NEOCORTICAL LONG-TERM POTENTIATION IN THE ADULT, FREELY MOVING RAT

By

CHRISTOPHER TREPEL, B.Sc.

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AUTHOR: Christopher Trepel, B.Sc. (University of Calgary)

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ABSTRACT

Long-term potentiation (LTP) is a lasting enhancement of synaptic transmission following high frequency electrical stimulation. It has been widely studied as a memory model, primarily because it has associative properties and is long-lasting. If LTP reflects a mechanism for information storage, then it should be readily induced within structures believed to encode long-term memory. The neocortex, however, has proven resistant to LTP induction. Most of our knowledge about neocortical LTP has come from anaesthetized and *in vitro* slice preparations. If LTP is to be considered seriously as a memory model, then it must be demonstrated in awake animals and shown to last for periods longer than a few hours.

This thesis provides the first parametric analysis of neocortical LTP in the freely moving rat including: (i) the variables governing the induction and decay of LTP; (ii) the critical role played by the NMDA receptor in LTP induction; (iii) GABAergic and cholinergic modulation of LTP and; (iv) the effects of maximal electroconvulsive shock on the induction of LTP.

Neocortical potentiation resulted in robust changes in an early and two late components of the evoked EPSPs, as well as the superimposed population spikes. Stronger potentiation effects were obtained by increasing the number of trains

delivered/session, the number of stimulation sessions, the pulse intensity of the trains, or the interval between train delivery. Moreover, the delivery of either 1 train/day, or very low intensity trains, resulted in LTP. An NMDA antagonist blocked potentiation and unmasked a long-term depression effect. GABAergic agonism or cholinergic antagonism retarded the development of LTP while cholinergic agonism enhanced LTP of a polysynaptic component. GABAergic antagonism slowed the development of LTP. Maximal electroconvulsive shock (MES) resulted in a blockade of LTP induction that was dependent on the interval between the trains and the MES stimulation. These results are discussed relative to *in vitro* data and considered in the context of the different information processing features of the neocortical and hippocampal learning systems.

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IT'S BETTER TO BURN OUT THAN TO FADE AWAY

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CHAPTER 1

GENERAL INTRODUCTION

The hippocampus and neocortex are believed to be critical for learning and memory processes. The individual roles played by these structures in memory formation, as well as the interactions between them, are discussed below.

1.1 Dual Memory Systems

The present belief that there are at least 2 anatomically and functionally distinct learning and memory systems is based upon observations made of patients who have suffered large, bilateral lesions of the hippocampus and related structures (Scoville and Milner, 1957; see also Zola-Morgan et al., 1986; Squire et al., 1989; Squire and Alvarez, 1995). Damage to the hippocampal system appears to compromise some types of learning and memory, termed *declarative*, but not others, termed *non-declarative*. Declarative memory (also termed explicit memory) refers to the conscious recollection of facts and events (Cohen and Squire, 1980). This type of memory is characterized as being fast, not always reliable (that is, retrieval failure and confabulation can occur) and flexibly accessible to multiple response systems (Squire et al., 1993). Non-declarative memory (Squire, 1992), by contrast, is considered slow (with the exception of priming), reliable and inflexible in the sense that retrieval cannot be performed by systems other

than those used for the original encoding. This type of memory influences behaviour without the need for conscious access to the stored information. Non-declarative tasks include gradually acquired skills that require several practice sessions (eg: Cohen and Squire, 1980), learning the structure common to a set of items (eg: Knowlton and Squire, 1993) and some forms of classical conditioning (Zola-Morgan and Squire, 1993). The available experimental data strongly support the belief that declarative tasks depend upon the hippocampal memory system, while non-declarative tasks are mediated by neocortical areas (for reviews see Zola-Morgan and Squire, 1993; McClelland et al., 1995).

Patients with hippocampal lesions also consistently display moderate to severe retrograde amnesia depending upon the extent of the damage (Squire, 1992). In addition, the retrograde amnesic effects are temporally-graded. Memory for a particular learning task is impaired if the hippocampus (and surrounding structures) are damaged during a period of time after the learning experience. This amnesia is not observed if there is a large interval between the original learning experience and the subsequent damage (Zola-Morgan and Squire, 1993).

Two theories were developed to explain the time-limited role of the hippocampus in this process. The first posits that the types of memory dependent upon the hippocampus are short-lived, either written over or decaying with the passage of time. A necessary conclusion of this theory, however, is that the ability to recall the recent past can never fall below the ability to recall the remote past, a conclusion that has been shown to be false (Zola-Morgan and Squire, 1990).

The second theory suggests that the hippocampal system plays a temporary role in

memory, because the information originally stored in the hippocampus gradually becomes independent of this structure (Squire et al., 1984). It is believed that after a learning experience there is a gradual reorganization of the information wherein the importance of the hippocampal system gradually diminishes as the information is transferred to a more permanent memory store, presumably in the neocortex. This view has received a great deal of experimental support since its original formulation, primarily from prospective animal studies of temporally-graded retrograde amnesia. One of the earliest demonstrations came from work in monkeys (Zola-Morgan and Squire, 1990). In this experiment, monkeys were trained to accomplish a series of simple object discriminations over the course of a 10 day period. At varying intervals following the training period (2-16 weeks), separate groups of monkeys underwent nearly total ablation of the hippocampal formation, including the subicular complex and entorhinal cortex. These authors found that the operated monkeys performed more poorly than normals only when the object pairs had been learned as recently as 2-4 weeks before surgery (Zola-Morgan and Squire, 1990). These data indicated that monkeys that had received hippocampal system lesions were able to remember remote information considerably better than they were able to remember recently acquired information.

Several researchers have replicated this effect using rat models. Winocur (1990) examined socially transmitted food preferences in hippocampally lesioned animals. In this paradigm, a naive rat is paired with a "demonstrator" animal that has recently consumed food flavoured in a particular way. The naive animal subsequently develops a preference for the food sampled by the demonstrator and, on the basis of this preference,

can learn to discriminate between two differently flavoured foods. Winocur (1990) trained rats on this task and then performed hippocampal lesions immediately, 2, 5 or 10 days after the learning protocol. While sham-operated rats' preference for the previously demonstrated diet was strongest immediately following surgery and declined steadily thereafter, rats that had been hippocampally-lesioned immediately after, or 2 days following exposure to the demonstrator, did not show preference learning. In fact, rats with hippocampal lesions did not show normal performance levels until the interval was extended to 5 days.

Kim and Fanselow (1992) made hippocampal lesions in separate groups of rats 1, 7, 14 or 28 days after training in a fear conditioning task. Rats were classically conditioned to fear both the presentation of a tone, as well as the context in which the tone-shock pairing took place (a distinctive testing box). Animals that were lesioned 1 day after training showed no signs of contextual fear, while animals that had longer intervals between training and surgery showed little or no impairment. As in primates, the rat hippocampus appears to have a temporally limited role in memory storage.

The same point has been made in other rodent species. Using mice, Cho et al. (1993) demonstrated that chemical lesions of the entorhinal cortex (EC) resulted in temporally-graded amnesic effects on discrimination problems as assessed using the radial arm maze. The entorhinal cortex is the major input to the hippocampus and, thus, its destruction results in the effective loss of hippocampal processing (Amaral and Witter, 1989). The mice were trained to discriminate between baited and unbaited arms on a series of five, 2-choice radial arm problems. The tasks were learned at different intervals

ranging between 0.5-8 weeks pre-surgery. While control animals showed significant forgetting with increasing retention intervals, the EC-lesioned mice showed greater retention for the tasks learned *less* recently (i.e., > 4.5 weeks pre-lesion). Cho et al. (1993) interpret these data to indicate that a 4 week period is required before memory for a 2-choice discrimination problem becomes independent of the entorhinal cortex (and, by association, the hippocampal system generally), and that after this time, an entorhinal cortex-independent structure becomes the storage site for the learned information.

Most recently, Kim et al. (1995) have shown that the well-studied eyeblink/nictitating membrane response in rabbits also shows a temporally-graded dependence on the hippocampal system. Tones (CSs) were paired with puffs of air (USs) delivered to the eye of a rabbit to elicit blink responses (URs). The CR is an eyeblink in response to the tone. Kim et al. (1995) trained rabbits on this task and then performed bilateral hippocampal aspiration lesions either 1 day or 1 month following learning. They reported that rabbits lesioned 1 day post-learning were severely impaired in the retention of the conditioned responses. Sham-control lesioned rabbits, and rabbits that received aspiration lesions 1 month after training, retained the conditioning at equivalent levels. These authors conclude that the hippocampus is necessary for the retention of recently learned, but not remotely learned, conditioned responses.

Most researchers assume that the neocortex is responsible for the long-term storage of information. It is less clear what role the hippocampus plays in the consolidation of long-term memory. One of the most compelling arguments for a role for the hippocampus in the consolidation of neocortical memories stems from computational

considerations. Modeling efforts have led researchers to conclude that the acquisition of new knowledge can easily disrupt the integrity of existing knowledge (an effect termed *interference*), resulting in some circumstances in the complete loss of the original information (*catastrophic interference*). One hypothesis about how this problem may be avoided has been forwarded by McClelland, McNaughton and O'Reilly (1995). These authors began with the assumption that the hippocampus is capable of rapid learning, while the neocortex must acquire information slowly. There are advantages and disadvantages associated with each. Rapid learning permits the fast acquisition of new information with little requirement for repetition, and thereby allows for immediate access to the new learning. However, for many learning tasks, such as paired-associate learning, the rapid acquisiton of new information results in strong intereference with previously learned material (Barnes and Underwood, 1959). Slow learning, while it can dramatically reduce these interference effects, would not permit us to make adaptive changes quickly enough to survive. McClelland et al. (1995) have attempted to resolve this paradox by analyzing neural network responses to *focused* and *interleaved* learning. Focused learning refers to the acquisition of new information which is presented to the system repeatedly without reference to previously stored data relevant to that information. By contrast, interleaved learning is a strategy in which new information is presented together with (interleaved with) old information. A slow learning rate is required to allow the new information to be interleaved with previously learned information and allows for the discovery of shared structure between newly learned and previously learned items and events. *Structure* is defined as any systematic relationship between

items or events that, when learned, can be used as a template to appropriately identify novel events and make appropriate responses. McClelland et al (1995) offer several reasons to explain why the discovery of structure depends on a slow learning rate. The first is based on our need to derive general rules or characterizations for items to be learned rather than simply memorizing the individual items themselves. There are a number of different sets of connection weight changes that could store a specific piece of information. Unfortunately, the pattern of connections that a network would most easily settle into is not likely to be suitable for the retention of previously learned information. Consequently, there is a risk that the previous information will be written over. This is one of the advantages of a slow learning rate—it allows time for the reinstatement of old representations which permits the old information to be maintained in the newly developing traces.

Although focused learning is far superior to interleaved learning when speed of acquisition of new information is at issue, the focused strategy fails utterly at preventing interference with existing memories in networks storing related information. In short, focused learning allows information to be obtained quickly, but this information must be sacrificed if new information is to be stored. In order to avoid interference and thereby retain previously learned material, the system must either be designed for the storage of orthogonal patterns of input or an interleaved strategy is required.

From these considerations, McClelland et al. (1995) have proposed a division of labours for the hippocampal and neocortical memory systems. The role of the hippocampus is to acquire limited amounts of information quickly while at the same time

storing it in a form that does not interfere with the information already stored in the neocortex. The hippocampus utilizes a sparse encoding strategy to minimize interference between old and new information. It sacrifices the ability to extract common structure across input patterns. Once stored, however, the memory can be reactivated and subsequently reinstated in the neocortex where it can be interleaved with existing exemplars so that it may be stored with minimal interference. This reinstatement allows for both the initiation of behavioural responses appropriate to the memory and provides an opportunity for the incremental adjustment of the appropriate neocortical connections. Eventually, the memory can become independent of the hippocampus.

The exact mechanisms of memory storage in either the hippocampus or neocortex have yet to be conclusively identified, but the strongest candidate theories are those involving changes in synaptic strength. Based on the theoretical arguments outlined above, hippocampal synapses would be expected to alter their connection strength quickly, while neocortical synapses should change more slowly (McClelland et al., 1995). Section 1.3 discusses the best known and understood model for the study of synaptic plasticity, long-term synaptic potentiation (LTP). In order to appreciate the physiological preparations commonly employed in the examination of neocortical LTP, however, we must first consider the anatomical features of the neocortex. The next section, then, covers the general anatomy of the neocortex including a more detailed examination of regions Par1 and Fr3 that were specifically used in the series of studies described in chapters 2-6.

1.2 Anatomy of the Neocortex

General Features

The rat cortex is divided into *isocortical* and *allocortical* areas. Those cortical regions showing a clearly defined, 6-layered structure fall into the former category, while regions displaying greater homogeneity are grouped into the latter. Evolutionary considerations have resulted in the isocortex being renamed *neocortex*, while the allocortex has been further divided into *paleocortical* (olfactory) and *archicortical* (limbic) regions (Stephan, 1975).

The principal cell types in the cortex are pyramidal, granule (or stellate) and fusiform (or spindle) neurons. The pyramidal cells are the output cells of the cerebral cortex. There is only one case where it appears that nonpyramidal cells, specifically a group of large spiny cells found in layer IVb of the visual cortex, project extracortically. This stellate cell population sends axons to the visual association area MT (medial terminal nucleus of the accessory optic tract) in the middle temporal sulcus (Lund, 1984). The layers of the neocortex are divided according to the types and packing density of the cells they contain, and are comprised of both somata and intrinsic and extrinsic axonal pathways (Brodmann, 1909; but see Vogt and Vogt, 1919 for lamination based upon myelin stains). The following terms have been applied to the cortical layers (White, 1989): I, molecular layer; II, small pyramid layer; III, layer of medium and large superficial pyramids; IV, granular layer; V, layer of large, deep pyramids; and VI, multiform layer (primarily fusiform neurons).

Lorento de Nó (1949) asserted that the most constant feature of cortical structure was the arrangement of axonal and dendritic branching within a cortical column. Layer V pyramidal cells extend basilar dendrites into their own layer and an apical dendrite to the superficial layers (usually layer I). The ascending dendritic shafts of layer VI fusiform cells terminate in layers I and IV, while their descending dendrites remain within layer VI. Layer IV granule cells possess many dendrites which extend in all directions, generally terminating within layer IV itself and up into layer III. The basilar dendrites of layer II/III pyramidal cells are generally contained within the same layer, while their apical dendrites extend into layer I. The axons of pyramidal and fusiform neurons, and many of the deeper granule cells, become projection, association or callosal fibers, and these axons additionally send collaterals to layers V and VI, forming horizontal connections. Many of these axons also send collaterals through layer IV, synapsing in layers II/III.

The neocortical areas all receive their major subcortical input from specific thalamic nuclei terminating in layer IV and lower layer III (Ribak and Peters, 1975). Moreover, additional inputs to layers I and VI are received from ventromedial and intralaminar thalamic nuclei. This general pattern holds true for areas with clear granular layers. In those without a clearly defined layer IV (e.g., motor areas, but see Skoglund et al., 1997), the main thalamocortical afferents project to layers I and III. The cortex is also connected to the thalamus both reciprocally, with efferents arising primarily from layer VI pyramidal cells, and nonreciprocally, through efferents projecting from layer V pyramidal neurons (Herkenham, 1978).

Callosal connections can be either homotopic or heterotopic depending on whether homologous or asymetrical loci are the targets. Layers II/III, V and VI are the output source of most callosal connections. The callosal connections terminate in all layers, though they are most densely packed in layers I-III and V-VI in most cortical regions (Isseroff et al., 1984; Carpenter, 1972). A circuit diagram depicting the basic anatomical arrangement of the neocortex is shown in Figure 1.1.

Parietal Area 1 (Somatosensory Cortex)

The somatosensory cortex of the rat has the 6 layer arrangement typical of the neocortex. The primary somatosensory area (SI) has been divided up into parietal area 1 (Par1), the ipsilateral forelimb area (FL) and the hind-limb area (HL) (Zilles and Wree, 1995). The rat primary somatosensory cortex is dominated by representations of the face and vibrissae. Parietal area 1 receives projections from contralateral cutaneous mechanoreceptors (Chapin and Lin, 1984) and proprioceptive sensory input. In Nissl stained sections, the parietal regions can be distinguished from the surrounding tissue by a clearly defined inner granular layer IV (Zilles et al., 1980), and by having the best developed myelination of any neocortical region (Zilles, 1990). Layer V of Par1 possesses upper (Va) and lower (Vb) regions that are populated by medium sized pyramidal neurons with a low packing density, and large, highly packed pyramidal cells, respectively.

In rats, the regions of the body which may be activated by light touch have been found to be represented by granular somatosensory cortex (Welker, 1976). Moreover,

there is a high degree of somatic organization in regions of the limb and face representations which forms a somatosensory *ratunculus* (analogous to the commonly described human *homunculus*). The face portion of the rat somatosensory cortex (Par1) contains the cortical *barrel field* representation of the mystacial vibrissae (Welker, 1976). Physiological studies have shown that this cortical region integrates information generated by spatially and temporally patterned whisker deflections (Simons, 1978; Simons and Carvell, 1989), and it has been suggested that the capacity of the rodent whisker system to make tactile discriminations is comparable to that of primates' use of their fingertips (Carvell and Simons, 1990). Those areas not occupied by the cortical barrels can be further divided into granular and dysgranular regions, indicating high and low-packing density-zones of the cells in layer IV (Donogue and Wise, 1982). The dysgranular regions send and receive dense projections from homologous sites in the contralateral hemisphere, while the granular regions respond to cutaneous inputs and receive only sparse inputs from contralateral sites (Zilles and Wree, 1995).

The connectivity of the parietal regions has been studied extensively. Parietal area 1 receives afferent connections from many thalamic nuclei, notably the ventroposterolateral (VPL) (Wise and Jones, 1978), ventroposteromedial (VPM) (Donoghue et al., 1979) and ventromedial (VM) (Herkenham, 1980) nuclei. Moreover, it receives substantial connections from contralateral Par1 (Akers and Killackey, 1978), ipsilateral Par2 and frontal areas 1-3 (Fr1-3) (Paperna and Malach, 1991). Par1 sends efferent outputs to many sites including the VPM (Wise, 1975), caudate-putamen (Wise and Jones, 1977), intralaminar nuclei (Wise and Jones, 1977), and several spinal cord

sites (Wise et al., 1979). Par1 is also reciprocally connected to the ipsilateral motor cortex (Akers and Killackey, 1978) and several visual cortical areas including Oc2M (occipital cortex, area 2, monocular), Oc1 (occipital cortex, area1) and Oc2L (occipital cortex, area 2, lateral) (Kolb, 1990).

Frontal Area 3 (Motor Cortex)

The frontal region of the rat is divided into three architectonically distinct regions: frontal areas 1 (Fr1), 2 (Fr2) and 3 (Fr3). Some authors do not distinguish Fr1 from Fr3 (e.g., Donoghue and Wise, 1982), though the current belief is predominantly in favour of such a division, as defined by cell packing density (for discussion see Zilles and Wree, 1995). The main feature delineating the frontal neocortical regions from the parietal areas is an agranular cortex, defined as the lack of a prominent inner granular layer IV. Relative to the other frontal areas, lower layer III of Fr3 has a higher cell packing density and is often considered comparable to the distinct layer IV of the sensory areas. Fr1 and Fr3 together comprise the primary motor cortex (M1) (Donoghue and Wise, 1982; Donoghue and Parham, 1983), which contains a well-defined population of giant layer V pyramidal cells called Betz cells whose axons project through the corticospinal tract, terminating in the intermediate and ventral zones of the spinal cord.

Frontal area 3 receives afferent connections from several thalamic nuclei including VPL, VM and the posterior thalamic nuclei (Beckstead, 1976; Cicirata et al., 1986). It also receives cerebellar afferents via the VPL and ventrolateral nucleus (VL) (Ghez, 1991). In addition, Fr3 receives inputs from the intralaminar region (Jones and

Leavitt, 1974), the locus coeruleus (Ungerstedt, 1971) and all neocortical areas (for review see Neafsey, 1990). The main outputs of Fr3 target Parl (Paperna and Malach, 1991), the spinal cord (Wise et al., 1979), the premotor and supplementary motor cortices (Ghez, 1991), the cranial nerve motor nuclei (Kolb, 1990), the caudate putamen (Donoghue and Herkanhan, 1986) and the pons (Ghez, 1991). The primary motor cortex is also reciprocally connected to homologous contralateral regions (Zilles and Wree, 1995).

