal summation of synaptic events over the course of milliseconds and metaplasticity could serve to integrate synaptic events that occur over longer time periods (Abraham, 1999). The longevity of LTP induced in weakly activated synapses, for example, can be greatly enhanced if induced during a time of increased protein synthesis resulting from previous strong synaptic activation (Frey et al., 1997).

Our goal in the present experiments was to determine whether or not LFS delivered prior to HFS would prime the induction of LTP of white matter inputs to sensorimotor cortex. In the neocortex of the awake, behaving rat, multiple sessions of HFS must be delivered over consecutive days to induce maximal LTP effects (Trepel and Racine, 1998) and multiple sessions of LFS are required for maximal LTD effects (Froc et al., 2000). Depotentiation is also observed in this preparation, and following the induction of LTP, 1 Hz stimulation delivered daily for ten days induces a depotentiation that is NMDAR-independent (Froc and Racine, in preparation). We have not yet, however, determined the interactions, if any, between the LTP- and LTD-inducing stimulation *during* the induction phase of these phenomena or the

effects of potentiating stimulation on newly depressed synapses. Consequently, these experiments were designed to assess the interaction between LTP and LTD inducing stimulation in the neocortex of the adult, freely moving rat.

MATERIALS AND METHODS

Animals and Surgery

Forty-six male Long-Evans rats (300-400 g) were used in the following experiments. Electrodes were surgically implanted, I/O measures were taken as described in chapter two and the electrodes were placed according to the coordinates described in chapter three. Rats were housed individually on a 12h/12h-light/dark cycle and tested during the light cycle. A two-week recovery period preceded experimental testing.

Stimulation and Recording

Input/output Tests and Long term Potentiation. To induce LTP, 60 high-frequency trains were delivered once per day for ten or fifteen days for experiments 1 and 2, respectively. Each 24 msec train consisted of 8-pulses at 300 Hz, and trains were delivered once every 10 seconds. Pulse intensity was 1260 μA. Daily input/output measures were recorded during the induction phase and for 7 days after the LTP induction procedure to confirm that the potentiation effects were long lasting. The procedures for I/O tests were as described in the previous chapters.

Long-Term Depression and Depotentiation. To induce LTD and depotentiation, low-frequency stimulation (1 Hz, 900 pulses, 1260 μA) was delivered daily for ten days immediately following baseline I/Os or the LTP induction regimen.

Experimental Design

The first experiment examined the effects of combined, daily HFS and LFS on

the induction of neocortical LTP and LTD. The second experiment dealt with the reversibility of established LTP by the subsequent application of LFS and the reversibility of established LTD by HFS.

Experiment 1. Following three baseline I/O measures, animals were randomly assigned to one of four groups based on response morphology. To establish an LTP baseline for comparison and verify the stability of evoked responses an LTP control group (n=10) received HFS alone for 15 days, and a second control group (n=5) received no conditioning stimulation. The experimental groups received 15 days of both HFS and LFS, differing only in the order of presentation. The HFS-LFS animals (n=5) received one session of high-frequency trains followed two minutes later by one session of low-frequency trains, every day for 15 days. The LFS-HFS animals (n=5) received LFS followed two minutes later by HFS each day. Amplitude measures were taken for both monosynaptic and polysynaptic field components. The conditioning phase of this experiment was extended from 10 to 15 days to allow all of the experimental groups to reach asymptotic levels of LTP.

Experiment 2. The second experiment examined depotentiation by comparing the effects of LFS (n=5) on synaptic activity previously potentiated to asymptotic levels and, conversely, the reversibility of LTD by subsequent HFS (n=6). Both groups received 20 consecutive days of stimulation (10 HFS and 10 LFS), differing only in the order of presentation. Additionally, to determine the stability of the neocortical-evoked response, an LTP control group (n=6) received HFS daily for 10 days and a fourth group (n=6) received no stimulation.

Data Analysis

Changes in evoked potential amplitude were quantified by changes in the peak amplitudes of field potential components relative to the last baseline I/O test. This was done at midrange intensities (250 and 500 μA), which typically reflect the largest potentiation and depression effects. For each animal the early and late components were measured at fixed latencies corresponding to the peak response amplitudes of each component. Within animals, depotentiation and LTP effects were measured and analysed at the same latencies. For the late components, the LTP effects, and thus the depotentiation effects, were maximal at longer latencies than the LTD effects. This is likely due to the fact that LTP recruits polysynaptic responses and broadens the field potential. The late component peaks and peak changes were most clear following the induction of LTP and LTD, so the control responses for these experiments were analysed using the mean latencies for each component from the experimental animals. Since LTD effects peaked at somewhat shorter latencies than LTP effects, late components were measured at two latencies: late1 (11.0 to 21.0 msec) and late2 (17.4 to 34.0 msec). Changes in response amplitudes (in mV) were analysed using mixed design ANOVAs and the Tukey HSD post-hoc test.

Histology

Rats were anaesthetised with urethane (2.0 g/kg) and perfused through the heart with formol-saline. Frozen brain sections were cut at 40 μm and stained with Cresyl violet to verify electrode placements.

RESULTS

Histological examination confirmed that the electrode tips were located in their intended targets for all animals. The field potentials were as characterized by Chapman et al. (1998) who showed that both early and late components are generated by current sinks in upper layer V. In baseline recordings, the mean peak latency of the early and late2 components of the field response was 7.5 msec (range 4.3 to 10.0 msec) and 23.1 msec (range 17.4 to 34.0 msec), respectively. The population spikes emerged most clearly following LTP induction, repeated over 2.0-9.0 msec, and were superimposed on the early monosynaptic component. The maximal LTD effects were typically observed at shorter latencies (late1 component: mean=14.7 msec; range 11.0 to 21.0 msec) than the maximal LTP effects (late2).

Alternating HFS/LFS Stimulation

All animals that received high frequency stimulation showed typical LTP effects (Figure 4-1) as previously characterized by Chapman et al. (1998) and Trepel & Racine (1998). The mean latency for the early monosynaptic and late2 polysynaptic components in this experiment was 7.3 msec (range 4.3 to 9.5 msec) and 23.1 msec (range 21.0 to 30.0 msec), respectively. Both response components showed changes in amplitude following LTP induction yielding a significant interaction between group and session (early component: $F(72,480)=3.46$, $p<0.001$; late2 component: $F(72,480)=5.41$, $p<0.001$). While there was a clear increase in the amplitude of the polysynaptic component, there was a decrease and often a reversal in

the amplitude of the monosynaptic component (Figure 4-1), attributable to a potentiation of population spike activity (Chapman et al, 1998). As in previous studies (Froc et al., 2000; Chapman et al., 1998; Trepel and Racine, 1998) the greatest potentiation effects were observed at a test pulse intensity of 250 μA. The LTP effect observed in the late component reached asymptotic levels on the eighth day of conditioning for the LTP control animals.