The first demonstrations that specific regions of the motor cortex control movements were made over 100 years ago (Fritsch and Hitzig, 1870; Ferrier, 1875). It has since been demonstrated repeatedly that the motor areas are organized somatotopically; that is, that distinct areas of this region control movement of specific areas on the contralateral side of the body. Precisely arranged motor maps have been described for humans (Penfield and Rasmussen, 1950), monkeys and rats (Woolsey, 1958).

In addition to possessing a precise macroanatomical arrangement, it is clear that there are mechanisms that serve to adaptively change the *synaptic* features of the neocortex in response to sensory stimuli. Section 1.3 discusses the most widely studied of these mechanisms: long-term synaptic potentiation.

1.3 Long-Term Synaptic Potentiation

High-frequency electrical stimulation applied to specific forebrain pathways can lead to long-lasting increases in the amplitude of responses subsequently evoked in the

targets of those pathways. This phenomenon is referred to as long-term synaptic potentiation (LTP) and is believed to be due to an intracellular cascade that commences with glutamate binding and increases in post-synaptic Ca^{2+} and ends with gene expression and *de novo* protein induction (Goelet et al., 1986; Sheng and Greenberg, 1990; Silva and Giese, 1994). LTP was first demonstrated in the perforant path, connecting the entorhinal cortex with the dentate gyrus of the hippocampus, in both an anaesthetized (Bliss and Lomo, 1973) and freely-moving (Bliss and Gardner-Medwin, 1973) preparation. Since that time, LTP has been shown to occur in a wide variety of subcortical and cortical sites (Wilson and Racine, 1983; Racine et al., 1994a; see Kuba and Kumamoto, 1990; Teyler et al., 1990; Tsumoto, 1992; Bliss and Colligridge, 1993 for reviews).

LTP has several basic properties that have made it theoretically interesting to memory researchers including cooperativity, associativity and input-specificity. Cooperativity refers to the condition that a minimum number of synapses be activated during a high-frequency tetanus to induce LTP (McNaughton et al., 1978; but see Zalutsky and Nicoll, 1992 for evidence that the mossy fiber system does not display cooperativity), and indicates that the lasting enhancements that are seen following LTP induction are dependent upon correlated input activity. Associativity is similar in that it refers to the fact that a "weak" input can be potentiated if it is activated concurrently with a "strong" input. The two inputs must be independent and converge on the same target (Levy and Steward, 1979; Kirkwood and Bear, 1994). This feature has been described as a cellular analogue to classical conditioning and is judged by some researchers to be the

most compelling link between LTP and memory (Malenka, 1994). Moreover, only the activated lines are potentiated, which means that the mechanism must be synaptic (Andersen et al., 1977; but see Schuman and Madison, 1994). The primary advantage of a synaptic mechanism, compared to a cellular mechanism, is the increase in potential storage capacity.

One of the hallmark features of LTP is its reliance on glutamate receptor activation. Glutamate receptors have been pharmacologically grouped into 5 classes (Hollmann and Heinemann, 1994), and are named after their most selective agonists. Glutamate receptors include the *N*-methyl-D-aspartate (NMDA), kainate (KA), alpha-amino-3-hydroxy-5-methyl-4-isoxazole proprionate (AMPA), 2-amino-4-phosphonobutyrate (L-AP4), and *trans*-1-amino-cyclopentane-1,3 dicarboxylate (ACPD, also known as metabotropic or mGluR) receptor subtypes.

Mechanistically, Glu receptors have been grouped into two classes, termed *ionotropic* and *G-protein coupled* receptors. The former includes the NMDA, KA and AMPA receptors. These are ligand-gated ion channels possessing a fast (msec) time scale. The latter group is comprised of the L-AP4 and the mGluR receptor classes. The L-AP4 receptor acts via a G-protein to increase the hydrolysis of cGMP, which thereby leads to the closure of ion channels conducting inward currents. The mGluR is linked to inositol phosphate/diacylglycerol formation and is subsequently able to initiate the release of calcium from internal stores.

The *induction* of LTP in both the CA1 region of the hippocampus (Coan et al., 1987; Malenka and Nicoll, 1993; Bliss and Collingridge, 1993) and in the neocortex

(Artola and Singer, 1987, 1990; Bear et al., 1992; Kirkwood and Bear, 1994) has been shown to be critically dependent upon activation of the NMDA receptor. This receptor acts to regulate a cation channel that is permeable to Ca^{2+}, Na^{+} and K^{+}. An important characteristic that distinguishes the NMDA receptor is that it generally contributes little to transmission because its associated ion channel is blocked in a voltage-dependent fashion by Mg^{2+} ions (Nowak et al., 1984; Mayer et al., 1984). For the NMDA receptor to become ion permeable, the postsynaptic membrane must be sufficiently depolarized to expel the Mg^{2+} block at the same time that glutamate binds with the receptor and opens the channel. This feature of the NMDA receptor has led many researchers to assert that it acts as a molecular coincidence detector (Singer and Artola, 1991), making it the focus of attention in attempts to identify the molecular basis of memory. Once the NMDA receptor is active it permits the entry of Ca^{2+}, which in turn activates calcium-dependent protein kinases (for reviews of the processes underlying LTP induction and the role of the NMDA receptor subtype see Bliss and Colligridge, 1993; Malenka, 1994).

Aroniadou and Keller (1995) examined the mechanisms of neocortical LTP in layers II/III following stimulation of vertical (stimulation in layers V/VI) or horizontal (stimulation in layers II/III) inputs in slices of rat motor cortex. The induction of LTP following stimulation of either pathway was prevented by blockade of either NMDA receptors or dihydropyridine-sensitive Ca^{2+} channels, suggesting that neocortical LTP may be supported by more than one mechanism. Similarly, Castro-Alamancos et al. (1995) found that application of AP5 blocks LTP in both SI and MI, while Lee et al. (1991) demonstrated that low-frequency layer VI stimulation could elicit LTP in layers

II/III if the levels of extracellular Mg^{2+} were lowered. LTP was not found, however, to be abolished by blockade of L-type (voltage-sensitive) Ca^{2+} channels (Castro-Alamanacos et al., 1995).

Despite the clear role played by the NMDA receptor in use-dependent synaptic plasticity, several brain sites also display an NMDA-independent LTP, most notably the hippocampal mossy fiber system (Nicoll and Malenka, 1995). Moreover, it has been demonstrated that LTP can be induced in visual cortex by prolonged (15 min) low-frequency (2 Hz) stimulation combined with the application of bicuculline (Komatsu et al., 1991). This form of neocortical LTP is believed to be mediated by low-threshold Ca^{2+} channels (Komatsu and Iwakiri, 1992).

The *expression* of LTP is primarily mediated by increases in AMPA-dependent synaptic responses (Muller and Lynch, 1988; Muller et al., 1988), though whether the locus of this effect is pre- or post-synaptic remains controversial. Presently, there is evidence of presynaptic increases in neurotransmitter release (Bekkers and Stevens, 1990; O'Dell et al., 1991; Schuman and Madison, 1991) and post-synaptic changes in AMPA receptors (Xiao et al., 1991; Foster and McNaughton, 1991; Maren et al., 1993). It is probably the case that both pre- and post-synaptic changes are responsible for the changes that take place following the induction of LTP (Stevens, 1993; Voronin, 1994).

Another property of LTP that has received attention by memory researchers is its longevity. This feature is arguably the most important, in that without it the remaining aspects of LTP would be insufficient to give it the status of a memory mechanism. The earliest work on hippocampal LTP demonstrated that potentiation effects could last for up

to 3 days in the chronically-prepared animal (Bliss and Gardner-Medwin, 1973). Since that time, a great deal of research has been directed at identifying the mechanisms underlying the maintenance of LTP. In the rat hippocampus, LTP is believed to be comprised of two (Racine et al., 1983) or three (Abraham and Otani, 1991) components: the first (LTP1) decaying over hours (Racine et al., 1983; Krug et al., 1984) the second (LTP2) and third (LTP3) decaying over days (Barnes, 1979; Racine et al., 1983; Jeffery et al., 1990) and weeks (Barnes and McNaughton, 1985; De Jonge and Racine, 1985; Bliss et al., 1987), respectively. LTP1 may be blocked by kinase inhibitors, but not by protein synthesis inhibitors, and is believed to involve the self-reinforcing covalent modification of proteins normally present in the neurons involved (Goelet et al., 1986; Lisman, 1989). The longer lasting forms of LTP, however, are believed to require both the synthesis of new macromolecules as well as alterations in gene expression that lead ultimately to enduring or even permanent changes in cellular and synaptic physiology. LTP2, which is blocked by translational inhibitors (Huang et al., 1996), depends on the transient activation of effector genes for memory lasting more than 24 hours, where the decay rate is determined by the half-lives of the induced mRNAs and proteins (Goelet et al., 1986). LTP3 requires the induction of an intracellular cascade that activates a gene sequence for memory lasting many weeks (Sheng and Greenberg, 1990; Abraham, Dragunow and Tate, 1991; Frank and Greenberg, 1994). This sequence is postulated to involve the activation of immediate early genes, leading ultimately to permanent changes in late, effector genes (Abraham et al., 1993; Dragunow, 1996).

The intracellular mechanisms underlying the maintenance of neocortical LTP

have also been examined, although every study has used the relatively short-lived *in vitro* preparation. As a result, much less is known about either the time-course or the mechanisms underlying LTP in the neocortex. The initial step in most models of both hippocampal and neocortical LTP maintenance involves intracellular Ca^{2+}, and Tsumoto et al. (1990) have shown that intracellular injection of a calcium chelator into layer II/III neurons of visual cortex prevents the induction of LTP. This has also been found in the motor cortex of anaesthetized cats (Baranyi and Szente, 1987). Thereafter, a number of molecular events have been shown to be correlated with the maintenance of LTP including the activation of Ca^{2+}-calmodulin dependent protein kinase (CaMKII) (Funauchi et al., 1992) and protein kinase C (PKC) (Baranyi et al., 1987; Jia et al., 1990).

In addition to work directed at identifying the physiological mechanisms governing the induction and maintenance of LTP, many researchers have attempted to identify possible similarities between LTP and memory (for reviews see Barnes, 1995; Maren and Baudry, 1995; Izquiedro and Medina, 1995; Eichenbaum, 1996; Martinez and Derrick, 1996). This research has generally developed along three lines: (a) demonstrations that real learning experiences produce LTP-like changes in synaptic efficacy; (b) blockade of learning following the saturation of LTP at the relevant synapses; (c) pharmacological/genetic manipulations that block both LTP and learning.

In the first study to examine behavioural LTP, hippocampal evoked potentials were monitored before and after exposing rats to complex, novel environments (Sharp et al., 1985). Although the initial data indicated that this paradigm could produce increases in synaptic strength, subsequent research attributed the increases to the elevations in brain

temperature associated with exploration (Moser et al., 1993). A more recent study, however, showed that a small residual potentiation effect remained even after factoring out variation in temperature, motor activity and EEG state (Moser et al., 1994).

Studies employing saturation have used both large numbers of high-frequency trains and/or electroconvulsive stimulation in an attempt to drive synaptic efficacy to asymptote, thereby preventing further increases and, presumably, learning. A typical experiment involves delivery of repeated trains to the perforant path to saturate LTP in the dentate gyrus followed by evaluation of performance on a spatial task, such as the Morris water maze (Castro et al., 1989). These data are controversial, with other researchers finding that animals are not spatially impaired following a variety of saturation paradigms (see Bliss and Richter-Levin, 1993 for brief review). One of the most recent experiments in this series paired high-frequency trains with electroconvulsive stimulation in an attempt to ensure a true saturation effect. It was concluded that LTP saturation may block learning under limited circumstances (Barnes et al., 1994).

Some of the most compelling data linking LTP and learning have come from studies using pharmacological agents to block both. The best known of these demonstrations have shown that NMDA receptor antagonists block spatial learning in the Morris water maze when administered i.c.v. at doses comparable to those that block LTP induction *in vivo* (Morris et al., 1986). There is, moreover, a growing body of literature indicating that genetic mutants which are incapable of sustaining LTP are also learning and memory deficient. Mice that have mutations in the alpha subunit of CaMKII (Silva et al., 1992), the *fyn* receptor tyrosine kinase (Grant et al., 1992), the metabotropic

glutamate receptor GluR1 (Aiba et al., 1994), or the $\epsilon 1$ subunit of the NMDA receptor (Sakimura et al., 1995) all show strong performance deficits in the Morris water maze. Recently, Wilson and Tonegawa (1997) engineered mice possessing a targeted deletion of the NMDAR1 gene in the CA1 field of the hippocampus. These mice were found to be strongly impaired in the Morris water maze as well as incapable of forming normal place fields, a characteristic feature of hippocampal neurons during exploration (Wilson and McNaughton, 1994). Moreover, LTP could not be induced in CA1 neurons of the mutants by tetanization of the Schaffer collaterals. These data offer substantial support for the idea that similar mechanisms underlie LTP and at least some forms of learning and memory.

1.4 Neocortical LTP

The neocortex is resistant to LTP induction when using traditional paradigms

If LTP reflects a primary and general mechanism for information storage, then one would expect to see it readily induced within the neocortex, where long-term memory is believed to be stored. However, the neocortex has proven resistant to the induction of LTP and this has called into question the validity of LTP as a memory model. If LTP is to be considered seriously as such, it is of crucial importance that the kinetics and mechanisms underlying LTP in the neocortex be described. There are several methodological factors which have slowed the progress of research on neocortical LTP (Tsumoto, 1992). Not the least of these is the complexity of the neocortical circuitry.

Nevertheless, there have been many recent demonstrations of neocortical LTP. Most of these, however, have been made using acute *in vivo* (Voronin, 1984; Keller et al., 1991; Tsumoto, 1992; Kimura et al., 1994) and *in vitro* slice (Kimura et al., 1989; Artola and Singer, 1990; Artola et al., 1990; Kirkwood and Bear, 1994) preparations, and have usually required the use of GABAergic blockers and/or young animals (Artola and Singer, 1987; Perkins and Teyler, 1988; Kato, Artola and Singer, 1991; Kirkwood and Bear, 1994, for reviews see Tsumoto, 1992; Bear and Kirkwood, 1993). The resistance of these preparations to LTP induction is highlighted by the fact that the intrinsic inhibition of neuronal tissue is already lower in *in vitro* than *in vivo* preparations (Dingledine et al., 1980).

These and other data also indicate that some aspect of early development facilitates the induction of LTP. Several authors have addressed this issue. Recording under anaesthesia in both juvenile and adult rats, Wilson and Racine (1983) monitored LTP induction in sensorimotor cortex following contralateral white matter stimulation. LTP could not be detected until post-natal day 18 (PND18), and then matured to adult levels by PND28. Tsumoto and Suda (1979) demonstrated that the probability of LTP induction in kitten visual cortex varies as a function of age, as LTP was readily induced in kittens 10 weeks of age and younger but not in kittens older than 14 weeks. Komatsu et al. (1981, 1988) showed that LTP could be induced in slices taken from kittens between 21 and 34 days of age, but not from slices taken before 14 days of age or older than 42 days. In slices of rat visual cortex, the age dependency of LTP induction has been shown to be layer specific. It is induced with highest probability between 11-15 days in layer V

and between 16-20 days in layers II/III (Perkins and Teyler, 1988; Berry et al., 1989). Recently, Kirkwood et al. (1995) demonstrated that LTP in rat visual cortical slices was most easily induced during the "critical period" (Olson and Freeman, 1980) of visual cortical development. Manipulations that delayed the onset or duration of the critical period also shifted the window for the induction of LTP. In all of these cases, the neocortex of adult animals has been shown to be uniformly resistant to the induction of LTP following stimulation of white matter afferents. It is important to note, however, that none of these demonstrations were made in chronically prepared animals.

The difficulties associated with the induction of neocortical LTP are even more apparent in the chronic preparation. Racine et al. (1994b) used 7 different protocols in an attempt to induce LTP in the adult, freely moving rat. These included the application of stimulus trains known to be effective in inducing LTP subcortically, manipulation of the pulse duration of the stimulus trains, variations in pulse frequency and train duration, co-activation of multiple cortical and/or thalamic inputs, the reduction of inhibition via the GABAergic antagonist picrotoxin, housing of animals in an enriched environment, delivery of stimulation trains as a cue within a learning task, and co-activation of cholinergic inputs by injection of the cholinergic agonist pilocarpine. None of these manipulations produced LTP, though the last produced a long-term depression (LTD) effect.

In vitro work performed in Mark Bear's lab has led to the development of a recent theory aimed at explaining the resistance of the neocortex to undergo LTP (Kirkwood and Bear, 1994). These authors found that LTP could be reliably induced in layer III of rat

visual cortex without the need to reduce inhibition, if layer IV was stimulated directly. They attributed the difference between white matter and layer IV stimulation to the presence of an inhibitory gate in layer IV. They assume the presence of two white matter inputs to layer III: one is monosynaptic, while the other is disynaptic and relays input through layer IV. The model proposes that high-frequency stimulation of the white matter results in blockade of the disynaptic pathway. The remaining monosynaptic path cannot provide sufficient depolarization to trigger NMDA-dependent LTP. Layer IV stimulation, however, allows monosynaptic activation of layer III along both paths, providing a strong enough input to exceed the LTP threshold. These authors thus postulate that layer IV acts as a "plasticity gate" for experience-dependent plastic change in layer III. This also accounts for the normal requirement for GABA antagonists in the bathing medium. GABA blockade allows the normally closed "plasticity gate" to remain open and follow at high frequencies. This would also explain the resistance of the adult neocortex to LTP induction.

Features of neocortical LTP using in vitro slice and anaesthetized preparations

Demonstrations of neocortical LTP reveal substantial variability, making it difficult to compare results between laboratories. Some similarities are evident, however, and Teyler et al. (1990) have provided a clear description of a white matter stimulation-evoked layer II/III response *in vitro* (see also Sutor and Hablitz, 1989). A representative response consists first of a fast EPSP (EPSP1) with a peak latency of 4-13 msec. This fast EPSP is comprised of 2 components (1a and 1b) which have a similar onset latency,

though 1b possesses a longer peak latency, decays more slowly and appears as the falling phase of the combined EPSP. A longer latency, more variable second EPSP (EPSP2) then follows with a peak latency between 15-30 msec or longer. The latter aspects of EPSP1 and all of EPSP2 are coincident with a fast, Cl^--mediated, depolarizing IPSP with a peak latency between 12-35 msec. The final component of the response is a slow, K^+-mediated, hyperpolarizing IPSP with a peak latency between 150-250 msec. EPSP1 is monosynaptic, while the other components are believed to be polysynaptic. Additionally, white matter stimulation often evokes an antidromic response from cells within a column as well as from reentrant axon collaterals. This antidromic response precedes all others when it is present.

Previous reports of neocortical LTP have shown that both EPSP 1a and b can be potentiated and that 1b may have a lower threshold for potentiation (Artola and Singer, 1987). EPSP2 shows a more variable potentiation effect and is occasionally visible only following potentiation (Berry et al., 1989). Reports using the *in vitro* slice indicate that potentiation of EPSP2 is generally more reliable and of larger amplitude when it can be recorded in the baseline response (Teyler et al., 1990).

A number of authors have examined the parameters and kinetics of LTP in the motor and somatosensory cortices, though all of the demonstrations have used anaesthetized animals or neocortical slices *in vitro*. In these neocortical regions, LTP of both extracortical afferents as well as corticocortical connections have been examined. Using anaesthetized cats, Asanuma and colleagues (Sakamoto et al., 1987) have shown that tetanic stimulation of the sensory cortex can induce LTP, lasting up to 15 minutes or

more, in a large percentage (about 75%) of presumed layer III motor cortex neurons. These authors have also reported associative LTP, lasting at least 73 minutes, of layer III motor cortex neurons following combined, high-frequency stimulation of both the sensory cortex and thalamic nucleus VL, but not following stimulation of the VL alone (Iriki et al., 1989). In the latter study, infragranular cells, which form synapses with sensory cortical afferents, did not show LTP following stimulation of the separate or combined inputs.

Similarly, Miyata and Kawakami (1995) investigated the role of corticocortical inputs from SI in the induction of LTP in secondary somatosensory cortex (SII). SII receives 2 VPL thalamic nucleus inputs: a direct one and one that arrives indirectly via SI. These authors were interested in determining the importance of the SI to SII connection in the induction of thalamically-driven LTP and delivered tetani to the VPL under two conditions: when SI was silenced by injection of local anaesthetic, and following recovery from anaesthesia. Their data indicate that the removal of the SI input blocks LTP induction in SII and indicates the importance of the corticocortical connections in the induction of thalamo-cortical LTP.