In contrast to HFS alone, when HFS was followed two minutes later by LFS the amount of LTP in the polysynaptic component was markedly reduced (Figure 4-2B). The change in the polysynaptic component was smaller and required fifteen days to reach asymptote compared to the LTP control group, which required only eight days to reach asymptote, yielding a significant interaction between stimulation session and group ($F(42,280)=6.93$; $p<0.001$). Furthermore, following ten days of conditioning, the LTP control group showed significantly more LTP of the late2 component than the HFS/LFS group ($F(20,170)=4.80$, $p<0.001$).

All conditioning groups showed an amplitude shift in the early component yielding a significant main effect of session ($F(72,480)=3.46$; $p<0.001$) (Figure 4-2A). Again, this early component amplitude shift was presumably due to the increase in the number and magnitude of population spikes superimposed on the monosynaptic component after LTP induction (Chapman et al., 1998). The early component potentiation effect was consistent across the HFS alone and combined stimulation groups, although there was a nonsignificant trend towards reduced potentiation in the HFS/LFS group. Both early and late components showed little decay following the

cessation of conditioning stimulation.

When the LFS *preceded* the HFS, there were no differences in either the early or late2 component amplitude changes compared to the LTP controls. The LFS that would normally produce significant LTD or depotentiation effects (Froc et al., 2000) was ineffective when followed immediately by HFS.

Potentiation (or depression) following prior Depression (or potentiation)

The Initial Potentiation and Depression. Typical LTP effects were produced by ten days of HFS in all groups. Again, the greatest potentiation effects were observed at an intensity of 250 μA and the LTP effects reached asymptotic levels by the last day of conditioning. The mean latency-to-peak for the early and late2 components was 7.8 msec (range 5.6 to 11.0 msec) and 23.0 msec (range 17.4 to 29.0 msec), respectively (Figure 4-3). HFS induced a similar amount of late component LTP across all experimental groups (Figure 4-5) as reflected in a significant effect of conditioning days ($F(12,156)=10.51$; $p<0.001$). There were no significant differences between the LTP effects observed in the LTP control, LTP-LTD and LTD-LTP groups at this latency, indicating that both the induction rate and the asymptotic levels of LTP were also similar between these groups. The LTP effects among the groups should also have been equivalent for the early component, but the potentiation was significantly greater in the LTP control group than in the LTP-LTD group ($F(10,80)=15.35$; $p<0.001$). The pattern of potentiation was otherwise similar between these groups.

Compared to controls, the greatest LTD-induced amplitude reduction was seen in the early (Figure 4-4A) and late1 components (Figure 4-4B) and occurred at an average latency-to-peak of 7.5 msec (range 5.6 to 11.0 msec) and 14.3 msec (range 11.0 to 16.1 msec), respectively. These amplitude reductions were reflected in significant interactions between group and session for both the early ($F(12,120)=7.89$; $p<0.001$) and late1 ($F(12,120)=5.12$; $p<0.001$) components and reached asymptotic levels by the fourth day of LFS.

Long-Term Potentiation followed by Long-Term Depression (Depotentiation). When ten days of low-frequency stimulation followed the induction of asymptotic LTP, there was a depotentiation of the late2 component (Figure 4-3B & 4-4C), as reflected in a significant interaction between group and session ($F(10,80)=2.09$; $p<0.05$). For comparison, the LFS phase in the LTD-LTP group had no significant effect on baseline responses at this latency (Figure 4-4C), but most rats showed minimal baseline activity at these latencies prior to LTP induction, so there was very little to measure. There were no depotentiation effects observed in the early component (Figure 4-3B & 4-4A).

The magnitude of the late1 component LTD in the LFS phase of the LTD-LTP group was similar to the magnitude of the depotentiation observed in the LTP-LTD group (Figure 4-3 & 4-4B).

Long-Term Depression followed by Long-Term Potentiation. Ten days of LFS induced an LTD in both early and late1 components of the LTD-LTP group. This

depression was very effectively reversed by the subsequent HFS (Figure 4-3A). The HFS, in fact, produced a significantly greater amplitude shift in the early component of animals in the LTD-LTP group compared to LTP controls ($F(10,90)=2.78$; $p<0.005$), and this difference was still evident following 7 days of decay (Figure 4-4A). The late1 component measure (Figure 4-4B) showed a significant decrease in the response amplitude during the LTD phase compared to controls ($F(12,120)=5.12$; $p<0.001$). Subsequently, ten daily sessions of HFS increased the response amplitude of this component from the depressed level to the baseline level that was observed prior to any conditioning (Figure 4-4B). This *potentiation* effect required only 3 sessions of HFS to reach asymptote, but did show some reduction back towards the depressed level following 7 days of decay. The magnitude of the late1 LTD effect in the LTD-LTP group was similar to the depotentiation observed in the LTP-LTD group and there was no difference between these groups following the seven-day decay period (Figure 4-4B).

The late2 component showed no depression effect in the LTD-LTP group and similar potentiation effects were observed in this component in the LTP phase of the LTP-LTD and LTD-LTP groups when the latter group was normalized to the last day of LFS (Figure 4-5).

After the seven day decay period, there was no significant difference between the mean response amplitudes observed in the LTD-LTP and LTP-LTD groups (Figure 4-4C), with the former group decaying somewhat more rapidly than normal, and the latter group showing a small increase in amplitude over the decay period.

Moreover, the faster decay observed in the experimental groups compared to the LTP controls was reflected in a significant group effect ($F(2,13)=5.39;p<0.05$).

DISCUSSION

Low frequency (LTD-inducing) stimulation can modulate the effects of high frequency (LTP-inducing) stimulation when the stimuli are both presented *during* the induction phase, and this effect is dependent on the order of presentation. It is, therefore, unlikely that the differential increases in postsynaptic Ca^{2+} normally associated with LTD and LTP (Lynch et al., 1983; Mulkey & Malenka, 1992; Malenka and Nicoll, 1993; Cummings et al., 1996; Hansel et al., 1996; Zhang & Poo, 2001) simply summate to determine the consequences for the synapse. While normal LTP effects require 8-10 days of conditioning to reach asymptotic levels of potentiation, the HFS/LFS group showed significantly less potentiation after ten days of conditioning, and required fifteen days to reach asymptote. The LFS appears to have suppressed the developing potentiation induced by the HFS, although it remains to be seen if the mechanisms of this effect and depotentiation of a fully induced LTP are the same. During the first few sessions, there is little measurable LTP to affect, so latent effects in a downstream effector pathway is the likely target. Since the HFS effect was stronger or more effective than the LFS, the depotentiation effect did not "clamp" the response amplitude at baseline levels, but the potentiation effect showed a significantly slower rate of change across days of stimulation. It is not clear whether the weaker LTD effect is attributable to the stimulation protocol or reflects a true difference between LTP and LTD. It is also not clear what we should expect from such interactions. Theoretically, it might be an advantage if potentiation and depression (depotentiation) were not equivalent. This would make it difficult to fully

erase stored information. The parameters of the LTP-LTD interactions, however, need to be more fully explored. Low-frequency paired-pulse stimulation, for example, has been shown to induce LTD in regions that were thought to be resistant to LTD induction by single pulse LFS (Kemp et al., 2000; Kemp and Bashir 1999, 1997).