Kimura et al. (1994), also using anaesthetized cats, demonstrated that the intracortical connections of the motor cortex could support LTP. These authors stimulated the superficial layers and recorded from layer V pyramidal cells. They found that 5/19 cells displayed LTP, lasting at least until the electrode moved away from the cell being recorded (up to 28 minutes). Sakamoto et al. (1987) reported similar proportions in motor cortex (4/22 cells that were held for more than 3 minutes) following tetanic

stimulation. Aroniadou and Keller (1995) examined LTP in layers II/III following stimulation of vertical (stimulation in layers V/VI) or horizontal (stimulation in layers II/III) inputs in slices of rat motor cortex. Tetanic stimulation of either pathway separately induced LTP in 60% of the field potentials and 73% of the intracellularly recorded PSPs. Further, cells with various intrinsic firing patterns (regular- and fast-spiking and bursting cells) showed LTP, in contrast to reports in visual cortex (Artola and Singer, 1987) that suggest the preferential involvement of bursting cells in LTP induction.

Using rat sensorimotor cortex slices, Bindman et al. (1988) found that pairing intracellular depolarization of target cells (eliciting 6-13 action potentials) with pre-synaptic volleys elicited from either ipsi- or contralateral white matter resulted in the induction of potentiation in 4/28 cells. In addition, 2 cells showed a depression effect while the remainder showed no effect. These authors then delivered tetani to the input lines of the cells that did not potentiate, producing a mix of LTP, depression and post-tetanic potentiation. Similarly, in bicuculline-treated slices from rat prefrontal cortex, Hirsch and Crepel (1990) applied tetanic stimulation to white matter afferents and induced LTP in 10/28 cells and LTD in 14/28 (the remainder showed no effect).

Much of the work on corticocortical LTP has examined changes in layer II/III pyramidal neurons following the combined stimulation of infragranular layers and homologous contralateral sites. Following this approach, Hess et al. (1996) and Hess and Donoghue (1996) applied theta-burst stimulation to the superficial horizontal pathway of the primary motor cortex (MI). They found that LTP could only be induced in this pathway if inhibition was reduced with bicuculline. However, coactivation of both the

horizontal and vertical (layers V/VI) pathways produced layer III LTP in the absence of GABAergic inhibition. These authors suggest that the induction of LTP in horizontal projections, as compared to those projecting vertically, may be more difficult and require additional inputs.

The current body of data indicates that neocortical LTP induction can be difficult to induce. One possibility is that longer durations of stimulation are required to induce neocortical LTP. Keller et al. (1991) tested a variety of LTP-inducing stimulation parameters in the somatosensory cortex of the anaesthetized cat. These authors found that the minimal tetanic stimulation capable of reliably inducing LTP was a 5 sec. train at 50 Hz, with shorter trains producing only short-lasting, transient potentiation effects. As previous studies had always used much shorter stimulation trains, this may explain the low probabilities with which LTP had been induced. It is important to note, however, that stimulation protocols with these parameters can be epileptogenic, even in slices. This is problematic because seizures are also capable of inducing potentiation.

Castro-Alamancos et al. (1995) compared LTP in the granular SI area and the agranular MI area. They recorded both extracellularly and intracellularly in layer II/III during layer IV stimulation and found that while granular cortex supported robust LTP, LTP induction in agranular cortex required the application of bicuculline. These authors also found that during the induction regimen, robust synaptic facilitation (a short-term enhancement of synaptic strength lasting approximately 1 second (Zucker, 1989)) could be produced in granular, but not agranular cortex. As NMDA receptors rely on a sufficiently depolarizing stimulus in order to become active, it may be that this

facilitation underlies the differences in LTP expression. Granular cortex may express LTP more easily than agranular cortex because it is more prone to express short-term facilitation which causes greater NMDA receptor activation.

Neocortical LTP in the awake animal

There have been even fewer reports of neocortical LTP in the freely-moving animal. This is troubling given the premise that long-term memory (memory lasting weeks or months) is believed to be stored in the neocortex. The paucity of data concerning truly long-lasting LTP in the neocortex (for brief discussion, see Teyler et al., 1990) is directly tied to the reliance researchers have had on anaesthetized and slice preparations, and the difficulties associated with finding a chronic preparation that supports neocortical LTP.

The first demonstration of a long-lasting potentiation effect in the adult, freely moving animal established that the neocortex could support *kindling*-induced potentiation (Racine et al., 1995a). In this study, adult rats were chronically implanted with recording electrodes in the ipsi- and contralateral sensorimotor cortices and thalamus, and stimulating electrodes in either layer V or the corpus callosum. Potentiation effects did not develop until after 5 and 10 daily kindling sessions, for sites contra- and ipsilateral to the site of stimulation, and did not reach asymptotic levels for 14 days or more. Both the mono- and polysynaptic component of the evoked response displayed potentiation. The monosynaptic component displayed an increasing surface-positive shift in response to stimulation, while the polysynaptic component always showed a clear potentiation effect

following 5 or more kindling stimulations. These effects were found to last several weeks following induction. Subsequent to this study, the same group reported that they had, for the first time, succeeded in inducing LTP in the sensorimotor cortex of the adult, freely-moving rat following the daily application of tetanizing stimulation to the corpus callosum (Racine et al., 1995b). Potentiation effects similar to those seen in the kindling-induced potentiation study became obvious in both the mono- and polysynaptic components of the evoked response after 5 days of stimulation, reaching asymptote by the 15th day. The population spikes superimposed on the early component also showed potentiation, growing in size and number. These potentiation effects were still evident 4 weeks following the cessation of the 25 day stimulation protocol.

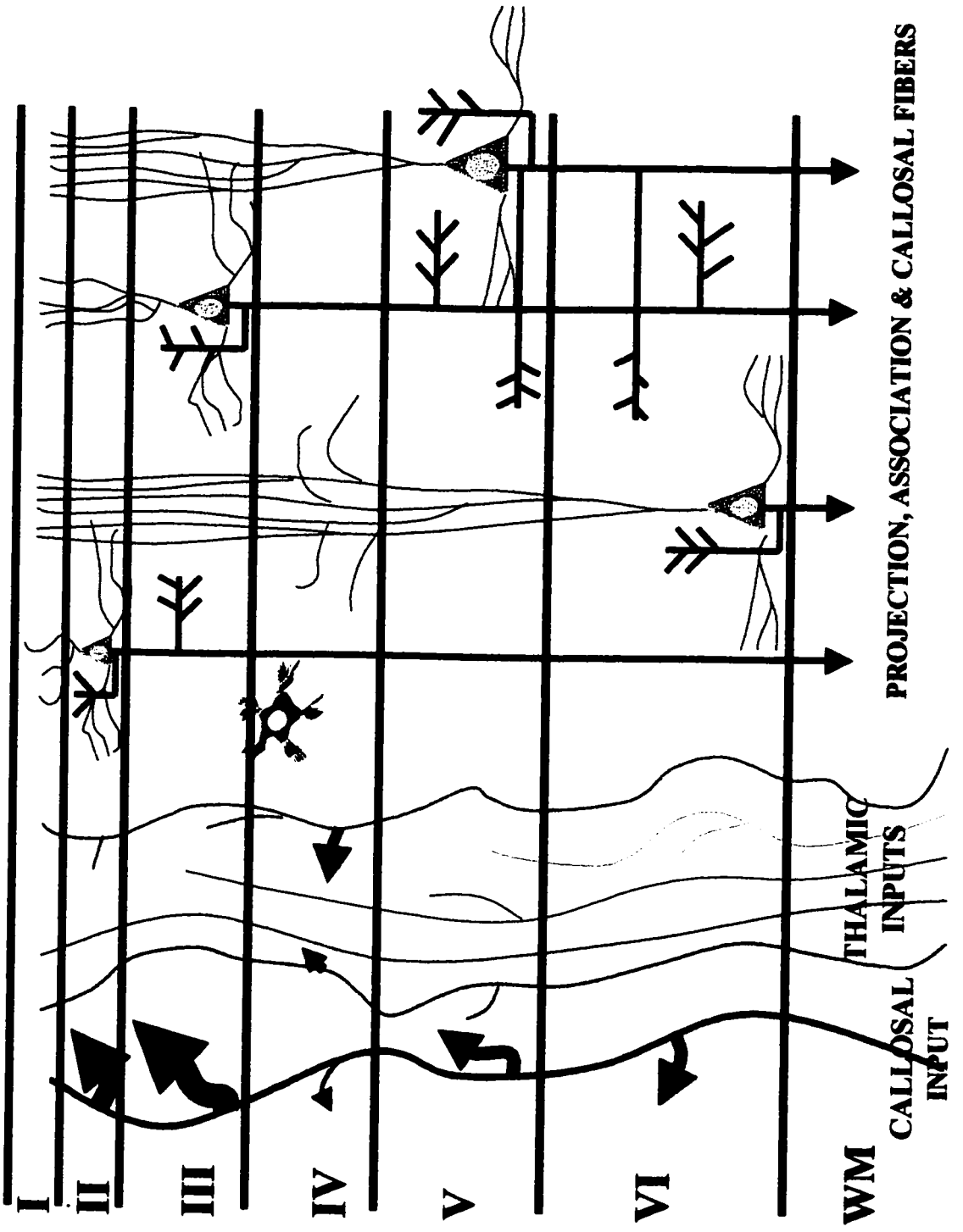
The hippocampal and neocortical learning systems have been distinguished on the basis of several characteristics. While the former has been characterized by rapid acquisition and transient storage, the latter is believed to learn more slowly but store for longer periods (Zola-Morgan and Squire, 1993; McClelland et al., 1995). Although the hippocampus is capable of the rapid learning of associations, often with only a single presentation, the neocortical system is believed to learn through many repetitions of similar acts of information processing, producing gradual, incremental changes in the synaptic connections between neocortical neurons (McClelland et al., 1995). The initial demonstrations of neocortical LTP in chronics fit rather well within this theoretical framework. Rather than being resistant to the induction of LTP, the neocortex can be quite plastic so long as the stimulation trains are spaced and repeated over a period of days. These data, however, stand in contrast to the results from acute and slice

experiments where neocortical LTP, like hippocampal LTP, can be induced following a single, brief session of high-frequency stimulation (Kirkwood et al., 1993; Kirkwood and Bear, 1994; Racine et al., 1994a). It is clear that a full understanding of neocortical LTP requires a thorough analysis of the induction and decay kinetics of neocortical LTP as it is expressed in the intact, awake animal. The experimental series described in the following chapters was designed to characterize and investigate long-term synaptic potentiation of white matter afferents to the sensorimotor cortex. The following experiments represent the first comprehensive description of this important phenomenon in the adult, freely moving animal (rat).

FIGURE LEGENDS

Figure 1.1 Circuit diagram depicting the basic anatomical arrangement of the neocortex (Par1 shown here). Layer II, III, V and VI pyramidal cells are shown, as is a layer IV stellate cell. Pyramidal cell dendrites (pink) extend both toward the pial surface as well as laterally within their laminae of origin, while axons (thick black lines emerging from the basal aspect of the pyramidal cells) are shown projecting to the white matter (WM). Axon collaterals extend from the main axonal projections. Stellate cell axons are shown extending within layer IV and into layer III. Inputs from the intralaminar thalamic nucleus (yellow), posterior thalamic nucleus (green) and ventral posterior thalamic nucleus (brown) are displayed, as are nonspecific ventromedial (red) and specific ventrolateral (light blue) thalamic nuclei inputs. The relative strength of the callosal and thalamic inputs are represented by the line thicknesses. The laminae are labelled by upper-case roman numerals (I-VI).

Figure 1.1


I
II
III
IV
V
VI
WM
PROJECTION, ASSOCIATION & CALLOSAL FIBERS
THALAMIC INPUTS
CALLOSAL INPUT

CHAPTER TWO

KINETICS OF NEOCORTICAL LTP INDUCTION AND DECAY

The variables governing the duration of LTP are not well understood. Several factors have been shown to be important for the maintenance of hippocampal LTP including the number of tetanizing trains delivered (DeJonge and Racine, 1985; Barnes and McNaughton, 1985; Abraham et al., 1993), the frequency of the pulses in the trains (Larson et al., 1986; Staubli and Lynch, 1987; Grover and Teyler, 1990), the age of the animal (Barnes, 1979) and the presence of anaesthesia (Jeffrey et al., 1990). These variables and others are believed to initiate and/or modulate the intracellular events which may ultimately lead to short- or long-lasting potentiation or depression effects. In both slice and intact preparations, for example, short-term potentiation has been shown to require a single, weak train of stimulation (Racine and Milgram, 1983; Malenka and Nicoll, 1993), while longer lasting LTP effects require considerably more (Racine, Milgram and Hafner, 1983; Huang and Kandel, 1994).

The development, maintenance and decay of long-lasting neocortical LTP cannot be quantified in acute and slice experiments because these experiments cannot run for the 10 to 20 days required to reach asymptotic levels of LTP, or even the 4 to 5 days required to see the onset of LTP, as expressed in chronic preparations (Racine et al. 1995b). In the rat hippocampus, there is a transient component of LTP that decays over hours (Racine and Milgram, 1983) and does not require protein synthesis (Frey et al., 1989). It is

possible that a comparable, transient component of neocortical LTP is the *only* component induced in slice and acute preparations. Consequently, in order to evaluate the induction and decay characteristics of LTP effects that last for days or weeks, it is necessary to induce LTP in a preparation that permits long-term monitoring.

It has been proposed that the neocortex utilizes a slow learning rate so that new and old information can be interleaved during the learning phase. This allows for the extraction of common structure in the new and old patterns and minimizes interference between them (McClelland et al., 1995).

The goal of the following experiments was to provide a parametric analysis of the induction and decay kinetics of neocortical long-term potentiation as it is expressed in the freely-moving rat. To that end, several variables were manipulated including the number of stimulation trains delivered in a session, the total number of sessions received, the intensity of the stimulation used to induce LTP, and the stimulus interval. The minimal number of stimulation trains and the minimal intensities required for LTP induction were also determined. Surprisingly, the neocortex was found to be highly reactive to these patterns of activation, even at very low intensities, providing that the stimulation trains were spaced. Moreover, the kinetics of neocortical LTP in the chronic preparation appear to follow rules similar to those believed to apply for the consolidation and decay of memory, offering physiological support for the hypothesis that an incremental consolidation process is essential for information storage in the neocortex.

MATERIALS AND METHODS

Surgery

One hundred and fourteen adult, male Long-Evans hooded rats were used in these experiments: 104 in the long-term potentiation studies and 10 in a control test for kindling effects. At the time of surgery, the animals weighed 325-450 g. They were housed individually, maintained on an *ad libitum* feeding schedule, and kept on a 12 h on/12 h off light cycle.

Twisted wire bipolar electrodes were prepared from Teflon-coated stainless steel wire (120 μm in diameter). The average tip separations were 1.0 mm and 0.50 mm for the recording and stimulating electrodes, respectively. The animals were anaesthetized with sodium pentobarbital (65 mg/kg) and received atropine methyl nitrate (1.2 mg/kg) to prevent respiratory distress. In each animal a bipolar recording electrode was implanted into the anterior neocortex (frontal area 3/parietal area 1) 2.0 mm anterior to Bregma and 4.0 mm lateral to the midline (Zilles, 1985; Paxinos and Watson, 1996). A callosal stimulation electrode was implanted 2.0 mm anterior to Bregma and 2.0 mm lateral to the midline. These two electrodes were adjusted during surgery to provide optimal response amplitudes. The resulting mean depths for the callosal stimulating and cortical recording electrodes were 3.0 mm and 1.8 mm ventral to dura, respectively. The electrodes were connected to gold-plated male pins that were inserted into a 9-pin miniature connector plug. This, in turn, was mounted onto the skull with dental cement and anchored with stainless steel screws. One of the screws served as the ground electrode. Data acquisition began 2 weeks following surgery.

Experiment 1: The effect of varying train number on the induction of LTP

For all LTP experiments throughout this thesis, three sets of field potential measures, spaced at 48 h, were taken to establish a series of baseline input/output (I/O) curves. Pulses of increasing intensity were delivered to the corpus callosum at a frequency of 0.1 Hz. Ten field responses were evoked, amplified, digitized (at 10 kHz) and averaged at each of ten intensities (16, 32, 63, 100, 159, 251, 501, 794, 1000, 1259 μA). Twenty-four hours following the third baseline I/O, the first set of high frequency trains was delivered to the corpus callosum.

For this experiment, varying numbers of 8-pulse, 24 ms trains (pulse frequency: 300 Hz) were delivered at a frequency of 0.1 Hz. Pulse duration and intensity were 0.1 ms and 1259 μA, respectively. Train sets were delivered to the animals according to random group assignment. Animals received 5, 10 or 60 trains each day for 10 days in the first (n=6), second (n=6) and third (n=6) groups, respectively, and received 1, 5, 10 or 60 trains each day for 25 days in the fourth (n=4), fifth (n=7), sixth (n=7) and seventh (n=7) groups, respectively. An eighth, control group (n=6) was implanted with stimulating and recording electrodes. Control animals had baseline I/Os taken as described for the experimental groups and had additional I/Os taken at five day intervals for 8 weeks but did not receive high frequency trains. Neocortical EEG activity was recorded on chart paper during train delivery to ensure that epileptiform discharges were not triggered. I/O tests were repeated every day immediately preceding delivery of trains. Following completion of the LTP induction phase, a single intensity was selected to

monitor responses over a 35 day decay phase. The intensity chosen was one which evoked responses showing the clearest LTP effects. For nearly all animals, this was the intensity that evoked a response that was approximately half the maximum amplitude expressed during the I/O tests. Ten test pulses were delivered at this intensity, and the responses averaged together, each day for 35 days to track the decay of the potentiated responses. Although most experimental animals received test pulses at either 159 (n=23) or 251 (n=9) μA, several animals had higher response thresholds requiring that test pulse intensities be set at 501 (n=4), 794 (n=2) or 1000 (n=1) μA. Additionally, 4 animals required test pulses of only 100 μA. In the absence of LTP-evoked late components, data from the 6 control animals were analysed at a pulse intensity of 159 μA, the mode intensity used for the experimental animals. When five weeks of decay data were collected, a final I/O was performed 24 hours following the last test pulse measure.

Experiment 2: The effect of varying train intensity on the induction of LTP

I/Os were collected during the baseline and induction phases as described above. Twenty four hours after the third baseline I/O, 30 high frequency trains were delivered daily to the corpus callosum (30, rather than 60, trains were delivered to gain some estimate of the minimal amount of low-intensity stimulation required for LTP induction). Trains were identical to those described above except that train pulse intensity was varied according to random group assignment. Animals received trains for 24 days with pulse intensities of 16 μA (single pulses at this intensity evoke early component responses that are only 5-10% of maximum amplitude), 32 μA (single pulses trigger responses 8-14% of

maximum), 159 μA or 501 μA in the first (n=7) second (n=5), third (n=6) and fourth (n=5) groups, respectively. A fifth, control group (n=6) was implanted with stimulating and recording electrodes and had I/Os taken every 2 days to monitor the stability of the baseline. Animals in the control group did not receive high frequency trains.

Experiment 3: The effect of varying stimulation session interval on the induction of LTP

I/Os were collected during the baseline and induction phases as described above. Twenty four hours after the third baseline I/O, the animals began their stimulation regimen, each session consisting of 60 high frequency trains. Stimulation session interval (ISI) was varied according to group designation. Animals received trains every 6, 12, 24 or 48 hours for 10 days in the first (6h, n=5), second (12h, n=5), third (24h, n=6) or fourth (48h, n=5) groups, respectively. A fifth, control group (n=5) was implanted with stimulating and recording electrodes. Control animals had baseline I/Os taken as described for the experimental groups and had additional I/Os taken daily but did not receive high frequency trains. EEG activity was monitored during train delivery to ensure that epileptiform discharges were not triggered. Daily I/O tests were repeated immediately preceding delivery of trains. Following completion of the LTP induction phase, 4 weekly I/Os were collected to monitor decay.

Experiment 4: Kindling controls

Although EEGs were monitored during all stimulation procedures to ensure that

epileptiform discharges were never evoked, the following experiment was run to confirm that none of the potentiation effects could be attributable to kindling.

Kindling is a progressive increase in the strength of epileptiform activity evoked by temporally spaced and repeated electrical stimulation (Racine, 1978). This phenomenon is accompanied by an increase in the amplitude of the evoked responses in the kindled pathways (Racine and Cain, 1991). To assess whether the repeated stimulations were resulting in kindling-related effects, a single afterdischarge (AD) was evoked in two groups of animals, one that had been fully potentiated (n=5) and another that was treated identically with the exception that they were not potentiated (n=5). Animals were implanted with stimulating and recording electrodes as described for the LTP experiments above. The kindling stimulation consisted of a 1 sec. train of biphasic pulses (pulse duration: 1 msec; pulse intensity: 800μA) at 60 Hz. These parameters were sufficient to evoke epileptiform discharge in all animals.

Analyses

Changes in the field potentials over LTP and decay sessions were measured by subtracting the final baseline responses from all other baseline and potentiated responses. This was done at the same intensity selected for monitoring responses during the decay period. All data-points were thus standardized to the third (final) baseline response. The early (average latency-to-peak: 8.1 ms), late1 (average latency-to-peak: 15.2 ms) and late2 (average latency-to-peak: 20.0 ms) components were measured separately in this manner at fixed latencies corresponding to the peak response of the components being analysed

(see Fig. 2.2). Control animals were then analysed using the means of these latencies from the experimental animals, because the peaks for the late components were not always clear prior to the induction of potentiation.

Population spikes were measured from the largest amplitude response in the I/O tests before and after LTP induction and after the decay phase. The height (in mV) of all population spikes within a response were summed to provide a measure of total spike height for that response. These measures were taken from field potentials recorded during the last baseline I/O, the I/O following completion of trains and (where applicable) the final I/O taken at the end of the 5 week decay period. The initial downward-going spike in each sweep, which appeared to potentiate in most cases (Figure 2.2), was excluded from the calculations because of uncertainties concerning the possible contributions of antidromic effects or distortions due to the stimulus artifact. Consequently, the spike measures likely underestimate the extent of potentiation of the population spikes.

Repeated measures ANOVAs were calculated for the LTP data. For Experiment 1 (train number), the LTP ANOVAs compared the means from every fifth day over the baseline, induction, and decay phases, to correspond to the control animals' I/O regimen. For Experiment 2 (train intensity), the LTP ANOVAs compared the means from every second day over the baseline and induction phases. For Experiment 3 (stimulus interval), an ANOVA was used to compare the means from every day over the baseline, induction and decay phases.

The afterdischarge durations evoked by the kindling stimulation (Experiment 4)

were measured and a between-subject t-test was calculated comparing the afterdischarge length of the potentiated and unpotentiated groups.

Following collection of all electrophysiological measures, animals were perfused and the brains were sliced and stained with Cresyl Violet to verify electrode placements.

RESULTS

Histology

The stimulating electrode was found to span the forceps minor corpus callosum in all animals. Localization of the recording electrode placements was completed in 74% (84/114) of animals as a result of damage caused to the brains during the histological procedures. In all cases, the ventral tips of the recording electrodes were placed in parietal area 1 or the medial-posterior aspect of frontal area 3 (Paxinos and Watson, 1986). The locations of the ventral tips of both the recording and stimulating electrodes are shown in Figure 2.1.

Response morphologies

Figure 2.2 shows representative sweeps taken from an animal that received 60 trains for 25 days, including examples of the cursor positions used to mark the latencies of the measured components. Field potentials appeared to have three main components: an early surface-negative response, reflecting monosynaptic activity (average latency-to-peak: 8.1 ms, range: 3.0-13.2 ms) (Racine et al., 1995b), and two larger (post-LTP) late responses, referred to here as *late1* and *late2*, likely indicating polysynaptic corticocortical activity (average latencies-to-peak: 15.2 and 20.0 ms, ranges: 8.2-27.0 and 10.0-32.0 ms, respectively). Prior to LTP induction, the main difference in response morphology across animals was in the presence or absence of a discernable late2 component. Before potentiation, only 31% and 47% of animals had a distinguishable late2 component at midrange (251 μA) and maximum (1259 μA) I/O pulse intensities,

respectively.

The late1 component generally showed a faster LTP onset and a slower decay than did the late2 component. Its peak, however, was often masked by the late2 component once that component was itself maximally potentiated. Superimposed on the early response in all cases were at least one, and usually multiple, population spikes which grew in amplitude, and often number, following potentiation.

Stimulation-induced behavioural and electrographic responses

Many animals showed an ipsilateral postural deflection during delivery of the trains. These animals did not appear to potentiate any differently from animals that did not show this reaction. Moreover, many animals displayed cortical spindle waves following the delivery of stimulation trains of 159 μA and above and test pulses of 501 μA and above. The delivery of 16 and 32 μA pulse trains did not produce spindle activity. The presence or absence of cortical spindles showed no clear relationship with the incidence or magnitude of the neocortical LTP effects. None of the animals showed any evidence of seizure activity during, or post-ictal depression following, any of the high-frequency stimulation protocols.

Experiment 1: The effect of varying train number on LTP induction

The induction of LTP was always associated with an amplitude change in both the early and late components. The change in the amplitude of the early component showed a significant interaction effect between session and group for both the 10 days of

trains ($F(27,180)=2.62$, $p<0.001$) and 25 days of trains ($F(48,312)=2.48$, $p<0.001$) groups. Animals that received 60 trains/day showed the strongest effect in both cases, though the groups all showed the same pattern of change. The enhanced population spike activity superimposed on the early response following trains ($F(1,48)=6.95$, $p<0.01$), suggests that the underlying EPSPs may also be potentiated. There was also a significant interaction between group and session for both the late1 and late2 components in both the 10 days of trains (late1: $F(27,180)=2.11$, $p<0.01$; late2: $F(27,180)=3.90$, $p<0.001$) and 25 days of trains (late1: $F(48,312)=1.68$, $p<0.01$; late2: $F(48,312)=1.63$, $p<0.01$) groups. Animals that received 60 trains/day displayed the largest increase, 5 and 10 trains resulted in approximately equivalent changes, and 1 train/day produced the smallest potentiation effect.

Figure 2.3 shows changes in the early and late component response amplitudes of animals receiving trains for 10 days and 25 days. Although the late component peaks were often not discernable prior to the delivery of trains, they became visible following the first 3 sets of trains in all animals except the 1 train/day group, for which the late1 component did not appear to develop. However, the late2 component became visible in the 1 train/day group following an average of 15 days of trains. The late1 component increased over the course of train stimulation, reaching asymptotic levels of potentiation following 7, 10 and 6 sets of trains for animals receiving 5, 10 and 60 trains/day, respectively, while the late2 component reached asymptotic levels of potentiation following 25, 17, 14 and 8 sets of trains for animals receiving 1, 5, 10 and 60 trains/day, respectively.

The decay rates of both early and late components over the five week decay period varied according to both the number of trains delivered and the number of days over which trains were delivered. The percent decay from peak potentiation, after a 5 week period for the early, late1 and late2 components, is shown in Table 2.1. It is clear that the changes in the early component of the response are longer lasting than those of the late components and, moreover, that the longer train stimulation regimens produced longer lasting potentiation effects in all three components.

The response thresholds increased following trains for most animals: the average pulse intensity required to evoke a minimal response was 50 μA prior to potentiation and increased to 90 μA following LTP induction. Following the 5 week decay period, the response thresholds returned to their pre-LTP values.

As the experimental groups tended to show the same percentage increases in population spike potentiation, they were collapsed together for the statistical analyses. There was a significant interaction between group and potentiation ($F(1,48)=6.95$, $p<0.01$) over the pre- and post-LTP conditions. Following the five week decay period, these measures had only decreased by 50% of the asymptotic level of potentiation (Fig. 2.4).

Experiment 2: The effect of varying train intensity on LTP induction

The extent of LTP induction increased with train intensity, but all train intensities were capable of producing potentiation effects. Figure 2.5 shows changes in the field potentials following the application of high-frequency trains of varying intensity.

Although LTP was reliably induced in the late1 and late2 components at *all* train intensities, the early component was differentially affected by train intensity. Animals that received 159 and 501 μA trains showed a strong surface positive shift of the early component, and the largest and most rapid change was induced by the highest intensity trains (501 μA). Animals that received 16 and 32 μA trains, however, showed no significant change in this component compared to controls. The change in the amplitude of the early component showed a significant interaction effect between session and group ($F(56,336)=5.41$, $p<0.001$). The early components of animals in the 159 and 501 μA groups were still potentiated 4 weeks following the LTP induction protocol.

There was also a significant interaction between group and session for both the late1 and late2 components (late1: $F(56,336)=5.10$, $p<0.001$; late2: $F(56,336)=12.72$, $p<0.001$). The delivery of 501 μA trains produced the strongest potentiation effects, followed by 159, 32 and 16 μA trains, respectively. As in the case for the early component, increasing the train intensity caused a more rapid increase in the late component potentiation effects. However, while every member of the 32, 159 and 501 μA groups showed potentiation following the stimulation protocols, only 43% (3/7) of the 16 μA animals did. Therefore, a threshold for the induction of potentiation appears to exist around the 16 μA mark.

As described in Experiment 1, the late component peaks were often not visible prior to LTP induction. Both late components became visible, and increased in amplitude, during the stimulation regimen (except in those 16 μA animals that did not express LTP of any component). The late1 component reached asymptotic levels of

potentiation following 12, 16, 20 and 20 sets of trains for animals in the 16, 32, 159 and 501 μA groups, respectively, while the late2 component reached asymptotic levels of potentiation following 12, 16, 20 and 16 sets of trains for animals in the 16, 32, 159 and 501 μA groups, respectively. While the late1 and late2 component potentiation effects had decayed fully 2 weeks following the cessation of trains in animals receiving 16 μA trains, animals that received 32, 159 or 501 μA trains still showed significant late1 and late2 component potentiation 4 weeks following the LTP induction protocol.

In contrast to the effects of higher intensity stimulation, the response thresholds did *not* increase after potentiation for animals receiving 16 or 32 μA trains.

As in the previous experiment, the stimulated animals showed an enhancement of the population spike amplitudes. There was a significant interaction between group and potentiation ($F(4, 24)=10.42$, $p<0.001$) over the pre- and post-LTP conditions. Animals receiving 501 μA trains showed the largest mean increase in population spike amplitude, followed by the 159 and 32 μA groups which showed equivalent increases, and finally the 16 μA group which showed only a slight increase in population spike amplitude following potentiation.

Experiment 3: The effect of varying stimulation session interval on the induction of LTP

As the pattern of development of the late1 component did not appear substantially different from the late2 component in the first 2 experiments, and because it is often obscured during the course of LTP by the late2 component, we chose not to

analyse it in this experiment. Hereafter, unless otherwise specified, the term *late1* has been omitted and the term *late2* has been replaced by simply *late*. The induction of LTP was always associated with amplitude changes in the early and late components. The surface positive shift of the early component showed a significant interaction effect between session and group ($F(64,336)=3.42$, $p<0.001$). Animals that received trains every 6h showed the largest early component shift, followed by the 12h, 24h and 48h groups, respectively. There was also a significant interaction between group and session for the late components ($F(64,336)=5.80$, $p<0.001$). For these components, animals that received trains every 24h showed the largest enhancements, followed by the 12h and 48h groups which showed approximately equal potentiation. The 6h group showed the least amount of late component potentiation.

Changes in the early and late component response amplitudes of animals receiving trains at the various inter-session intervals can be seen in Figure 2.6. The early component amplitude shift was largest for all groups on day 10 of the induction regimen and did not appear to have yet reached asymptotic levels. By contrast, the late component increased over the course of the induction protocols and reached an asymptotic level of potentiation following 6 days of the induction protocol for animals in the 6, 12 and 24h groups, respectively, and following 5 days of the induction protocol for the 48h group.

In order to determine the relative contributions of the amount of stimulation received versus the spacing of the stimulation, it is necessary to compare the groups following equivalent numbers of stimulation sessions. By contrasting the eighth stimulation session for the 24h (day 8 of the LTP-induction regimen) and 6h (day 2 of the

LTP-induction regimen) groups (Figure 2.6), it is clear that the stimulation interval, rather than the *amount* of stimulation *per se*, is a critical variable mediating the large LTP induction effects observed. Figure 2.7 contrasts representative sweeps from animals in the 6h, 12h and 24h conditions for 2 time-points (following 4 and 8 stimulation sessions) following the delivery of equal numbers of high-frequency trains. Following 4 stimulation sessions, animals in the 6h group showed no population spike or late potentiation, while animals in the 12h and 24h groups showed increasing population spike potentiation. By contrast, early component amplitude shifts are evident in all groups. After 8 stimulation sessions, 3/5 of the 6h animals show late component potentiation, while all other groups showed further increments in late component in all animals. The 48h group did not appear different from the 12h group following 4 stimulation sessions.

There was a significant interaction between group and session for the population spike measures ($F(8,40)=2.19$, $p<0.04$). Relative to controls, all groups showed population spike potentiation, with the 6h group showing the greatest amount of potentiation, followed by the 24 and 48h groups which showed approximately equal population spike enhancement, and the 12h group which showed the least amount of potentiation. After a 4 week decay period, the 12, 24 and 48h groups continued to show population spike potentiation, though some decay was evident by this time, while the 6h group continued to show a strong enhancement. The population spike measures for these groups are shown in Figure 2.8.

The decay rates of both early and late components over the 4 week decay period

varied according to group. The percent decay from peak potentiation, for the early and late components, is shown in Table 2.2. It appears that the large amount of stimulation received by the 6h animals, relative to the other experimental groups, resulted in the slowest decay rate. Similarly, the only group which showed complete decay by the end of the follow-up testing was the 48h group, which received the least stimulation. The slow decay rate of the 6h group resulted in a crossover of the late component decay curves (Figure 2.6) at the 2 week follow-up (decay) point.

Experiment 4: Control for kindling effects

No epileptiform afterdischarges were induced in response to stimulation in any of the preceding experiments. To confirm that there were no kindling effects in our potentiated animals, I tested for increases in the strength of evoked afterdischarges in a separate group of control and potentiated animals. The kindling stimulation reliably induced a rapid onset tonic-clonic seizure as described by Racine et al. (1995a). The afterdischarge durations of animals that were fully potentiated were not significantly different from animals that did not receive high-frequency trains ($t(8)=0.96$, $p<0.36$). Both groups of animals displayed similar seizure behaviours with no apparent differences in onset, duration or severity. These data, in combination with the demonstration of potentiation effects following either 1 train/day or minimal white matter stimulation, clearly indicate that there are no kindling-related contributions to the LTP effects reported here.

DISCUSSION

These data confirm that the spaced and repeated delivery of high frequency trains to the corpus callosum results in a long-lasting potentiation of neocortical evoked field potentials in the adult, freely moving rat. Moreover, it is apparent that the number of trains delivered per session, the number of daily sessions over which trains are delivered, the intensity of the pulses in the stimulation trains, and the stimulation session interval, alter the strength and/or duration of the LTP effects. The delivery of either larger numbers of stimulation trains or stimulation trains of increasing pulse intensity results in a more rapidly developing potentiation compared to the delivery of fewer trains. It is also the case that animals receiving trains for 25 days showed a slower rate of decay as compared to animals that received trains for only 10 days. Further, by manipulating the interval between the stimulation sessions it was established that the development of neocortical LTP relies heavily on the spacing characteristics of the stimuli.

The stimulation session interval experiment demonstrated that for both the early and late components of the neocortical evoked response, the greatest potentiation was expressed by animals receiving trains with a 24h inter-session interval (ISI). Animals that received as much as four times that amount of stimulation (6h group) showed substantially less LTP at equivalent time points (Figures 2.6 & 2.7). By contrast, the amount of stimulation received by the animals did seem to affect the *decay* of LTP. Animals that received the greatest amounts of stimulation (6h group) showed the slowest rate of decay, especially of the population spikes which showed no apparent decay 4

weeks following LTP induction, while animals that received the least stimulation (48h group) had decayed back to baseline by the end of the follow-up period.

A surprise finding of these studies was that it was possible to potentiate animals with the delivery of only 1 train/day, or by using trains with pulse intensities as low as 16 or 32 μA. In contrast to previous reports of the resistance of this structure to undergo plastic change (Racine et al., 1994b; Kirkwood and Bear, 1994), these results indicate that the neocortex is, in fact, highly reactive so long as the stimulation trains are *spaced and repeated.* Indeed, it appears that the neocortex may actually be more responsive than the hippocampus. In our hands, for example, hippocampal LTP is rarely induced by stimulation trains as low as 16 μA, and we have never seen long-lasting potentiation effects in response to one 24 ms train per day (Racine et al., 1983; Huang and Kandel, 1994). It appears that the neocortex is at least as plastic as the hippocampus so long as the stimulation used to induce LTP follows the rules appropriate to the system.

Previous studies have shown that monopolar field recordings in slices of *visual* cortex following white matter stimulation usually evoke a response with two components. The first, early, positive component has been reported to have a latency ranging from approximately 2.3 ms (Kirkwood and Bear, 1994) to 6.0 ms (Berry et al., 1989) to peak. At least the earliest of these may correspond to our initial, spike-like component. The latency of the second, negative-going component has been reported to lie between 8.8-22.4 ms (Berry et al., 1989). The earliest of these may correspond to our presumed monosynaptic component, which we have found to follow stimulation frequencies over 100 Hz. In our chronic preparations we have also found components with still longer

latencies, and many of these are seen most clearly after the induction of LTP. The emergence of new, late components appears unique to the chronic preparation and may reflect the recruitment of intact polysynaptic circuitry.

In contrast to the clear enhancements seen in both the early and late components following high-intensity stimulation, the potentiation effects following *low*-intensity stimulation appear to be restricted to the polysynaptic components of the evoked responses. We believe that this is due to the potentiation of the monosynaptic component at low intensities being restricted to the region most heavily innervated by the stimulated axons. This is probably quite close to the site of the stimulation electrode. The enhanced polysynaptic responses seen at our recording sites would then reflect the increased input from these low-threshold, monosynaptically activated sites.

Another interesting result is the increase in field potential threshold seen at the low intensity end of the I/O tests. We have also seen this effect in response to dentate gyrus potentiation, although not as reliably as for the neocortical responses. These threshold increases are *not* seen when low intensity potentiating stimulation trains are used. The higher intensity stimulation trains trigger considerably more cell discharge, indicated by the population spikes seen in the train-evoked field potentials. They would also be expected to activate inhibition, at least recurrent inhibition, more strongly. Stripling et al. (1988) have reported potentiation of a long latency potential in the pyriform cortex which they argued represented an inhibitory component in the field response. If both inhibitory and excitatory systems are susceptible to potentiation, the net effect on the field potential may be dependent upon the overall level of activation.

Perhaps the balance shifts in favor of inhibition at low test pulse intensities. This explanation would seem to work best if the threshold for the potentiation of inhibition was higher than for the potentiation of excitation, *and* the altered inhibition included feedforward mechanisms (which could account for the altered thresholds). These results might also be explained by some form of axonal damage caused by the high intensity stimulation. This explanation is unlikely, however, as the threshold effects are obviously reversible.

The population spike is a compound action potential which is superimposed on the population EPSP and reflects the number of cells synchronously firing action potentials. We speculate that the increase in the amplitude and number of population spikes during potentiation is primarily responsible for the early surface positive shift and reversal in polarity of the early component. The same thing happens with the hippocampal field potentials, except that there is generally only one, brief population spike present in those responses. In any case, unless there is a general change in the *excitability* of the target cells, the increased population spike activity suggests that at least some of the underlying EPSPs must be potentiated.

By contrast to the early component, the late components show a relatively simple potentiation effect in all animals. This potentiation may reflect a passive driving of polysynaptic responses, from a monosynaptically potentiated site, or a potentiation effect expressed within the polysynaptic connections themselves. The augmented cell discharge from monosynaptically-activated neurons, reflected in the enhanced population spike activity, would be expected to drive stronger polysynaptic responses. Artola and Singer

(1987) have identified a subpopulation of "bursting" layer II-IV cells which support white matter-evoked LTP, even in the absence of bicuculline. These cells appear to possess lower intrinsic inhibition, as compared to "regular spiking" cells, and so may preferentially contribute to neocortical LTP induced by white matter stimulation. As well, recordings made in motor cortex have shown that both regular and fast-spiking cells, in addition to bursting cells, will display LTP following both middle layer (II/III) or white matter stimulation (Aroniadou and Keller, 1995). As our recording sites are within motor cortical regions, the multiple population spikes that were often seen in our field potentials may reflect either the bursting activity within the subpopulation identified by Artola and Singer (1987), or the potentiation of the larger populations of motor cortical regular or fast-spiking neurons following deep layer stimulation. One or both of these possibilities may, in turn, be responsible for driving the polysynaptic components.