While the previous exposure to LFS did not affect the rate of induction or the magnitude of subsequent LTP induction in the LFS/HFS group, seven days following the cessation of the HFS, the LTP decay in the LFS/HFS group was greater than normal, reaching a level similar to that seen in the HFS/LFS group, indicating that the longevity of the LTP effect may have been adversely affected by the prior exposure to LFS.

It was shown in the previous chapter that while LTP in the sensorimotor neocortex of the awake, behaving rat is NMDAR dependent, LTD and depotentiation are not. LTD is dependent on metabotropic glutamate receptors (mGluRs) in area CA1 of the rat hippocampus (Nicoll and Malenka, 1998; Manahan-Vaughan, 1997; Nicoll et. al., 1998; Oliet et al., 1997; but see Selig et al., 1995b), dentate gyrus (O'Mara et al., 1995; but see Martin and Morris, 1997) and visual neocortex (Haruta et al., 1994; Kato, 1993) suggesting that LTD and depotentiation in our preparation may also be mGluR-dependent.

In an alternative hypothesis Bortolotto et al (1994) suggest that LFS can restrict the subsequent induction of LTP through the activation of mGluRs. The authors proposed that a molecular switch linked to metabotropic glutamate receptor

activation regulates the induction of LTP. Although ([RS]-α-methyl-4-carboxyphenylglycine (MCPG), an mGluR antagonist, blocks the induction of NMDA-dependent LTP at 'naïve' synapses, it does not block the induction of further LTP once submaximal LTP has been induced. The authors propose that mGluRs activate this molecular switch, which is dependent on protein kinase activity and stays on for at least six hours once activated, unlike NMDARs, which must be activated each time for the induction of NMDA-dependent LTP. Moreover, this switch can be reset by LFS. Thus, LFS delivered prior to and following (depotentiation) LTP induction can reset the switch thereby preventing the subsequent induction of LTP. LFS (900 pulses delivered at 2 Hz) in this preparation induced depotentiation but not LTD. These results contradicts the BCM theory, which states that the prior induction of LTD by LFS should lower the threshold for LTP. Clearly, in this case, LFS is resetting the mGluR-dependent switch thereby preventing the subsequent induction of LTP. While LTP in our preparation requires NMDA receptor activation, the role of prior mGluR activation is unknown.

Prior mGluR activation has been shown to prime the induction of LTP (Cohen et al., 1998). Prior mGluR agonism or synaptic activation of mGluRs also facilitates the longevity of subsequent LTP effects and this increased persistence is protein synthesis-dependent (Raymond et al., 2000). Conversely, priming stimulation of group II mGluRs has been found to inhibit the subsequent induction of LTD (Mellentin and Abraham, 2001).

While LTP induction is primarily dependent upon protein kinase activity (Malenka and Nicoll, 1999; Wang and Kelly, 1995; Lisman, 1989), LTD induction effects, at least in the short term, are commonly dependent on protein phosphatases (Kemp and Bashir, 2001; Mulkey et al., 1993; Lisman, 1989). The final change in synaptic weight following patterned activation of afferents may therefore depend on the balance of kinase and phosphatase activities (Coussens and Teyler, 1996). For example, calcineurin, a calcium- and calmodulin-dependent protein phosphatase inhibits the induction of LTP and facilitates LTD (Mulkey et al., 1994) in the rat hippocampus. Stanton (1995) has shown that direct, transient chemical activation of protein kinase C can substitute for prior synaptic activity to prime the induction of LTD and suppress LTP induction in CA1. The PKC-sensitive phosphorylation site(s) may raise and lower the LTP and LTD stimulus thresholds, respectively, in an activity-dependent manner (Stanton, 1995).

AMPA receptors are also known to be involved in the expression of both LTP and LTD. While LTP is thought to involve an increase in functional AMPAR number (Isaac et al., 1995; Liao et al., 1995; Durand et al., 1996; but see Grosshans et al., 2002), LTD and depotentiation have been linked to decreases in AMPARs (Carroll et al., 1999). It may be the final balance between opposing processes mediating the number of functional AMPARs in the postsynaptic membrane that is reflected in the amplitude of the evoked response.

The second experiment in this study shows that asymptotic levels of LTP and LTD are both reversible. Neither the magnitude nor the induction rate differed

between the LTP induced on *naive* synapses and that induced following the induction of asymptotic LTD. Following the induction of asymptotic LTD by LFS, HFS reversed this depression by potentiating the evoked responses back to baseline levels. This subsequent LTP effect, however, decayed below baseline levels in the seven days following the cessation of conditioning suggesting that the prior LTD was not eliminated, but rather masked by the LTP. The LTP longevity may have also been reduced. Perhaps a molecular switch has been altered by the LFS resulting in a less durable LTP. There was no difference in the asymptotic level of LTP or the rate of change during induction of LTP between the experimental groups and the LTP control group.

Similar to the results reported in the previous chapters, depotentiation effects were not evident in the early component. While LTD induction is evident in the early component, depotentiation is not. It is possible that the measuring techniques employed in this experiment were not sensitive enough to detect changes in the monosynaptic component following depotentiation. Determination is doubly difficult, because both LTD and LTP move the early component in the same direction. LTP enhances the population spikes, which have a polarity reversed relative to the population EPSP, and LTD simply depressed the population EPSP. However, the population spike components should be depressed by depotentiation, but they are not, at least not significantly (Figure 4-3B). In any case, the monosynaptic components appear to be more resistant to depotentiation than to LTD. Interestingly, de novo LTD, but not depotentiation, is inducible in calcineurin Aα

knockout mice (Zhuo et al., 1999). According to Lee et al. (2000), LFS-induced depotentiation and LTD at naïve synapses both involve dephosphorylation of the GluR1 subunit of AMPARs, but at different sites. While LTD induction from baseline involves dephosphorylation at the cyclic-AMP-dependent protein kinase (PKA) site (Ser845), depotentiation involves the calcium/calmodulin-dependent protein kinase II (CaMKII) site (Ser831). Furthermore, AMPA receptor endocytosis is dependent upon dephosphorylation of Ser845 (Ehlers, 2000), suggesting that LTD induction may be the result of AMPAR internalization. Increased expression of AMPARs has been associated with the induction of LTP (Liao et al., 2001; Durand et al., 1996; Isaac et al., 1995; but see Grosshans et al., 2002).