We can exclude an involvement of kindling mechanisms in our neocortical potentiation effects for several reasons: First, we monitored EEG during all stimulation sessions and never saw any sign of epileptiform spiking. Second, to counter the argument that kindling, an increase in epileptogenic reactivity, might be produced by *non*-epileptogenic stimulation in our neocortical sites, we tested the effects of LTP induction on the strength of epileptiform discharge evoked through the same electrodes. There were no significant differences between potentiated and control animals. Third, although neocortical thresholds for kindling are much higher than those measured in the hippocampus (Racine, 1972; 1975), we have found neocortical LTP to be inducible with very low intensity stimulations. Fourth, kindling-induced potentiation can be induced in

our neocortical site, as it can in hippocampus, but it actually progresses more *slowly* than does neocortical potentiation induced by lower intensity stimulation trains (Racine et al., 1995a,b). Fifth, in sites such as the hippocampus, amygdala and pyriform cortex, it is often the case that stimulation just below threshold for the induction of epileptiform activity can leave the system at least partly epileptogenic in the sense that isolated epileptiform spikes can often be induced by test pulses for a period of several minutes. We have never seen this in our neocortical sites. Although one cannot exclude the possibility that neocortical LTP represents some form of pathology, that is true of LTP in general. The very low intensities at which these effects can be induced suggest that the mechanisms engaged may be involved in normal physiological processes.

One of our most interesting discoveries is that the neocortex appears to be following quite different rules for LTP induction than does the hippocampus and most other subcortical sites. The hippocampal and neocortical learning systems have been distinguished on the basis of several characteristics. While the former has been characterized by rapid acquisition and transient storage, the latter is believed to learn more slowly but store for longer periods (Zola-Morgan and Squire, 1993; McClelland et al., 1995). The different potentiation and decay rates for hippocampal and neocortical systems are consistent with the proposed functional differences between these structures. The spaced, repeated presentations of the LTP-inducing stimulation trains may be effective because the neocortical system is operating with a relatively slow "learning rate." The results of the inter-session interval study are entirely compatible with this possibility. As the neocortex is believed to learn through many stimulus repetitions,

producing gradual, incremental changes in the synaptic connections between neocortical neurons, the shortened stimulus intervals may be compromising this process by forcing the system to integrate the inputs rapidly (McClelland et al., 1995). The fact that the 48h group showed less potentiation than the 24h group indicates that too long an interval may also serve to weaken the LTP induction process. It has been argued that a system designed to extract shared structure from multiple input patterns requires a slow learning rate in order to avoid catastrophic interference (McCloskey and Cohen, 1989; McClelland et al., 1995). These data are consistent with the idea that the temporal spacing of the inputs and a slow learning rate are critical for the prevention of interference.

FIGURE LEGENDS

Figure 2.1. Chronically implanted recording and stimulating electrode placements are indicated by the filled circles. The stimulating electrodes are within the corpus callosum, while the recording electrodes are all within neocortical regions. In cases where placements approximately overlap, only one point is shown. FR1: frontal area 1, FR3: frontal area 3, PAR1: parietal area 1, CC: corpus callosum, CP: caudate/putamen, LV: lateral ventricles, RF: rhinal fissure.

Figure 2.2. Representative examples of neocortical field potentials evoked by corpus callosum stimulation are shown above. Included are examples of the cursor positions used to measure the amplitude changes at the early, late1 and late2 component latencies. This set of sweeps is from an animal that received 60 trains/day for 25 days and shows a baseline response (solid), a response 24 h following completion of the LTP induction protocol (short dashes), and after a 30 day decay period (long dashes). Vertical calibration: 1.0 mV; horizontal calibration: 10 ms.

Figure 2.3. Long-term potentiation following varying numbers of trains over 10 or 25 days. The mV differences between the last baseline and all other sweeps are plotted in these figures for the baseline (pre-LTP), the LTP induction (LTP) and post-LTP (post-LTP) periods. **Top left:** Changes in the early component (average latency-to-peak: 8.1 ms, range: 3.0-13.2 ms) over days for groups receiving spaced, high frequency

stimulation for 10 days. All groups showed a shift in the surface-positive direction (indicated as a negative mV shift from baseline) relative to baseline and controls. These changes showed only partial recovery 35 days after potentiation. Increasing the number of trains delivered each day tended to result in both a greater amplitude shift as well as a slower rate of decay. **Middle left:** Changes in the late1 component (average latency-to-peak: 15.2 ms, range: 8.2-27.0 ms) for groups receiving 10 days of trains. Animals receiving 60 and 10 trains/day showed a large increase which had not fully recovered five weeks following trains, while animals receiving 5 trains/day displayed a modest increase that decayed fully once the stimulation regimen was completed. **Bottom left:** Changes in the late2 component (average latency-to-peak: 20.0 ms, range: 10.0-32.0 ms) for groups receiving 10 days of trains. All groups showed a large enhancement. Increasing the number of trains delivered per day increased the rate and amplitude of the LTP effect. Animals that received 5 trains/day decayed back to baseline prior to the end of the decay period while animals receiving 60 trains/day had not fully decayed 5 weeks following trains. **Top right:** Changes over days in the early component of animals receiving trains for 25 days. All groups showed a marked shift in response amplitude during the induction phase which only partially decayed over the post-LTP decay phase. With increased numbers of trains delivered per day, a greater amplitude shift was seen. Sixty and 1 train/day produced the largest and smallest amplitude shifts, respectively. **Middle right:** Changes in the late1 component over days for groups receiving 25 days of trains. Although animals receiving 60 and 10 trains/day displayed a long-lasting amplitude increase, animals receiving 5 trains/day decayed back to baseline prior to the cessation of

the stimulation protocol while animals that received 1 train/day did not display potentiation above baseline on any day. **Bottom right:** Changes in the late2 component over days for groups receiving trains for 25 days. Animals in the 60, 10 and 5 trains/day groups showed a clear potentiation, with animals that received 60 trains/day showing the largest amplitude increase. One train/day produced the weakest potentiation, differing from controls only near the end of the induction protocol. Sixty and 10 trains/day groups did not fully decay following the 5 week decay period. The 1 and 5 trains/day groups decayed back to baseline prior to the end of the decay period.

Figure 2.4. Total population spike height measures following varying numbers of trains are shown for time-points prior to LTP induction (pre-LTP), 24 h following the induction protocols (post-LTP) and following the 5 week decay period (post-decay). Both control (white) and experimental (black) groups are shown. While controls show no evidence of change over the course of the experiment, the LTP groups show significant potentiation following LTP induction. These measures remained enhanced even after 5 weeks.

Figure 2.5. Long-term potentiation following low-intensity stimulation for 24 days. The mV differences between the last baseline and all other sweeps are plotted in these figures for the baseline (pre-LTP) and the LTP induction (LTP-induction) periods. **Top:** Changes in the early component over days. While animals receiving 159 and 501 μA trains showed a shift in the surface-positive direction (indicated as a negative mV shift from baseline) relative to baseline and controls, animals receiving 16 and 32 μA trains

did not differ from controls over the LTP induction protocol. For animals that did show an effect, increasing the stimulation intensity caused a larger potentiation effect. **Middle:** Changes in the late1 component over days. All groups displayed an increase in the amplitude of the late1 component over the course of the induction protocol. The potentiation effect increased as the train intensity used to induce LTP increased, with animals receiving 501 μA trains showing the largest increase, followed by the 159 and 32 μA groups which showed approximately equal changes, and finally the 16 μA group which showed the smallest change. **Bottom:** Changes in the late2 component over days. All groups showed a significant enhancement. Increasing the train intensity increased the rate and magnitude of the LTP effect. Note that while only 43% (3/7) of animals receiving 16 μA trains displayed late2 component potentiation, the group curve is clearly enhanced relative to controls.

Figure 2.6. Long-term potentiation following various inter-session intervals. The mV differences between the last baseline and all other sweeps are plotted in these figures for the baseline (pre-LTP), LTP induction (LTP-induction) and post-LTP (decay) periods. **Top:** Changes in the early component over days. All animals showed a shift in the surface-positive direction (indicated as a negative mV shift from baseline), with animals receiving the most trains (6h group) showing the largest overall shift during the induction period. However, comparison of time points where animals have received equivalent amounts of stimulation (eg: day 8 for the 24h group vs. day 2 for the 6h group) shows clearly that, in fact, the amount of LTP induced is dependent upon the spacing between

the stimulations. **Bottom:** Changes in the late component over days. All groups showed a significant enhancement. It is clear by analysis of this component that the 24h spacing is the one best suited to inducing LTP in the neocortex, especially compared to the 6h group which received 4 times as much stimulation over the same period of time but showed the smallest amount of late component potentiation.

Figure 2.7. Representative sweeps recorded at equivalent stimulation time-points from 3 inter-session intervals. Baseline (solid) and partially (dashed) potentiated (4 or 8 stimulation sessions, for the same animal) are shown. Although the animals had all received the same amount of stimulation, animals in the 6h group (A) showed no population spike or late component potentiation after 4 stimulation sessions, while animals in the 12h (B) and 24h (C) groups showed substantial enhancement of both measures. By contrast, early component amplitude shifts were evident in all groups. After 8 stimulation sessions, 3/5 of the 6h animals (D) showed late component and population spike potentiation, while the 12h (E) and 24h (F) groups continued to show increasing potentiation. All groups underwent robust early component potentiation. Horizontal calibration: 10 ms; vertical calibration: 1 mV.

Figure 2.8. Total population spike height measures following varying inter-session intervals are shown for time-points prior to LTP induction (pre-LTP), 24 h following the induction protocols (post-LTP) and following a 4 week decay period (decay). While controls showed no evidence of change over the course of the experiment, the LTP

groups showed significant potentiation following LTP induction. Animals that received the largest numbers of trains showed the largest overall increase in population spike enhancement by the end of the induction period. These measures remained enhanced even after 4 weeks, with the 6h group showing no evidence of decay.

TABLE LEGENDS

Table 2.1. Percent decay of the early (Early Component % Decay) late1 (Late1 Component % Decay) and late2 (Late2 Component % Decay) field potential components from the varying train parameter experiment 5 weeks post-LTP induction. The early component response showed less decay (20.0-59.0%) than either the late1 (34.5-100%) or late 2 (47.7-100%) components by the end of the 5 week decay period. Several responses had decayed back to baseline levels prior to the 5 week measure, returning to baseline by decay day: **a:** 1; **b:** 25; **c:** 21; **d:** 7 (*: did not potentiate above baseline). The experimental conditions are designated according to the number of trains delivered per day / number of days trains were delivered (# Trains / # Days).

Table 2.2. Percent decay of the early (Early Component % Decay) and late (Late Component % Decay) field potential components from the inter-session interval experiment 4 weeks following LTP induction. The early component response showed less decay (48.4-66.7%) than the late (48.7-100%) component by the end of the 4 week decay period. The late response of the 48h group had decayed back to baseline levels as measured on the final day of the decay period (**a**). In the case of the 6h group, the late1 component did not show any decay (*). The experimental conditions are designated according to the inter-session intervals.

Figure 2.1

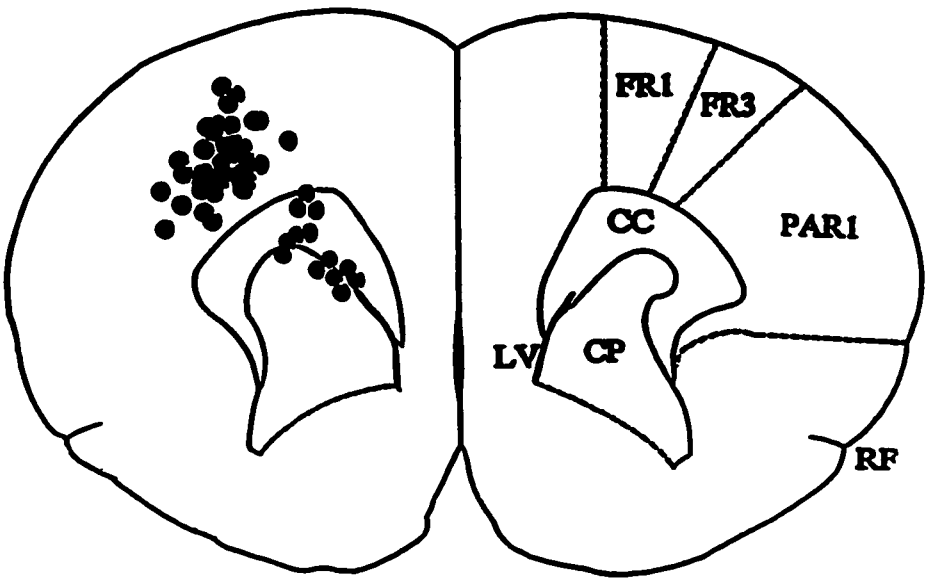


Figure 2.2

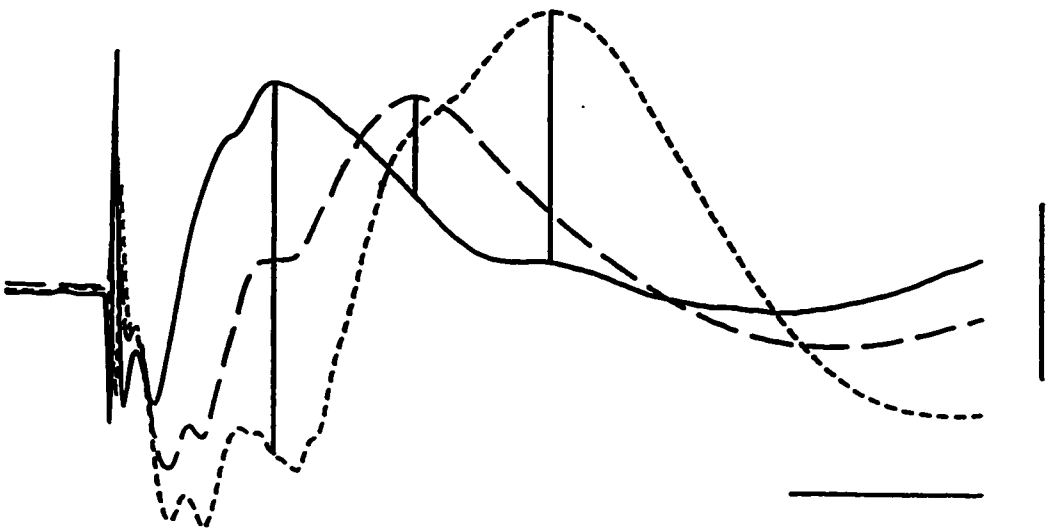


Figure 2.3

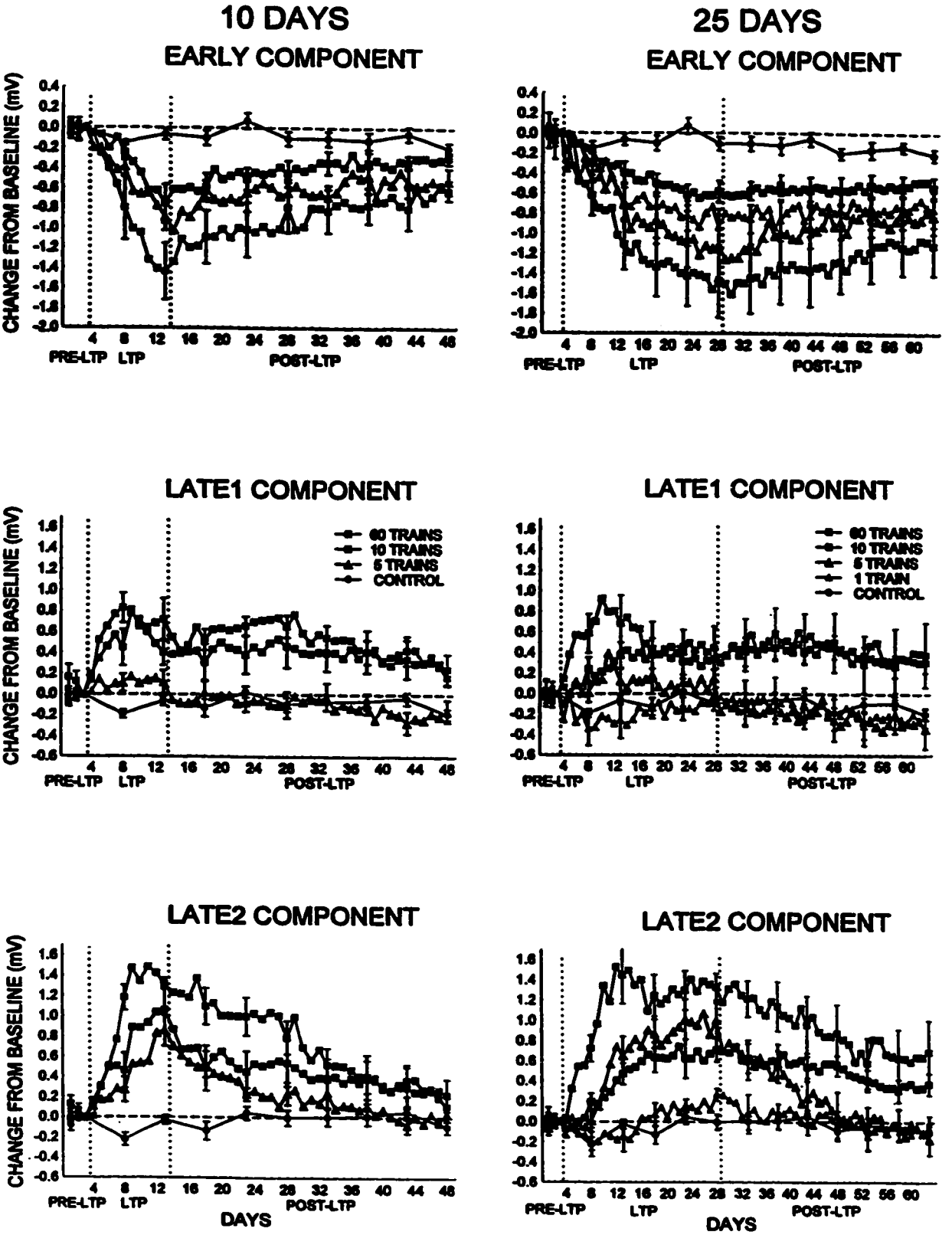

10 DAYS
EARLY COMPONENT
25 DAYS
EARLY COMPONENT
CHANGE FROM BASELINE (mV)
PRE-LTP
LTP
POST-LTP
LATE1 COMPONENT
LATE1 COMPONENT
60 TRAINS
10 TRAINS
5 TRAINS
1 TRAIN
CONTROL
LATE2 COMPONENT
LATE2 COMPONENT
DAYS

Figure 2.4

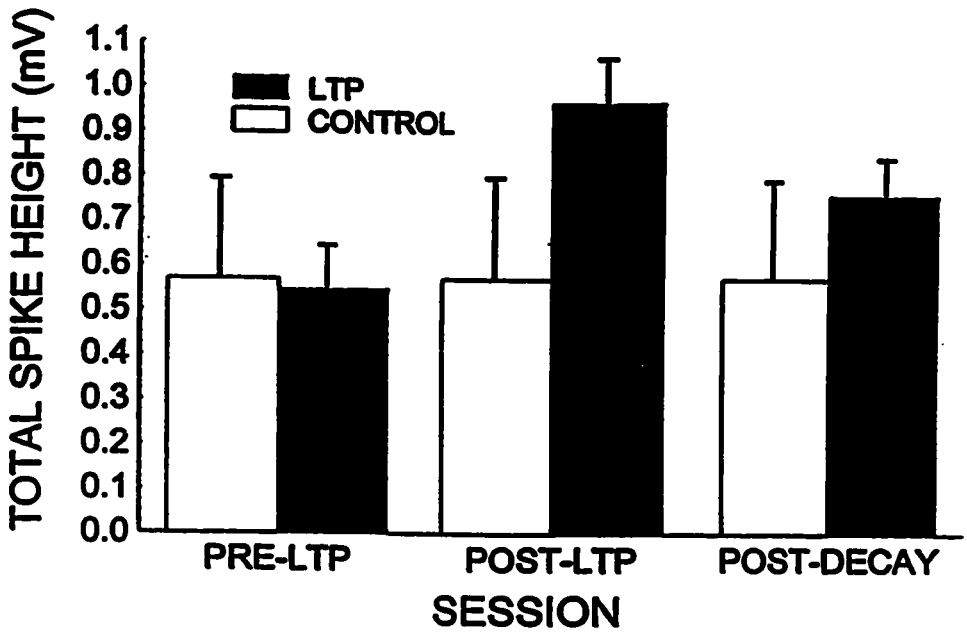

TOTAL SPIKE HEIGHT (mV)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
LTP
CONTROL
PRE-LTP
POST-LTP
POST-DECAY
SESSION