LTD mechanisms could be presynaptic, postsynaptic, or both. LTD has been associated with a decrease in quantal size (Oliet et al., 1996; Luthi et al., 1999), a decrease in glutamate release (Bolshakov and Sieglebaum, 1994), and a postsynaptic decrease in AMPA receptor expression (Carroll et al., 2001; Daw et al., 2000; Man et al., 2000; Carroll et al., 1999a; Carroll et al., 1999b; Luthi et al., 1999). Changes in NMDAR expression and/or function could also play a role (Montgomery & Madison, 2002; Grosshans et al., 2002). LFS has been shown to decrease protein levels of the NR1 subunit of the NMDAR in vivo (Heynen et al., 2000) and LTD of the AMPAR-mediated excitatory postsynaptic current (EPSC) has been reported concomitantly with depression of the NMDAR-mediated EPSC (Selig et al., 1995a; Xiao et al., 1995,1994; but see Carroll et al., 1999b). In paired recordings between CA3 pyramidal neurons, Montgomery and Madison (2002) have shown that while *de novo*

LTD is NMDAR-dependent, depotentiation requires mGluR activation. Given that mGluR-LTD does not occlude NMDAR-LTD (Oliet et al., 1997) these two forms of LTD may involve different expression mechanisms. These mechanistic differences between LTD and depotentiation suggest that the molecular *state* of the synapse has been altered by the induction of LTP (Montgomery & Madison, 2002; Lee et al., 2000). However, in the neocortex of the awake behaving rat, LTP was NMDAR-dependent but LTD and depotentiation were not (Froc & Racine, in preparation). Chemical activation of mGluRs in cultured hippocampal neurons can produce an LTD that is associated with long-lasting loss of AMPA receptors from the membrane surface (Xiao et al., 2001). It remains to be seen whether or not LTD or depotentiation are mGluR dependent in our chronic preparation.

The HFS had a greater effect on the early component amplitude when ten days of LFS *preceded* HFS, suggesting that prior exposure to the LTD-inducing stimulation had some enduring effect on subsequent LTP induction. Surprisingly, this effect was not observed in the late component.

While more research is needed to ensure that the optimal induction protocols are being used, it is clear from these data that LTD and LTP are capable of modulating one another in the awake behaving rat. That the effects of HFS and LFS could, at least partially, reverse each other, suggests that they can act cooperatively to modify the functional state of cortical networks.

FIGURE CAPTIONS

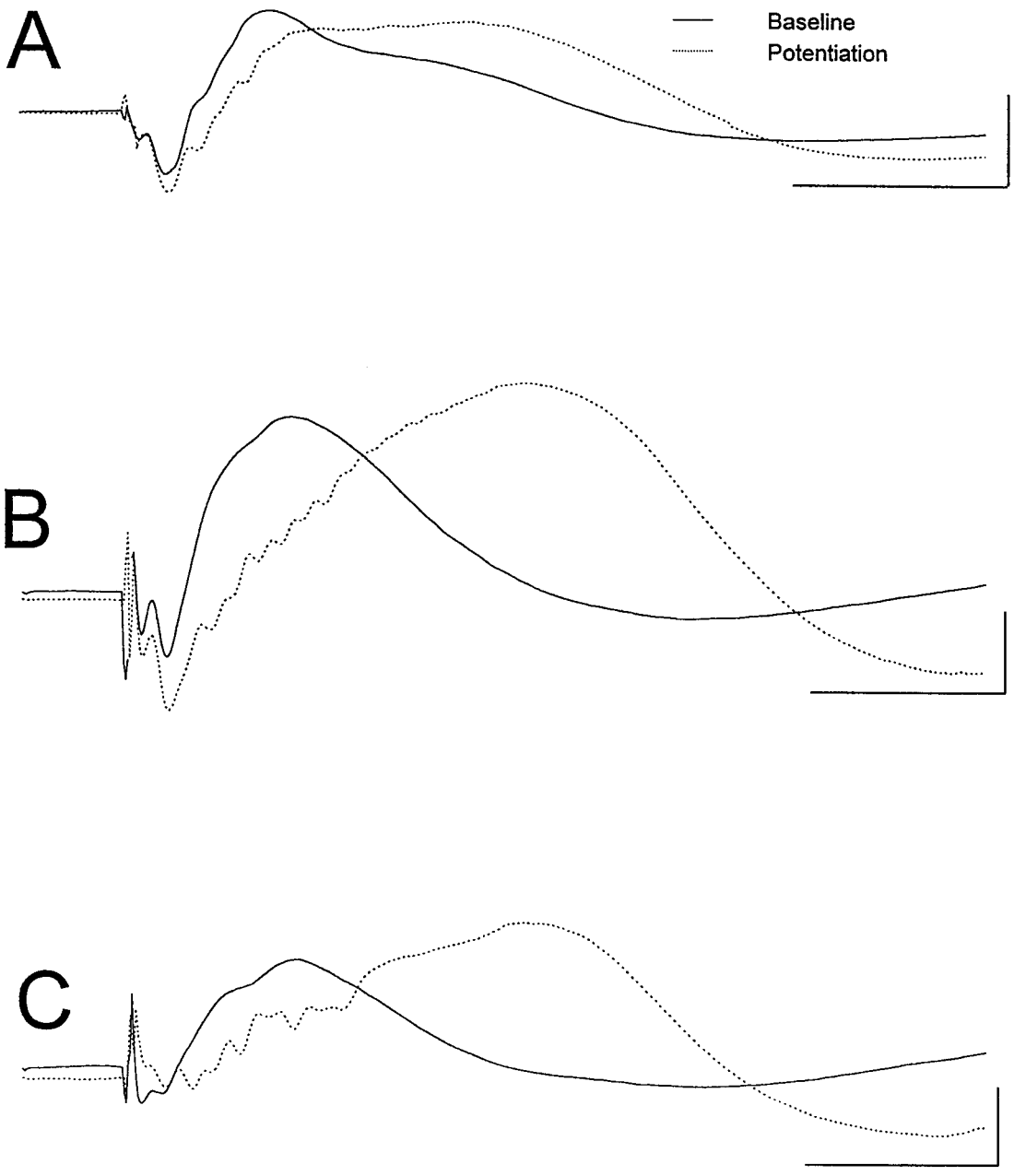
Figure 4-1. HFS/LFS and LFS/HFS stimulation are compared to HFS-Alone. Somatosensory cortex field responses are shown here representing baseline responses compared to 10 days of (**A**) combined HFS-LFS stimulation, (**B**) combined LFS-HFS stimulation and (**C**) HFS alone (vertical calibration: 1.0 mV; horizontal calibration: 10 msec). Baseline (solid), post conditioning (dashed) are shown in representative animals. While HFS alone and LFS-HFS induce a similar LTP effect, 10 days of combined HFS-LFS stimulation induces significantly less LTP.