Figure 2.5

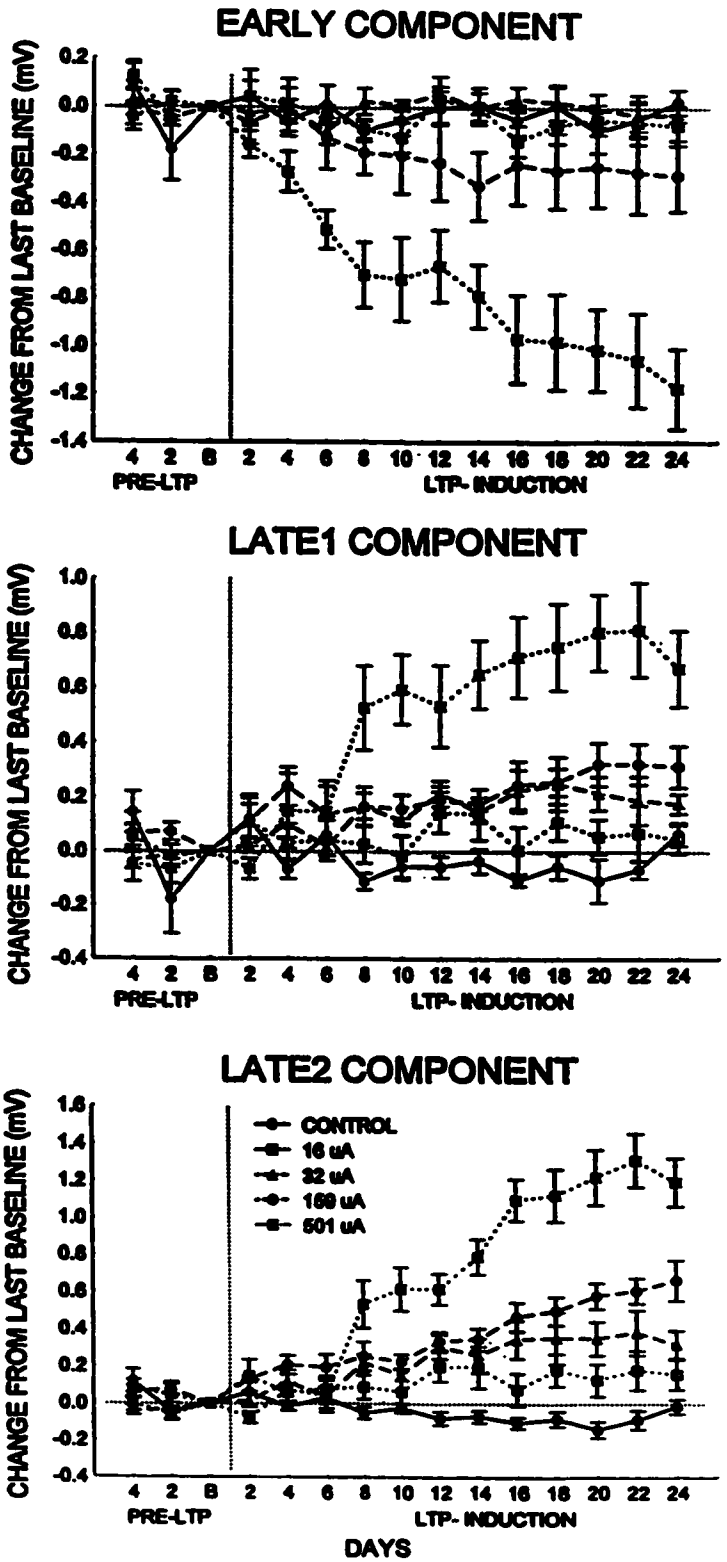

EARLY COMPONENT
CHANGE FROM LAST BASELINE (mV)
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
4 2 B 2 4 6 8 10 12 14 16 18 20 22 24
PRE-LTP
LTP- INDUCTION
LATE1 COMPONENT
CHANGE FROM LAST BASELINE (mV)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
4 2 B 2 4 6 8 10 12 14 16 18 20 22 24
PRE-LTP
LTP- INDUCTION
LATE2 COMPONENT
CHANGE FROM LAST BASELINE (mV)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
CONTROL
16 uA
32 uA
159 uA
501 uA
4 2 B 2 4 6 8 10 12 14 16 18 20 22 24
PRE-LTP
LTP- INDUCTION
DAYS

Figure 2.6

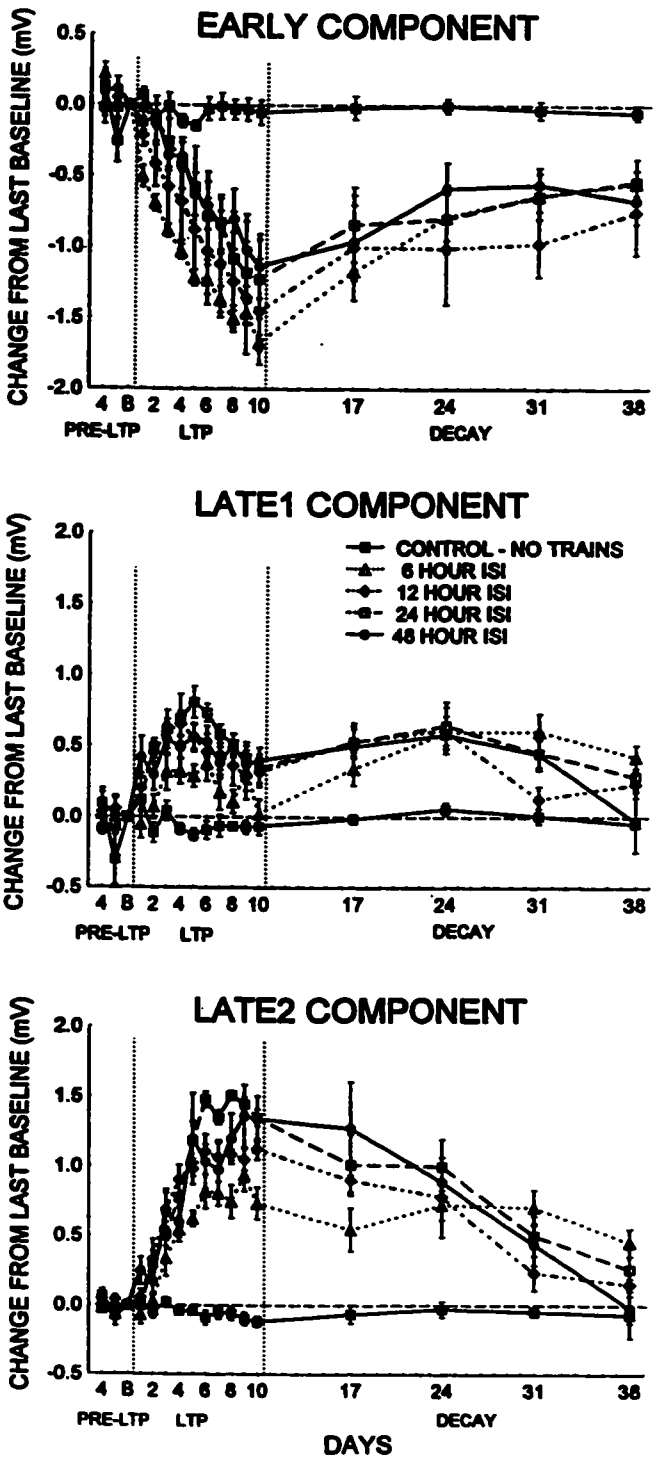

EARLY COMPONENT
CHANGE FROM LAST BASELINE (mV)
0.5
0.0
-0.5
-1.0
-1.5
-2.0
4 B 2 4 6 8 10 17 24 31 38
PRE-LTP
LTP
DECAY
LATE1 COMPONENT
CONTROL - NO TRAINS
6 HOUR ISI
12 HOUR ISI
24 HOUR ISI
48 HOUR ISI
2.0
1.5
1.0
0.5
0.0
-0.5
LATE2 COMPONENT
DAYS

Figure 2.7

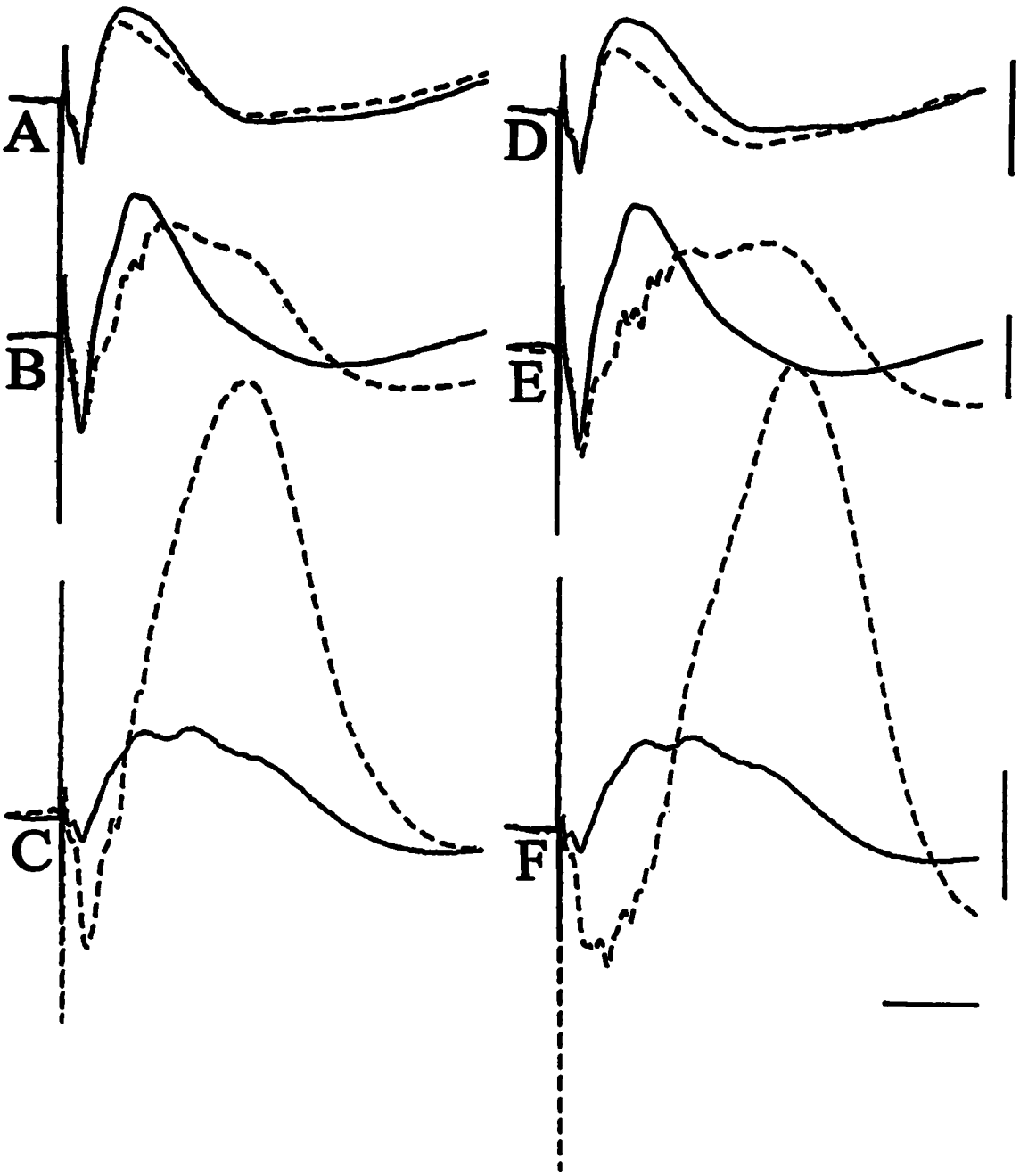


Figure 2.8

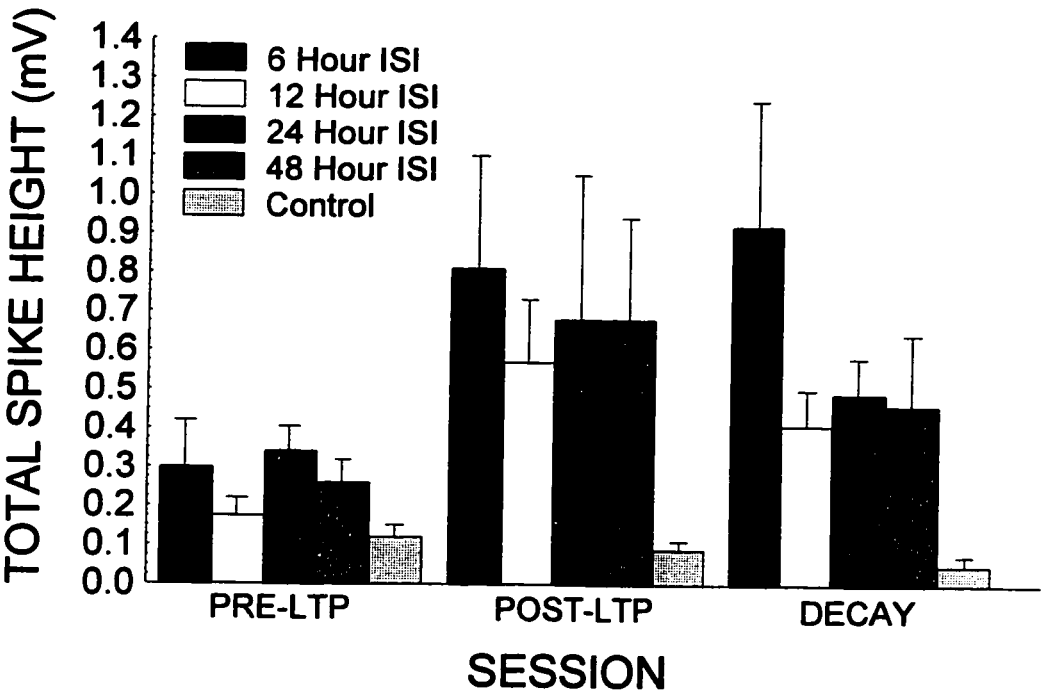

TOTAL SPIKE HEIGHT (mV)
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
6 Hour ISI
12 Hour ISI
24 Hour ISI
48 Hour ISI
Control
PRE-LTP
POST-LTP
DECAY
SESSION

Table 2.1

Group (# Trains / # Days)	Early Component % Decay	Late1 Component % Decay	Late2 Component % Decay
60 / 10	57.1	80.0	92.2
10 / 10	59.0	67.4	78.1
5 / 10	46.7	100[a]	100[d]
60 / 25	28.9	58.9	55.9
10 / 25	21.1	34.5	47.7
5 / 25	31.0	100[a]	100[c]
1 / 25	20.0	*	100[b]

Table 2.2

Group (Inter-train Interval)	Early Component % Decay	Late Component % Decay
6h	66.7	48.7
12h	48.4	87.2
24h	53.8	82.8
48h	59.2	100[a]

CHAPTER THREE

ROLE OF NMDA RECEPTORS IN NEOCORTICAL LTP INDUCTION

The *N*-methyl-D-aspartate (NMDA) receptor subclass of the excitatory amino acid glutamate regulates a cation channel permeable to Ca^{2+}, Na^{+} and K^{+}, and possesses binding sites for glycine, Zn^{2+}, PCP, MK-801, and Mg^{2+}, all of which serve to modulate its activity. The importance of the NMDA receptor in LTP induction stems from several properties, the most important being the voltage-dependent block of its ion channel by Mg^{2+} (Nowak et al., 1984; Mayer et al., 1984). This feature is what allows the NMDA receptor to act as a molecular coincidence detector (Singer and Artola, 1991; Bliss and Collingridge, 1993). In order for the NMDA receptor channel to open, the membrane must be depolarized to a level sufficient to remove the Mg^{2+} block coincident with glutamate binding. These requirements serve to explain the characteristics of LTP described in the introduction. *Cooperativity* follows from the need for sufficient depolarization to remove the Mg^{2+} block. This must be provided by a strong stimulus if adequate depolarization is to occur. *Associativity* is similar except that sufficient depolarization is provided by a separate, converging set of inputs (or depolarizing current injected directly into the cell). *Input-specificity* stems from the requirement that the presynaptic terminal provides glutamate in quantities that permit the activation of

adequate numbers of NMDA receptors (for a discussion of these features of LTP, see Bliss and Collingridge, 1993). Once the channel is open, Ca^{2+} enters the cell and, in combination with Ca^{2+} released from intracellular stores, activates a variety of Ca^{2+}-dependent protein kinases which ultimately result in the enhanced synaptic responses characteristic of LTP.

The NMDA receptor has been shown, using both *in vitro* slices and anaesthetized animals, to be critical for LTP induction in several neocortical regions including frontal (Sutor and Hablitz, 1989), sensorimotor (Lee et al., 1991; Aroniadou and Keller, 1995; Castro-Alamanacos et al., 1995), somatosensory (Kawakami and Ashida, 1993), prefrontal (Hirsch and Crepel, 1991) and visual (Artola and Singer, 1987, 1990; Kirkwood and Bear, 1995) cortex. In addition, however, the NMDA receptor has been implicated in a variety of other neural plasticity phenomena. For example, Durand et al. (1996) demonstrated in rats that during the first postnatal week, the glutamatergic system becomes functional following the transformation of pure NMDA-based synapses into combined AMPA/NMDA-based synapses. These authors also showed that an NMDA-dependent associative LTP mediates this "functional synapse induction", thus indicating a role for the NMDA receptor in the development of nascent synapses. In addition, Constantine-Paton and her colleagues (Cline et al., 1987; Cline and Constantine-Paton, 1989; Cline and Constantine-Paton, 1990) have examined the role of the NMDA receptor in synapse stabilization and axon survival processes. Using the 3-eyed frog model, they have shown that the infusion of NMDA antagonists into the optic tectum disrupts the normal growth of retinal ganglion cells into eye-specific stripes (Cline et al., 1987; Cline

and Constantine-Paton, 1989) while infusion of NMDA increases eye-specific segregation (Cline et al., 1987). They have presented a model based on their data whereby increases in retinally-driven correlated activity lead to increases in NMDA receptor activation, which in turn initiates the stabilization of synapses that are coactive. Cline and Constantine-Paton (1990) indicate that, because retinotectal synapses are transient, the NMDA-based stabilization of synaptic connections can be equated to increasing the lifetime of a synapse.

This receptor has also been shown to be important in mammalian visual development and ocular dominance plasticity. The changes in cortical physiology typically associated with monocular deprivation (Bear et al., 1990; Daw, 1994), reverse suturing (Gu et al., 1989), and recovery of function following dark rearing (Bear et al., 1990) are all substantially reduced or prevented by blocking the NMDA receptor. In addition, NMDA receptors are found in larger numbers during the critical period for visual cortical plasticity than in either immature animals or adults (Bode-Greuel and Singer, 1989; Fox and Daw, 1993; Gordon et al., 1996), and synaptic transmission in layer IV is most sensitive to NMDA receptor blockade at the peak of the critical period (Fox et al., 1991).

The role played by the NMDA receptor in learning and memory phenomena has been studied most intensely by researchers interested in exploring the role of LTP in spatial learning. Morris and his colleagues (Morris et al., 1986; Morris, 1989; Davis et al., 1992) have shown repeatedly that hippocampal NMDA antagonism at doses comparable to those that block LTP *in vivo* block spatial learning in the Morris water

maze (but see Saucier and Cain, 1995; Bannerman et al., 1995). Further, transgenic mice mutant for either the ϵ1 subunit of the NMDA receptor (Sakimura et al., 1995) or with a targeted deletion of the NMDAR1 gene in the CA1 field of the hippocampus (Wilson and Tonegawa, 1997) both display strong spatial deficits. The importance of the NMDA receptor has also been shown for other learning and memory phenomena including fear conditioning (Kim et al., 1991), olfactory memory (Staubli et al., 1989) and alternation learning (Highfield et al., 1996).

While NMDA receptor activation is clearly important for the induction of long-term potentiation in both the hippocampus and neocortex, there have been demonstrations of NMDA-independent LTP in both structures. The best studied model of NMDA-independent potentiation is hippocampal mossy fiber LTP (for review, see Johnston et al., 1992). Although LTP in this system appears relatively insensitive to NMDA receptor blockade, it is compromised by lowered extracellular Ca^{2+} levels (Higashima and Yamamoto, 1985) and intracellular Ca^{2+} chelation (Williams and Johnston, 1991). These observations have led to the assertion that presynaptic voltage-gated Ca^{2+} channels at mossy fiber synapses may serve a function similar to that played by postsynaptic NMDA receptors in hippocampal CA1. In support of this, Castillo et al. (1994), using a variety of Ca^{2+} channel blockers, concluded that the induction of mossy fiber LTP is critically dependent on presynaptic Ca^{2+} entry. Similarly, it has been demonstrated that NMDA-independent LTP can be induced *in vitro* in visual cortex following low-frequency stimulation, provided that bicuculline is added to the medium (Komatsu et al., 1991). This form of neocortical LTP is believed to be mediated by low-threshold Ca^{2+} channels

(Komatsu and Iwakiri, 1992). These examples emphasize the possibility that several mechanisms may underlie LTP in the neocortex.