Figure 4-2. The effects of combining HFS and LFS daily. The mean amplitudes (± SEMs) of the (**A**) early and (**B**) late components are shown here. Values indicate the change in the amplitude relative to the last baseline I/O test. The early component showed a clear amplitude shift (positive-going direction from layers I-V) following the conditioning stimulation in all groups. There was little decay in this component for all groups seven days after the cessation of the conditioning. The late component was clearly enhanced in all groups following conditioning. The HFS/LFS group showed significantly less potentiation following ten days of conditioning and required 15 days to reach asymptote. While the LTP effects showed some decay seven days following the conditioning, it did not return to baseline.

Figure 4-3. LTP, depotentiation, LTD, and LTD-reversal. Somatosensory cortex field responses are shown here representing (**A**) LTD and LTD-reversal and (**B**) LTP and depotentiation (vertical calibration: 1.0 mV; horizontal calibration: 10 msec). Baseline (solid), potentiation/depression (dashed) and depotentiation/LTD reversal (long dashes) are shown in representative animals. While HFS causes a complete reversal of LTD back to baseline and potentiates a longer latency component, LFS only partially but significantly depotentiates LTP.

Figure 4-4. The effects of ten days of LFS delivered before and after ten days of HFS. The mean amplitudes (± SEMs) of the (**A**) early, (**B**) late1 and (**C**) late2 components of evoked somatosensory cortex responses. The early component changes induced by HFS are greater when LTP induction is preceded by LTD induction. The late2 component shows little depression following ten days of LFS, which does not affect the subsequent induction of LTP. Following seven days of decay, this LTP decays to the depotentiated level observed in the LTP-LTD group. Ten days of LFS delivered after the induction of maximal LTP induces a clear depotentiation effect. The late1component shows a smaller LTP effect that is depotentiated by subsequent LFS. At this latency, maximal depression is observed and this depression can be reversed back to baseline levels by HFS. The LTP-LTD and LTD-LTP groups are not significantly different from the control groups following seven days of decay.

Figure 4-5. Comparison of maximal LTP induction across stimulated groups. The mean amplitudes (± SEMs) of the late2 components of evoked somatosensory cortex responses. The three baseline measures for the LTD-LTP group are taken from the last three days of the LTD induction phase. LTP-LTD, LTP controls and LTD-LTP groups are superimposed to show the similarities in the induction of LTP by HFS regardless of prior synaptic activity. There is significantly more decay in the experimental groups compared to the LTP controls.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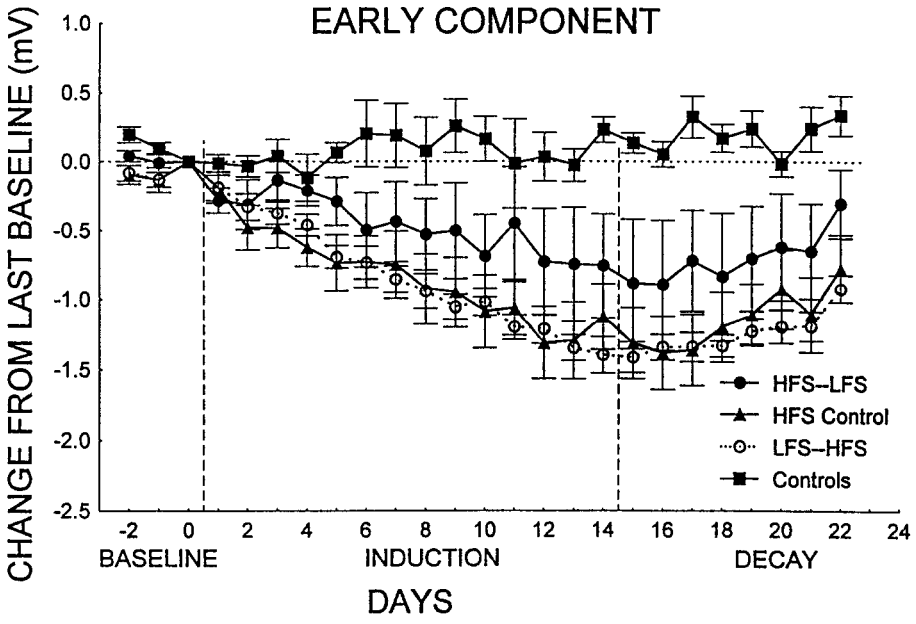
Baseline
Potentiation
B
C

A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-1.5
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-2.5
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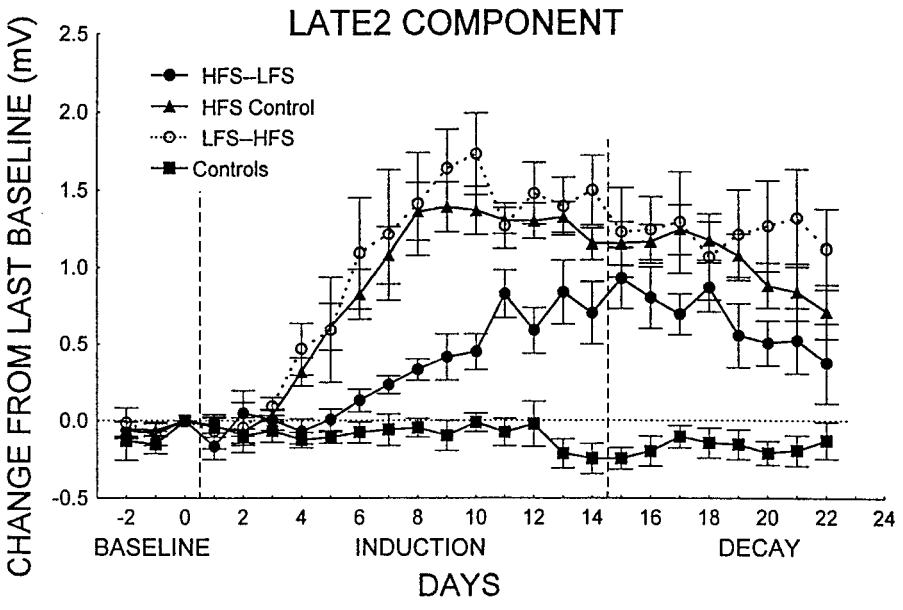
INDUCTION
DECAY
DAYS
HFS--LFS
HFS Control
LFS--HFS
Controls