The mechanisms governing LTP induction in the *chronic* neocortical preparation, including the role of the NMDA receptor, have not been examined. The following experiment was designed to assess the role played by the NMDA receptor in the induction of neocortical LTP in this novel preparation.

MATERIALS AND METHODS

Surgery, baseline measures and induction of neocortical LTP

Thirteen male Long-Evans hooded rats from the McMaster University Breeding Colonies were used in these experiments. At the time of surgery, the animals weighed 325-450 g. All animals were implanted with recording and stimulating electrodes and had baseline measures taken as described in chapter 2. LTP-inducing stimulation sessions were delivered daily and consisted of sixty 8-pulse trains as described in chapter 2 (pulse intensity: 1259 μA).

LTP induction and NMDA blockade

Animals in which the effect of NMDA antagonism on LTP was tested were implanted with stimulating and recording electrodes and had baseline measures taken as described in chapter 2. The competitive NMDA antagonist CPP (3-[(±)-2-Carboxypiperazin-4-yl]-propyl-1-phosphonic acid) (1.0 and 10.0 mg/kg i.p., Cambridge Research Biochemicals) was dissolved in 0.9% saline vehicle solution. On the first day of experimentation, animals were injected with one of 1.0 mg/kg CPP (n=3), 10.0 mg/kg CPP (n=3) or saline (n=4), followed 150 minutes later by the delivery of 60 high-frequency trains as described in chapter 2 (pulse intensity: 1259 μA). On subsequent days the injection was preceded by an I/O test, and followed 150 minutes later by 60 high frequency trains. Additionally, a fourth group (n=3) received 10.0 mg/kg CPP followed by an I/O test every 24 hours for 10 days, but did not receive trains. These daily LTP

induction regimens were continued for 10 days. Following this, I/O tests were run weekly for two weeks.

Analyses

Changes in the field potentials over LTP and decay sessions for both experimental and control animals were measured by subtracting the final baseline responses from all other baseline and potentiated responses as previously described. Population spikes were measured from the largest amplitude response in the I/O tests before and after LTP induction and after the decay phase. Repeated measures ANOVAs, comparing all induction days, were calculated for the field measures, while a population spike ANOVA compared the third baseline and final LTP induction days. Following collection of all electrophysiological measures, animals were perfused and the brains were sliced and stained with Cresyl Violet to verify electrode placements.

RESULTS

Histology

Localization of the stimulation and recording electrode placements was completed in all animals. The ventral tips of the recording electrodes were placed in parietal area 1 or the medial-posterior aspect of frontal area 3, while the stimulating electrode spanned the forceps minor corpus callosum in all animals (Paxinos and Watson, 1986).

Behaviour

Animals injected with the competitive NMDA antagonist CPP were sluggish for several hours following the injection, struggled weakly when picked up and rarely vocalized, although the startle reflex was still present in response to a loud noise (hand clap). Twenty-fours hours after the injections (immediately preceding the I/Os), rats struggled and vocalized normally when handled. Animals groomed normally and gained weight throughout the experiment and appeared completely healthy 24 hours following the cessation of the CPP injection protocol.

The effect of NMDA blockade on LTP induction

Administration of CPP prevented the induction of LTP in a dose-dependent fashion. Figure 3.1(a) shows changes in the response amplitude of the late component for animals receiving saline, 1.0 mg/kg CPP ($CPP_{T1.0}$) or 10.0 mg/kg CPP ($CPP_{T10.0}$) in combination with high-frequency trains, or 10.0 mg/kg CPP ($CPP_{NT10.0}$) *without* high-

frequency trains. There was a significant interaction effect between late component potentiation and group ($F(36,108)= 9.46$, $p<0.001$). This effect was primarily due to the reliable potentiation evoked in the saline and $CPP_{T1.0}$ groups compared to the lack of potentiation or an actual depression in the $CPP_{NT10.0}$ and $CPP_{T10.0}$ groups. While 1.0 mg/kg CPP appeared to have little effect on the induction of late component LTP, 10.0 mg/kg CPP not only blocked LTP but may have unmasked an underlying depression effect (Figure 3.1). There was also a significant interaction between the population spike measure and group ($F(3,9)=27.31$, $p<0.001$). Although the saline and 1.0 mg/kg CPP groups showed significant potentiation of the population spikes following LTP induction, animals receiving either 10.0 mg/kg CPP and high-frequency trains, or 10.0 mg/kg CPP without trains, showed no potentiation. Figure 3.2(a-c) shows representative sweeps from animals that received saline or CPP injections in conjunction with the stimulation protocol.

All groups receiving trains displayed a similar surface positive shift of the early component yielding a significant main effect of session ($F(36,108)=12.07$, $p<0.001$). For animals that received 10.0 mg/kg CPP, however, there was no potentiation of the population spikes. This result, concurrent with the apparent amplitude *decreases* seen in the late component in the 10.0 mg/kg CPP group (figures 3.1 and 3.2), is consistent with the possibility that the CPP may have unmasked a depression effect. A comparison of the sweeps in figure 3.2(c) (CPP-treated) and 3.2(d) (same animal without CPP) makes this difference clear. There is a surface positive shift in the early surface negative component in both cases, but only the sweep in 3.2(d) appears to be potentiated. As shown in figure

3.2, the re-administration of high-frequency trains to animals that had received 10.0 mg/kg CPP in conjunction with trains, one week following the completion of the drug/stimulation regimen, resulted in a significant potentiation of both the early ($F(12,24)=5.35$, $p<0.001$) and late ($F(12,24)=11.88$, $p<0.001$) component. This group also showed significant population spike potentiation following clearance of the drug ($t(2)=-4.33$, $p<0.04$). These results suggest that the drug protocol did not result in long-lasting deleterious effects. Figure 3.2(b) shows the changes in the late component occurring over days following clearance of the drug, while figure 3.2(d) shows representative pre- and post-LTP sweeps once the drug had cleared.

DISCUSSION

In agreement with studies utilizing the NMDA antagonist AP5 (Artola and Singer, 1987; Sutor and Hablitz, 1989; Artola and Singer, 1990; Kirkwood et al., 1993), these data demonstrate that the competitive NMDA antagonist CPP blocks the induction of neocortical LTP in a dose-dependent fashion if present during the stimulation sessions. This experiment provides the first demonstration that an NMDA mechanism contributes to neocortical LTP in the freely moving animal. Further, the capacity of these receptors to mediate LTP induction is not permanently harmed by chronic antagonism, as LTP of the population spikes and late components was readily induced one week following the CPP regimen and lasted for several weeks. The blockade of potentiation by NMDA antagonists does not preclude the possibility that other forms of potentiation are present in these systems, as an NMDA blocker would also be expected to affect purely voltage-dependent processes as well (Cavus and Teyler, 1996). Komatsu et al. (1991) provided an example of an NMDA-independent LTP in visual cortex following prolonged 2 Hz stimulation.

NMDA blockade appeared to unmask a depression of both the early and late components. We are inferring a depression of the early component based upon the block of population spike enhancement and the reduction in early component response amplitude. The late component effects were more clear, as they shifted from a net enhancement to a net depression in the peak amplitude. These results concur with previous studies using cortical slices where AP5 has been shown to both block LTP as

well as unmask a long-term depression effect (Hirsch and Crepel, 1991). Artola et al. (1990) have suggested that neocortical potentiation and depression effects result from greater and lesser levels of activation, respectively, of a common substrate. It may be that NMDA blockade reduced the intracellular Ca^{2+} levels to the point that they fell within the range required for depression, but not potentiation. Further research will be required to determine if such a "dual threshold" mechanism is responsible for the effects seen here.

FIGURE LEGENDS

Figure 3.1. **A:** Changes in the late component for groups receiving either saline, 1.0 mg/kg CPP, 10.0 mg/kg CPP in conjunction with trains (CPP(T)), or 10.0 mg/kg CPP without trains (CPP(NT)). The mV differences between the last baseline and all other sweeps are plotted in these figures for the baseline (pre-LTP) and LTP induction (LTP) periods. While there appear to be few differences between the potentiation induced when trains are delivered in the presence of either saline or 1.0 mg/kg CPP, 10.0 mg/kg CPP blocks the induction of late component LTP in addition to unmasking a depression (relative to animals receiving CPP in the absence of trains). **B:** The blockade of neocortical LTP resulting from the administration of 10.0 mg/kg CPP is reversible, however, and a strong potentiation of the late component may be induced following a one week "washout" period during which time the drug has been allowed to clear.

Figure 3.2. **A-C:** Representative sweeps taken pre- and post-LTP induction for groups that received saline, 1.0 mg/kg CPP or 10.0 mg/kg CPP, respectively, in conjunction with the delivery of high frequency trains. Notice that while saline (A) and 1.0 mg/kg CPP (B) show similar levels of potentiation, 10.0 mg/kg CPP (C) may have unmasked a *depression* effect, consistent with a blockade of both the early and late component potentiation, as well as the prevention of population spike potentiation. Once the drug has been allowed to clear, the re-application of high frequency trains produces what appears to be a normal LTP effect (D). Vertical calibration: 1.0 mV; horizontal

calibration: 10 ms.

Figure 3.1

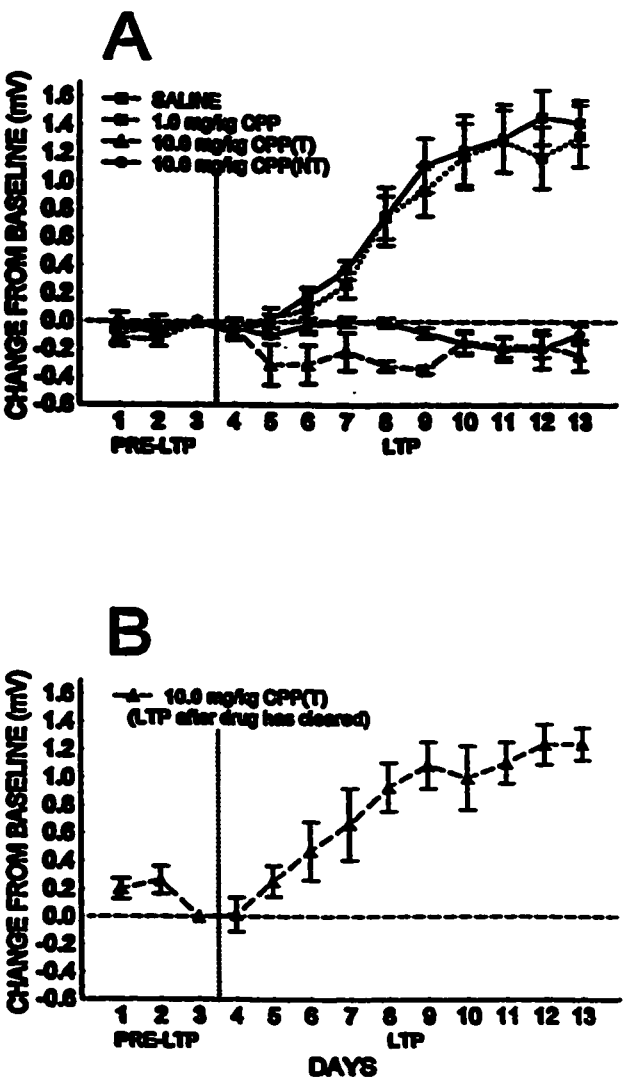
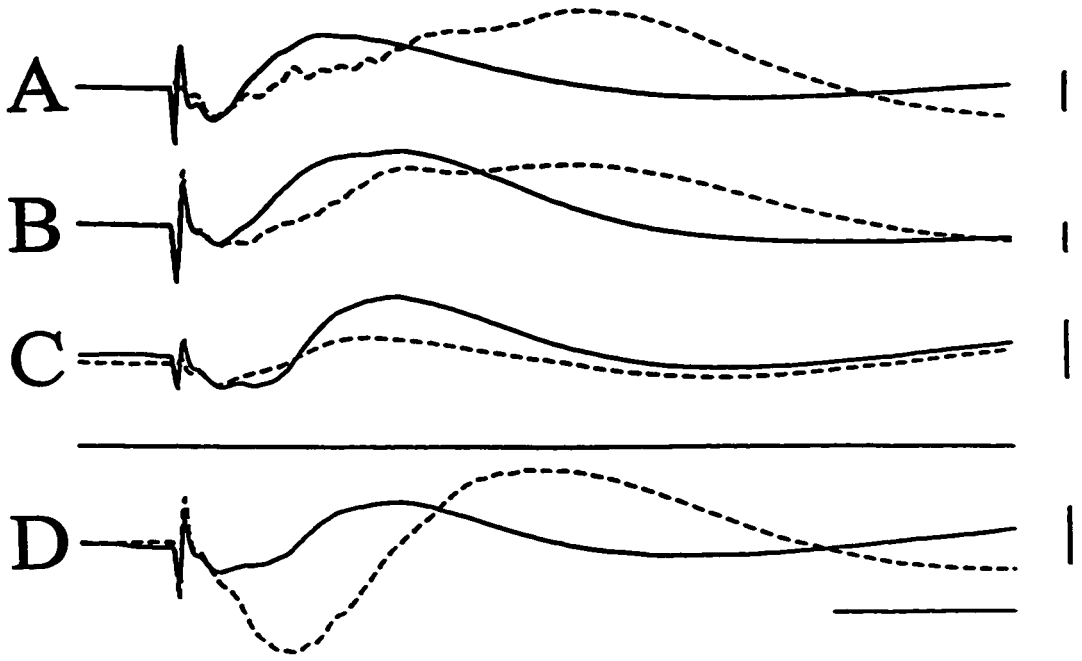

A
SALINE
1.0 mg/kg CPP
10.0 mg/kg CPP(T)
10.0 mg/kg CPP(NT)
CHANGE FROM BASELINE (mV)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
1 2 3 4 5 6 7 8 9 10 11 12 13
PRE-LTP
LTP
B
10.0 mg/kg CPP(T)
(LTP after drug has cleared)
CHANGE FROM BASELINE (mV)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
1 2 3 4 5 6 7 8 9 10 11 12 13
PRE-LTP
LTP
DAYS

Figure 3.2



CHAPTER FOUR

GABAergic MODULATION OF NEOCORTICAL LTP

The inhibitory neurotransmitter γ-aminobutyric acid (GABA) is the primary mediator of inhibition in the neocortex, producing both fast and slow IPSPs (Connors et al., 1988; Benardo, 1994). As in the hippocampus, the chloride-dependent $GABA_A$ response mediates the fast (ligand-gated) IPSP (Connors et al., 1988; McCormick, 1989), while the $GABA_B$ response is responsible for the slow (G-protein coupled) IPSP (Avoli, 1986; Connors et al., 1988). These receptors have also been distinguished on the basis of their bicuculline-sensitivity and insensitivity, respectively.

GABA is known to modulate the excitatory properties of glutamate in the hippocampus. Following drop application of picrotoxin onto the CA1 field of the hippocampus, a population spike is evoked in response to a previously subthreshold test pulse (Wigstrom and Gustafsson, 1983). Diazepam (valium), on the other hand, reduces the amplitude of baseline evoked potentials in hippocampal CA1 (Del Cerro et al., 1992). Moreover, the sustained depolarization that follows a tetanus is believed to be the result of a suppression of GABA-mediated synaptic inhibition. This process is governed by $GABA_B$ autoreceptors which, when activated during high-frequency stimulation, serve to reduce the amount of GABA released per impulse thereby shifting the balance of excitation and inhibition (reviewed briefly in Bliss and Collingridge, 1993).

It is clear that $GABA_A$ receptors also play an important regulatory role in

potentiation phenomena. Douglas et al. (1982) provided one of the first demonstrations that LTP was modulated by inhibitory inputs. These authors recorded field potentials in the dentate gyrus while stimulating the ipsilateral perforant path and contralateral hilus. Contralateral hilar (commissural) stimulation produced a strong inhibition of dentate granule cells which prevented the population spike normally evoked by perforant path stimulation. Douglas et al. (1982) showed, in both acute and chronically-prepared animals, that if high-frequency trains applied to the perforant path are preceded by contralateral hilar stimulation, the inhibition evoked by the latter is sufficient to block the induction of LTP. A maximum 50 msec inter-train interval was used to separate the commissural and perforant path stimuli, and LTP of both the EPSP and population spike was prevented. This blockade was also obtained with ipsilateral hilar stimulation.

Another means of examining local inhibition in the hippocampus is to record in hippocampal CA1 and stimulate the alveus to evoke recurrent inhibition. Using this preparation, Grunze et al. (1996) recorded *in vitro* an IPSP in CA1 pyramidal neurons which showed potentiation following tetanization of the alveus. This LTP was NMDA-dependent and was, additionally, more sensitive to NMDA blockade by APV than the excitatory LTP induced by stimulation of the Schaffer collaterals. These experiments also showed that baseline inhibitory transmission was reduced by APV, indicating the presence of NMDA receptors on inhibitory interneurons, an observation that had been reported previously for the hippocampus (Hablitz and Lanfmoen, 1986).

Tetanic stimulation of the hippocampal stratum radiatum evokes a triphasic depolarization/ hyperpolarization/ late depolarization sequence in CA1 pyramidal

neurons. Recording both extra- and intracellularly *in vitro*, Taira et al. (1997) found that the late depolarization often produced extended spiking. Both the late depolarization and the associated spiking could be specifically and completely blocked by application of the $GABA_A$ antagonist picrotoxin, indicating that GABAergic mechanisms are important for post-tetanic excitation. Activation of GABAergic interneurons likely enhances pyramidal cell excitation, facilitating LTP induction.

Stelzer et al. (1994) made intracellular recordings of orthodromic IPSPs generated in guinea pig hippocampus *in vitro*. Their results showed that $GABA_A$ receptor function was impaired following LTP induction, especially on pyramidal cell apical dendrites. This was reflected both as a reduction in the amplitude of dendritic IPSPs, as well as in synaptic $GABA_A$ conductances in somata and dendrites. Concurrently, interneuron excitatory postsynaptic potentials were found to be increased following LTP induction, an effect that was prevented by perfusion with the NMDA receptor antagonist AP5. The combined effect of these changes is to lower the overall level of GABAergic inhibition, indicating that $GABA_A$-ergic-mediated disinhibition plays an important role in hippocampal LTP.

The disinhibition hypothesis has also been examined in the context of neuropeptidergic regulation. One important route by which GABAergic mechanisms regulate the induction of LTP in the hippocampus is through opioid receptor activation. Opioids are costored with glutamate in several hippocampal pathways, including the perforant path-dentate pathway. Using antagonists against several opioid receptor subtypes, Bramham and Sarvey (1996) showed that endogenous opioids regulate LTP by

modulating GABAergic inhibition. Recordings made of dentate gyrus field potentials revealed that the delivery of high-frequency trains to the lateral perforant path during blockade of either μ- or δ-opioid receptors prevents LTP. However, when slices were pre-treated with picrotoxin, opioid receptor blockade did not prevent LTP induction, indicating that disinhibiting slices through GABAergic blockade eliminates opioid-dependent regulation. These data support the hypothesis that neuropeptides serve to fine tune the modulation of LTP, specifically through inhibitory pathways.

Hippocampal LTP has been shown to be blocked by benzodiazepines (Del Cerro et al., 1992; McNamara et al., 1993) and facilitated during GABAergic antagonism by either picrotoxin or bicuculline (Wigstrom and Gustafsson, 1983, 1985). Recording intracellularly from layer II/III pyramidal cells, Connors et al. (1988) examined the effects of focal membrane picrotoxin application. They found that GABAergic antagonism selectively blocked fast-IPSPs, allowing fuller expression of coincidently active short-latency EPSPs. The unmasking of these EPSPs may be important for neocortical LTP induction *in vitro*, as suggested by the need for GABAergic antagonists in this preparation (Bindman et al., 1988; Kimura et al., 1989; Bear and Kirkwood, 1993; Kirkwood and Bear, 1994).

$GABA_A$ agonists, especially benzodiazepines such as valium, have been shown to interfere with learning and memory task performance, producing a strong anterograde amnesia in both rodents (Venault et al., 1986; Izquierdo et al., 1990) and humans (for review see Lister, 1985), and blocking spatial learning in rats (McNamara et al., 1993). On the other hand, $GABA_A$ antagonists, such as picrotoxin, have been shown to facilitate

memory consolidation (Breen and McGaugh, 1961; Castellano and McGaugh, 1989; Izquierdo and Medina, 1991). These data indicate that GABAergic neurotransmission is likely to be an important component in learning and memory processes.

The role of GABAergic mechanisms in the induction of neocortical LTP in the freely moving animal is unknown. The following experiments were designed to assess the role of $GABA_A$-ergic inhibition in the induction of neocortical LTP.