B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CHANGE FROM LAST BASELINE (mV)
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1.0
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0.0
-0.5
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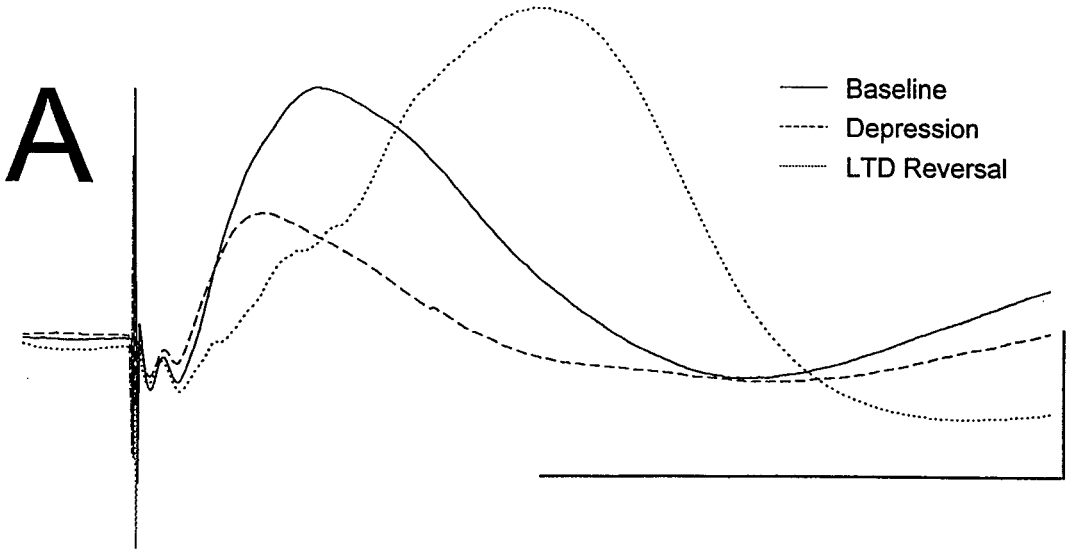
BASELINE
INDUCTION
DECAY
DAYS
HFS--LFS
HFS Control
LFS--HFS
Controls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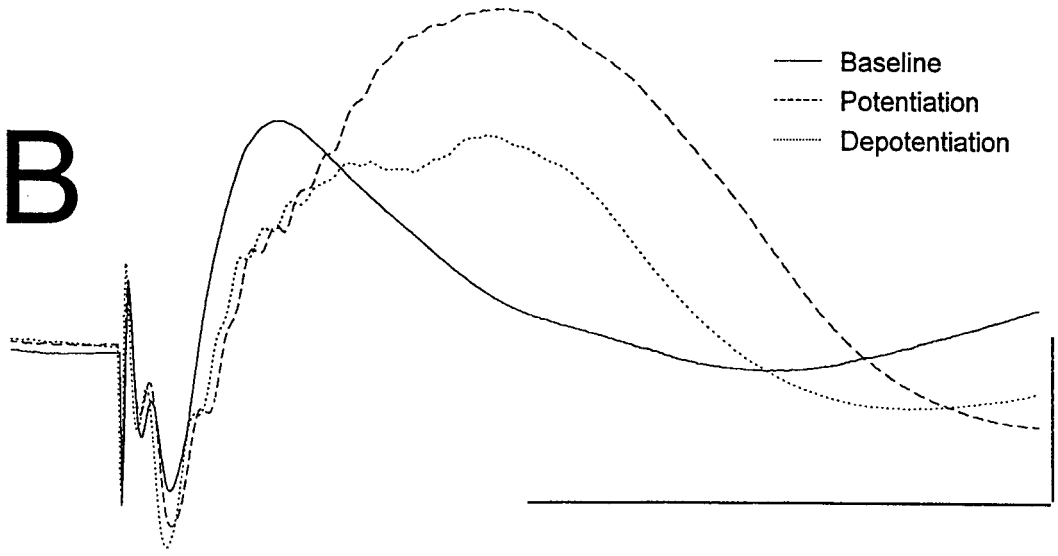
LTD Reversal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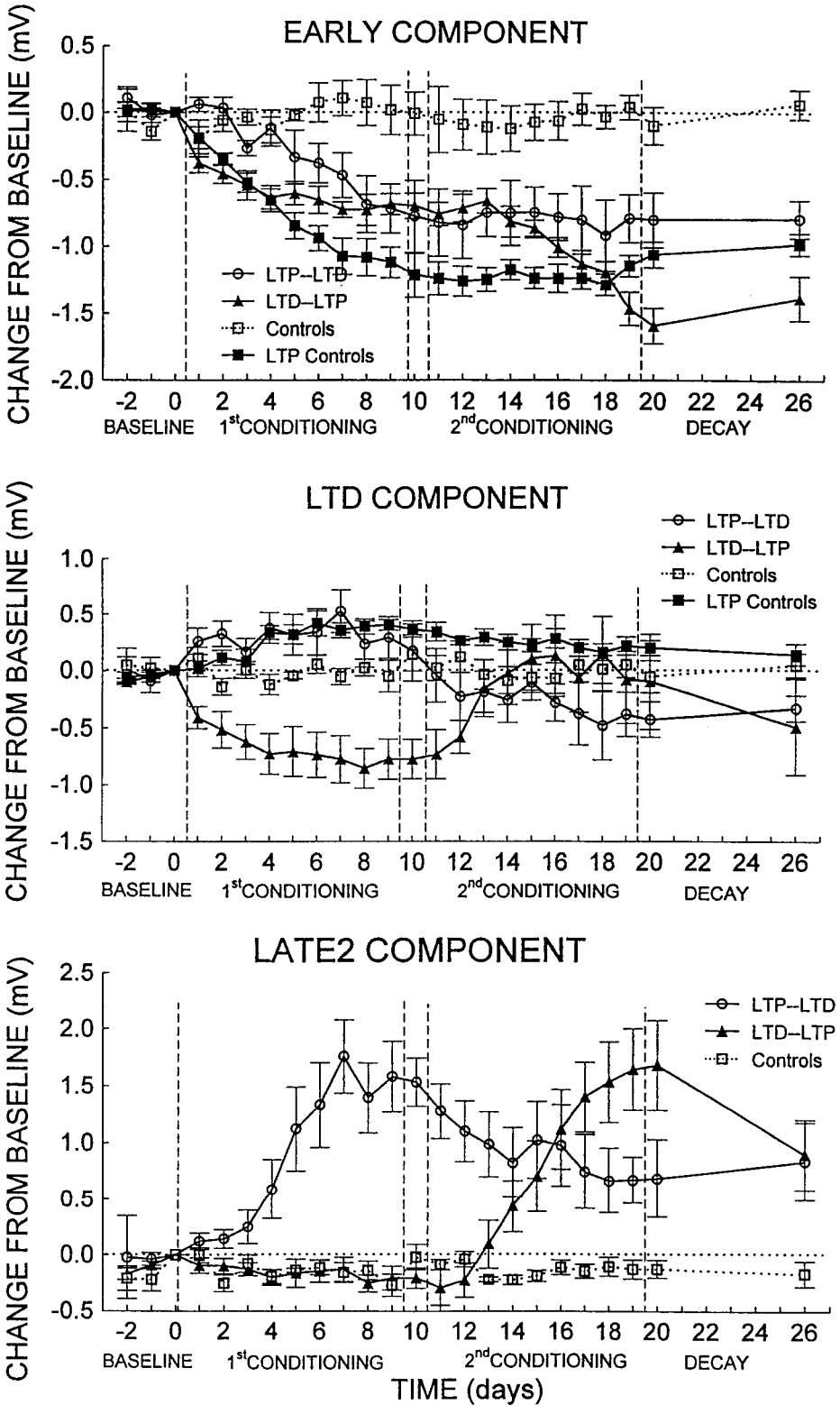
Depotentiation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CHANGE FROM BASELINE (mV)
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-0.5
-1.0
-1.5
-2.0
LTP--LTD
LTD--LTP
Controls
LTP Controls
-2 0 2 4 6 8 10 12 14 16 18 20 22 24 26
BASELINE
1st CONDITIONING
2nd CONDITIONING
DECAY
LTD COMPONENT
CHANGE FROM BASELINE (mV)
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0.5
0.0
-0.5
-1.0
-1.5
LTP--LTD
LTD--LTP
Controls
LTP Controls
-2 0 2 4 6 8 10 12 14 16 18 20 22 24 26
BASELINE
1st CONDITIONING
2nd CONDITIONING
DECAY
LATE2 COMPONENT
CHANGE FROM BASELINE (mV)
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2.0
1.5
1.0
0.5
0.0
-0.5
LTP--LTD
LTD--LTP
Controls
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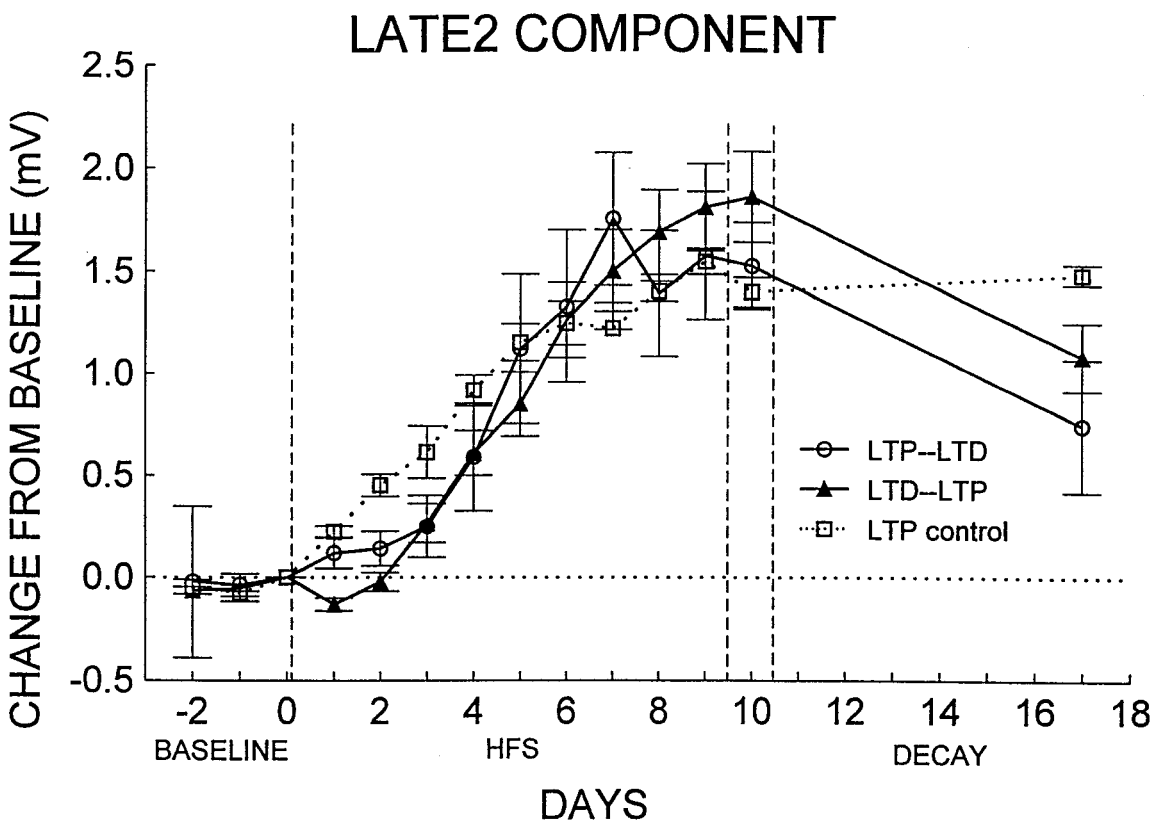
BASELINE
1st CONDITIONING
2nd CONDITIONING
DECAY
TIME (days)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CHANGE FROM BASELINE (mV)
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1.0
0.5
0.0
-0.5
-2
0
2
4
6
8
10
12
14
16
18
BASELINE
HFS
DECAY
DAYS
LTP--LTD
LTD--LTP
LTP control

CHAPTER FIVE

General Discussion

LTP has garnered widespread support as a model phenomenon suitable for the investigation of the mechanisms underlying memory storage in the mammalian brain, and it is currently the most widely studied model of use-dependent synaptic plasticity. If LTD and depotentiation play integral roles in the long-term storage of memory, it should be possible to produce them in the neocortex of the awake behaving animal. Furthermore, if LTD, depotentiation and LTP work in concert to store and maintain information, the relationship and interactions between these synaptic phenomena must be understood.

In chapter 2 it was shown that the HFS protocol used by Racine et al. (1995) to induce LTP in the neocortex of the behaving rat, with stimulation applied to the white matter, could be used to induce LTP in a homologous site in the contralateral hemisphere, with stimulation applied directly to the cortex. Furthermore, in accordance with the established literature, prolonged LFS was found to reverse previously established LTP in this system and induce a LTD in naïve animals. While the LTP induced by contralateral stimulation was similar to that induced by ipsilateral stimulation, depotentiation effects were much greater and longer lasting with ipsilateral stimulation. Thus, LTD and depotentiation effects may require an even greater cooperativity among co-active afferents than does LTP. Also, ipsilateral and contralateral stimulation may recruit different patterns of subcortical

neuromodulatory inputs to the cortex, which may produce different effects on LTP and LTD. Layer I, for instance, contains various diffuse neuromodulatory systems including cholinergic, noradrenergic and serotonergic projections from subcortical structures (Vogt, 1991). Any number of these systems may be involved in modulating the induction of LTD or depotentiation. Interactions between different pathways in the altering of synaptic efficacy have been demonstrated many times. For example, Hess and Donoghue (1996) reported that, in the absence of bicuculine, LTP of horizontal connections could only be induced by conjoint stimulation of horizontal and vertical inputs. Iriki et al. (1991) showed that thalamocortical input from the ventrolateral nucleus to the motor cortex could only be potentiated when coactivated with the corticocortical input from the somatosensory cortex. Amplitudes of both corticocortical and thalamocortical EPSPs were potentiated after combined tetanic stimulation and this potentiation occurred exclusively in neurons that were located in the superficial layers (II/III) and that received direct input from both the somatosensory cortex and ventrolateral nucleus. This corticocortical connection between the somatosensory and motor cortices is critical for learning new motor skills (Pavlides et al., 1993). While contralaterally and ipsilaterally induced LTP were quite similar, the induction of LTD and depotentiation in our preparation may require a more focussed thalamo- and corticocortical activation.

The population spike, and the monosynaptic and polysynaptic components all expressed potentiation and depression, but depotentiation appeared to be expressed primarily within the polysynaptic component. It is still unknown, however, whether

an altered polysynaptic component amplitude reflects an altered monosynaptic drive into the polysynaptic circuitry, changes at the polysynaptic connections themselves, or some combination of the two. In vitro LTD and depotentiation can be induced in horizontal motor cortex pathways (Hess & Donoghue, 1996). Layer V pyramidal cells are known to project, via horizontal collaterals, for long distances within layers III and V, while layer III horizontal fibres are primarily restricted to the superficial layers (Aroniadou & Keller, 1993). If polysynaptic potentiation and depression are mediated by changes within horizontal fiber pathways, depotentiation may be restricted to the synaptic connections within these pathways.

Analogous to LTP induction in the neocortex, LTD induced by ipsilateral white matter stimulation was found to be greater in magnitude and longevity when multiple trains were delivered rather than a single train. However, unlike neocortical LTP (Trepel & Racine, 1998) the multiple LFS sessions used to induce LTD were equally effective whether spaced over hours or days. The requirement for multiple stimulation sessions suggests that LTD reflects a gradual, cumulative process involving mechanisms distinct from those involved in rapid single session effects. The spacing of the trains, however, is not as critical for LTD induction as it is for LTP induction.

In chapter 3 we confirmed that, consistent with the hippocampal literature, LTP in the neocortex is NMDAR dependent. However, both LTD and depotentiation were found to be NMDAR independent. In fact, pharmacological blockade of NMDARs during the induction of LTP by HFS produced an LTD effect that was

remarkably similar to the LTD observed following multiple sessions of LFS suggesting that the frequency or pattern of activation is not the final determinant of the direction of synaptic weight change. Moreover, these data are consistent with the idea of a dual threshold model for the induction of LTP and LTD whereby lower levels of synaptic depolarization cause a small net increase in postsynaptic calcium levels that trigger LTD, and larger depolarizations permit larger calcium flux through NMDARs leading to LTP. LTD and depotentiation effects have also been shown to depend on metabotropic glutamate receptors, which release calcium from internal stores via second messenger pathways (Kato, 1993; Haruta et al., 1994; O'Mara et al., 1995). Antagonism of mGluRs has been shown to block visual cortical LTD (Kato, 1993; Haruta et. al., 1994).

The long-term storage of memory in the neocortex is thought to involve a gradual process of incremental changes in synaptic strength across a network of neurons through repeated reactivation of the neocortical engram (McClelland et al., 1995). Current experimental evidence suggests that LTP maintenance is comprised of an early stage that lasts for several hours and involves protein kinases and the phosphorylation of key substrates, such as the AMPAR, and a later phase, which lasts longer than 24 hours and involves protein synthesis (Krug et al., 1984; Otani et al., 1989; Nguyen & Kandel, 1996; Bailey et al., 1996). In our preparation, LTP requires 8-10 days to reach asymptote and more than one day just to see the onset of the effect, suggesting that the timescale for the maintenance of LTP in the neocortex of the awake behaving animal may be different than that established for the hippocampus.

Furthermore, the reversal of established neocortical LTP, depotentiation, follows different rules than that of its hippocampal counterpart. Contrary to the established literature regarding the hippocampal depotentiation (Fujii et al., 1991; O'Dell & Kandel, 1994; Staubli & Chun, 1996), depotentiation in the neocortex in vivo is not restricted to the time immediately following the induction of asymptotic LTP. We have successfully induced depotentiation 7 days following the saturation of LTP, immediately following LTP induction, and during the LTP induction phase. This suggests that neocortical depotentiation is not simply blocking the "consolidation" of LTP. The presence of a depotentiation effect suggests that LTP decay is not simply a passive process but may involve an active resetting of connection weights. Villarreal et al (2001) showed that blocking NMDARs *after* inducing LTP prolongs LTP and the retention of spatial memory.

In our hands, depotentiation rarely returns the potentiated response back to baseline levels, regardless of the delay period between LTP induction and LFS. This resistance to complete reversal may protect newly learned tasks and memories from being too easily abolished (particularly in the neocortex). Alternatively, we may not have discovered the optimal induction parameters. Depotentiation may reflect a process by which new memories can be incorporated into a network of neurons already storing previously encoded, but little utilized, information. Given the lack of NMDA-dependency for these neocortical LTD and depotentiation effects, it is possible that mGluRs may represent the neocortical mechanism by which memories are actively erased.

Chapter 4 addressed the effects of combined HFS and LFS during and immediately following the induction of LTD and LTP to determine the interactions between these synaptic phenomena. We found that combining LFS with HFS during the induction phase attenuates the LTP effect only if the LFS follows the HFS, suggesting that the postsynaptic mechanisms involved in the induction of LTD and depotentiation are only effective modulators of synaptic potentiation when the HFS induced changes are already underway (ie depotentiation). Conversely, the prior LFS has no effect on subsequent LTP associated mechanisms. When LFS precedes HFS, the postsynaptic Ca^{2+} increments induced by each pattern of activation may simply summate leading to the same level of LTP induction.

While theory has driven much of our current understanding of the processes involved in the long-term storage of memory in biological systems, biological plausibility for these theories can only be borne out by experiments that provide evidence of these processes in biological systems. These experiments show that activity-dependent bidirectional changes in synaptic strength occur in a biological system known to support the long-term storage of information in accordance with the learning rules delineated by Hebb (1949). It remains to be seen whether these activity-dependent phenomena share mechanisms with learning and memory.

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