MATERIALS AND METHODS

Surgery, baseline measures and induction of neocortical LTP

Twenty-nine male Long-Evans hooded rats from the McMaster University Breeding Colonies were used in these experiments. At the time of surgery, the animals weighed 300-400 g. All animals were implanted with recording and stimulating electrodes and had baseline measures taken. LTP-inducing stimulation sessions were delivered daily and consisted of sixty 8-pulse trains (pulse intensity: 1259 μA).

EEG activity was monitored following the administration of picrotoxin, and during train delivery, to ensure that epileptiform discharges were not triggered.

GABAergic agonism and antagonism

The GABAergic agonist diazepam (valium) (5.0 mg/kg i.p., Sabex Inc., Canada) and antagonist picrotoxin (3.0 mg/kg i.p. in dH_2O, Sigma Chemical Co., USA) were administered in conjunction with high-frequency trains as follows: On the first day, animals were injected with valium (n=6), picrotoxin (n=5) or saline (n=7), followed 4 minutes later by the delivery of 60 high-frequency trains. On subsequent days the injection was preceded by an I/O test and followed 4 minutes later by 60 high frequency trains. Additionally, a fourth (n=5) and fifth (n=5) group received daily 5.0 mg/kg valium or 3.0 mg/kg picrotoxin injections, respectively, immediately following an I/O test, but received no trains. These daily LTP induction regimens were continued for 10 days. Following a one week "wash-out" period, 60 trains/day were again delivered for 10 days

to animals that had received either valium and high-frequency trains or valium alone. This was done to demonstrate that any suppression of LTP by repeated valium treatments was reversible. I/O measures were collected every day during the induction protocol as described above.

Analyses

Changes in the field potentials over LTP and decay sessions for both experimental and control animals were measured by subtracting the final baseline responses from all other baseline and potentiated responses. Population spikes were measured from the largest amplitude response in the I/O tests before and after LTP induction. Repeated measures ANOVAs, comparing all induction days, were calculated for the field measures, while a population spike ANOVA compared the third baseline and final LTP induction days.

Following collection of all electrophysiological measures, animals were perfused and the brains were sliced and stained with Cresyl Violet to verify electrode placements.

RESULTS

Histology

The stimulating electrode was found to span the forceps minor corpus callosum in all animals. Localization of the recording electrode placements was completed in 83% (24/29) of animals. Damage caused to the brains during the histological procedures in the remaining animals precluded a precise determination of electrode placement. In all cases, the ventral tips of the recording electrodes were placed in parietal area 1 or the medial-posterior aspect of frontal area 3 (Paxinos and Watson, 1986).

Response morphologies

Valium reduced the amplitude of all components of the evoked responses and depressed the population spikes. This effect was clear both in control and potentiated animals. The polysynaptic components appeared most vulnerable to this effect as they were completely eliminated in some animals and substantially reduced in all the others. All components had recovered completely 24 h following the valium injections. By contrast, picrotoxin did not appear to have any effect on normal synaptic transmission, though it did appear to increase the amplitude of, and broaden, the *train*-evoked response.

Behaviour

Animals injected with valium were sluggish for approximately 30-60 min. following the injection, struggled weakly when picked up and rarely vocalized. Twenty-

fours hours after the injections (immediately preceding the I/Os), rats appeared normal when handled. Animals groomed normally and gained weight throughout the experiment and were active and healthy 24 hours following the cessation of the valium injection protocol. Animals injected with picrotoxin did not differ from control animals at any time during the experiment and remained active and healthy throughout.

The effect of GABAergic agonism and antagonism on LTP induction

Figure 4.1 shows changes in the response amplitudes of the early and late components for animals receiving high-frequency trains in conjunction with saline (SAL/T), 5.0 mg/kg valium (DZM/T) or 3.0 mg/kg picrotoxin (PTX/T), as well as for animals that received either 5.0 mg/kg valium (DZM) or 3.0 mg/kg picrotoxin (PTX) alone. There was a significant interaction effect between session and group for the early component ($F(48,288)=1.87$, $p<0.001$). SAL/T animals showed a robust early component potentiation that reached asymptote on day 8 of the induction regimen. The PTX/T group also showed an early component amplitude change, though it was smaller than that displayed by the SAL/T group and reached asymptote by day 5. The early component of the DZM/T, DZM or PTX groups did not appear to potentiate and a small amplitude shift, similar to the downward drift in amplitude evidenced by control animals (see Figure 2.3), was displayed by all 3 of these groups. The changes in the early component were reflected in the population spike changes, which also showed a significant interaction between session (pre- vs. post-LTP) and group ($F(4,24)=4.76$, $p<0.01$). Both SAL/T and PTX/T animals showed a large increase in population spike amplitude, though the effect

was larger for the SAL/T group. The other 3 groups, however, did not show any significant change from baseline and, in fact, the PTX animals showed a small *decrease* in population spike height by the end of the induction regimen. As in previous experiments, the increases in population spike amplitude for the SAL/T and PTX/T groups suggest that the underlying EPSPs are also potentiated. Similarly, the small decrease in population spike height in the PTX group indicates that the repeated administration of picrotoxin may slightly depress the EPSPs underlying the early component. Figure 4.2 shows changes in the population spike heights for all groups pre- and post-LTP. Figure 4.3 shows representative pre- and post-LTP sweeps from animals in all groups. It is clear that the SAL/T animals showed greater early component and population spike potentiation than the other groups (4.3(a)), followed by the PTX/T animals (4.3(b)). As shown in Figure 4.3, the drop in the early component amplitude of both the DZM/T (4.3(c)) and DZM (4.3(e)) animals appears to be a true depression. For example, we never saw an early component response *reversal* during the valium protocols, though this is a fairly common occurrence in our neocortical LTP studies. Further, we did not see significant population spike enhancement following these manipulations. It is clear by examination of the representative sweep (4.3(d)) that administration of picrotoxin alone had no effect on the polysynaptic response component(s).

Similar group differences were expressed by the late component which showed a significant interaction between group and session ($F(48,288)=11.88$, $p<0.001$). Both SAL/T and PTX/T animals showed robust late component potentiation (Figures 4.1 and

4.3(a, b)). This potentiation was larger and developed more rapidly in the SAL/T group, which reached asymptote following 8 days of trains. The PTX/T group, in contrast, had not yet reached asymptotic levels during the 10 day induction period. Although the PTX/T group potentiated more slowly, the potentiation evidenced by these two groups was nearly equivalent by the end of the induction regimen. By contrast, the PTX and DZM animals showed no late component potentiation. The DZM/T animals did not potentiate during the first half of the induction period, and then showed a weak and variable (3/6 animals) potentiation during the last half of the induction period. Figure 4.3 shows representative sweeps for each experimental condition before and after LTP induction.

The average response thresholds increased following trains for all groups. The average pulse intensities required to evoke a minimal response prior to potentiation were 19.2, 34.1 and 26.7 μA for the PTX/T, SAL/T and DZM/T groups, respectively. These thresholds increased to 50.6, 68.3 and 37.1 μA, respectively following LTP induction. While the PTX group showed no change over the course of the drug regimen, the DZM group showed a moderate change, increasing from 28.6 μA to 38.0 μA by the end of the drug protocol. These changes are typical of our neocortical LTP experiments (see, for example, chapter 2, experiment 1).

Figure 4.4 shows that the administration of high-frequency trains one week following the initial induction regimen to either the DZM/T or DZM animals resulted in early and late component potentiation of both groups. There was a significant interaction between session and group for the early component ($F(12,108)=2.90$, $p<0.001$). The

potentiation of the early component, however, appeared to be slowed in the DZM/T group, and achieved only half the magnitude of the DZM group. The early component of the DZM/T group reached asymptote following the fifth stimulation session, while the DZM group showed continual increments in potentiation throughout the induction protocol. The differences in early component potentiation were reflected in the population spike measures (Figure 4.5) which showed a significant interaction between session and group ($F(1,9)=9.01$, $p<0.01$). These differences were also apparent by examination of representative pre- and post-LTP sweeps from the two conditions following the one week "wash-out" period (Figure 4.6), where it could be seen that the DZM group showed considerably more population spike potentiation than did the DZM/T group.

The late component also showed a significant interaction effect between session and group ($F(12,108)=2.41$, $p<0.01$). For this component, however, there was a clear cross-over effect as the DZM/T group, which initially potentiated more rapidly than the DZM group, reached asymptote by the fourth stimulation session, while the late component amplitude of the DZM group continued to increase until the seventh day, overtaking the DZM/T group on day 6. As was the case for the early component, the pattern of potentiation in the DZM/T group suggested the presence of some form of suppression, while the DZM group appeared to potentiate normally. Representative sweeps (Figure 4.6) showing pre- and post-LTP time-points revealed the morphological similarity in the late components of both groups, with no obvious abnormalities in either.

DISCUSSION

Previous research has demonstrated that long-term potentiation may be modulated by $GABA_A$-ergic ligands. Whereas benzodiazepenes such as diazepam have been shown to block LTP in both the hippocampus and pyriform cortex (Del Cerro et al., 1992), $GABA_A$-ergic antagonists facilitate the induction of hippocampal LTP (Wigstrom and Gustafsson, 1983; Abraham et al., 1987). Moreover, there is considerable evidence that these same agents are capable of regulating memory. GABAergic agonists have been shown to impair retention of both maze learning (Tomaz et al., 1982) and inhibitory avoidance (Decker et al., 1990) in rodents, and compromise declarative (episodic) task acquisition in humans (Lister, 1985), while GABAergic antagonists such as picrotoxin and bicuculline have been shown to enhance acquisition and retention for a variety of tasks in rodents (Breen and McGaugh, 1961; Brioni and McGaugh, 1988; Castellano and McGaugh, 1989). The present experiment shows that neocortical LTP is blocked by valium administered prior to the application of tetanizing stimulation, and so is in agreement with the hippocampal LTP data. In contrast to previous reports (e.g.: Wigstrom and Gustafsson, 1983, 1985; Abraham et al., 1987), however, we show that picrotoxin *retards* the development of neocortical LTP. In interpreting this result it is important to note that previous experiments employed only the *in vitro* slice preparation. Several features of LTP *in vitro* have been shown to differ from that induced *in vivo*, not the least of which being that potentiation of excitatory inputs to inhibitory interneurons has been demonstrated *in vivo* (Buzsaki and Eidelberg, 1982; Kairiss et al., 1987) but has

not been shown *in vitro* (Abraham et al., 1987). It may well be the case that the slowed potentiation resulting from the administration of picrotoxin was the product of competition between excitatory and inhibitory pathways or synapses, both of which were potentiating and vying for expression. Another possibility is that the continual suppression of inhibition by picrotoxin resulted in the inhibitory interneurons becoming more excitable as a result of some form of compensatory mechanism. It seems unlikely that such an effect would completely counter the picrotoxin-induced suppression of inhibition, but it might play a contributing role.

There is some behavioural evidence suggesting that GABA *antagonism* may impair learning. Recent work has demonstrated that post-training injection of subthreshold doses of picrotoxin into the striatum induces a retrograde amnesia in inhibitory avoidance tasks (Chavez et al., 1995), and Nabeshima et al. (1988) provided evidence that administration of either picrotoxin or bicuculline immediately post-training attenuates both passive avoidance and conditioned suppression learning in mice. These data and others (e.g.: Kornhuber, 1973; Kim and Routtenberg, 1976) indicate that under some circumstances, antagonism of the GABAergic system may compromise learning and memory processes.

When we re-potentiated animals that had been exposed to valium and high-frequency stimulation (DZM/T), and potentiated for the first time animals that had been exposed to valium alone (DZM), we found that the previously stimulated animals showed a weaker potentiation effect. This was manifested as a slower early component potentiation, and a lower asymptote of the late component (60% of that achieved by DZM

animals). The normal rate and level of LTP induction in the DZM group indicates that the drug regimen by itself did not interfere with the ability to support potentiation. One possibility is that the delivery of trains in the presence of valium resulted in the preferential potentiation of the inhibitory GABAergic circuitry. The subsequent induction of excitatory LTP may then have been suppressed by this enhanced inhibition. Alternatively, some form of long-term *depression* of the excitatory synapses may have been induced during the initial exposure to high-frequency stimulation and valium which then inhibited the expression of LTP during the second phase of the experiment. In support of this, Hirsch and Crepel (1991) found that blockade of NMDA receptor activation by APV unmasked a long-term depression in 87% (14/16) of prefrontal cortex neurons tested. The reduction in the early component amplitude of DZM/T animals in the absence of population spike enhancement (Fig 4.3c) is consistent with a possible depression.

Both the weaker LTP effect obtained by the picrotoxin and train regimen, as well as the retarded potentiation expressed by the DZM/T animals during the second stimulation protocol, raise the possibility that the inhibitory circuits themselves may have potentiated. A number of authors have examined the role of inhibitory processes in hippocampal LTP (e.g.: Kano et al., 1992; Tomasulo and Steward, 1996). Buzsaki and Eidelberg (1982) made extracellular unit recordings of physiologically-identified basket cells and found both that these interneurons were directly excited by their inputs (therefore permitting feedforward inhibition of pyramidal cells) and that the majority tested (8/11) showed increased probabilities of cell discharge for up to 2 h following a

LTP-inducing tetanus. Similarly, Taube and Schwartzkroin (1987) recorded the intracellular activities of inhibitory interneurons in the CA1 region of the rat hippocampus before and after LTP induction. They found that 9/12 cells showed a 110-150% or greater enhancement of the evoked EPSP following high-frequency stimulation of the stratum radiatum. These authors assert that a direct potentiation of the interneurons, rather than one mediated by recurrent pyramidal cell collaterals, is likely since stratum radiatum stimulation evoked interneuron discharges several milliseconds in advance of pyramidal cell activation.

Direct evidence for the long-term potentiation of inhibitory synapses was provided by Komatsu and Iwakiri (1993) who showed that repetitive stimulation of an inhibitory input to layer V cells in visual cortex results in an input-specific LTP of the evoked IPSPs. Komatsu (1994) also demonstrated that this effect is more robust in young animals than in adults. Thus, there is strong evidence that interneurons have the capacity to support synaptic plasticity similar to that seen in pyramidal cells. Moreover, Buzsaki and Eidelberg (1982) have shown that interneurons are capable of showing potentiation following levels of activation that are subthreshold for pyramidal cells, and so may be able to support potentiation even during a valium-induced reduction of excitation.

If our protocol did enhance the inhibitory circuitry present in this system, it may be that the enhanced inhibition resulting from the initial potentiation was acting at odds with the expression of subsequent LTP at excitatory synapses. This would explain the delay in the development of, as well as the reduction in, early component potentiation. Tomasulo et al. (1991) observed that the induction of LTP in the presence of $GABA_A$-

ergic blockade caused a reduction of evoked population spike amplitude in the dentate gyrus. These authors hold that this decrease is the result of an increase in feed-forward inhibition following LTP induction. Concurrently, however, we saw an initially faster (though reduced level of) potentiation of the late component in the DZM/T group. This may be mediated by the enhancement of inhibitory connections to interneurons in this region. If this were the case, the overall level of inhibition would be *reduced* allowing potentiation to initially proceed more rapidly. Although the early and late component changes appear at odds, two factors must be considered: first, the early (monosynaptic) and late (polysynaptic) components must be generated at different regions (laminae) of the cortex and second, inhibition is mediated, with different outcomes, by both $GABA_A$ and $GABA_B$ receptors whose regional distributions are different. While the $GABA_A$ receptor is concentrated most heavily in layers II/III of motor, and layers II-IV of primary sensory, cortex (Zilles and Wree, 1995), $GABA_B$ receptor binding sites are most heavily concentrated in layers I-III in parietal regions (Chu et al., 1990). Therefore, the apparently contradictory impact of LTP induction may be explained by potentiation having different effects at discrete regions of the cortex. In this context, it is important to note that the $GABA_B$ receptor has been shown to regulate the induction of hippocampal LTP (Davies et al., 1991). The activation of $GABA_B$ autoreceptors on GABAergic terminals is known to reduce the magnitude of IPSPs by inhibiting the release of GABA (Davies and Collingridge, 1993; Mott et al., 1993). One component of $GABA_B$ activation is, therefore, disinhibitory and results in net excitation.

These data provide further support for the role of the GABAergic system in the

regulation of LTP in the mammalian brain, and are the first demonstration that the GABAergic system modulates neocortical LTP in the freely-moving animal. However, the complex effects we obtained make it unlikely that the results of the GABAergic manipulations reported here are all the product of direct actions resulting from the application of valium or picrotoxin. A number of neuromodulatory systems have been shown to affect both potentiation and memory phenomena, and to interact with GABAergic transmission. One of these neuromodulators is acetylcholine (ACh). GABA has been shown to affect cholinergic transmission in a number of ways including reducing ACh turnover (Wood, 1986), and regulating the release of ACh from presynaptic terminals (Decker and McGaugh, 1991). It is of interest, therefore, to examine the role of neuromodulators such as ACh in an attempt to converge on common features underlying LTP and memory. Chapter five examines the role of the cholinergic system in the induction and decay of neocortical LTP.

FIGURE CAPTIONS

Figure 4.1. The effects of GABAergic agonism and antagonism on the induction of long-term potentiation. The mV differences between the last baseline and all other sweeps are plotted in this figure for the baseline (pre-LTP) and LTP induction (LTP) periods. **Top:** Changes in the early component for groups receiving either saline, picrotoxin or valium and trains, or picrotoxin or valium alone. While animals that were administered either picrotoxin or saline in conjunction with high-frequency stimulation showed a strong surface positive shift (indicated as a negative mV shift from baseline), all other groups show only the downward drift indicative of control animals. **Bottom:** Changes in the late component over days. The groups receiving either saline or picrotoxin and trains showed marked potentiation, although the potentiation evidenced by the picrotoxin group was slower to develop. The group curve for animals that received valium and trains make it clear that $GABA_A$ agonism blocks the development of late component potentiation.

Figure 4.2. Total population spike height measures are shown for time-points prior to LTP induction (pre-LTP) and 24 h following the induction protocols (post-LTP). Animals that received either saline or picrotoxin and LTP-inducing stimulation showed population spike enhancements, while animals in all other groups showed no change. Note that, in agreement with the early component effects, picrotoxin appears to have *inhibited* the development of population spike potentiation.

Figure 4.3. Representative sweeps taken pre- and post-LTP induction for groups that received saline (A), picrotoxin (B) or valium (C) and high-frequency stimulation, or picrotoxin (D) or valium (E) alone. The groups that received saline or picrotoxin in conjunction with trains showed robust early and late component potentiation and clear population spike potentiation. By contrast, animals that received picrotoxin alone showed no change, while the 2 valium groups showed small reductions in early component amplitude which, in conjunction with a blockade of both any late component effects or population spike enhancement, is suggestive of a *depression* effect like that seen following the administration of the NMDA antagonist CPP (see Figure 3.2). Vertical calibration: 1.0 mV; horizontal calibration: 10 ms.

Figure 4.4. Potentiation following the repeated administration of the GABAergic agonist valium. Following a one week "washout" period after the drug has been allowed to clear, animals that received either valium and trains, or valium alone, all showed clear potentiation effects. The mV differences between the last baseline and all other sweeps are plotted in this figure for the baseline (pre-LTP) and LTP induction (LTP) periods. **Top:** Changes in the early component. Note that animals in which LTP had been previously blocked now show an early component effect that asymptotes at approximately half the magnitude of animals that had received valium in the absence of trains. **Bottom:** Changes in the late component. As seen for the early component, the late component potentiation of animals that received valium and trains appeared to asymptote at approximately half the magnitude of the group that received valium alone.

The latter group, by contrast, achieved normal levels of both early and late component potentiation.

Figure 4.5. Population spike measures for animals in the valium conditions following a 1 week "washout" period. Total population spike height measures are shown for time-points prior to LTP induction (pre-LTP) and 24 h following the induction protocols (post-LTP). Although both groups showed population spike enhancement following the LTP regimen, animals that initially received valium in the absence of high-frequency stimulation show considerably greater potentiation.

Figure 4.6. The differences in the magnitude of the potentiation effects expressed by the 2 valium conditions following the 1 week "washout" period are made clear by these representative sweeps contrasting baseline (solid) and fully potentiated (dashed) responses for the same animals. **A:** An animal that initially received valium alone. In addition to a large late component potentiation effect, considerable population spike enhancement was seen following 10 days of high-frequency trains. **B:** By comparison, this animal that initially received both diazepam and trains showed a less defined late component potentiation effect and less population spike potentiation. Vertical calibration: 1.0 mV; horizontal calibration: 10 ms.

Figure 4.1

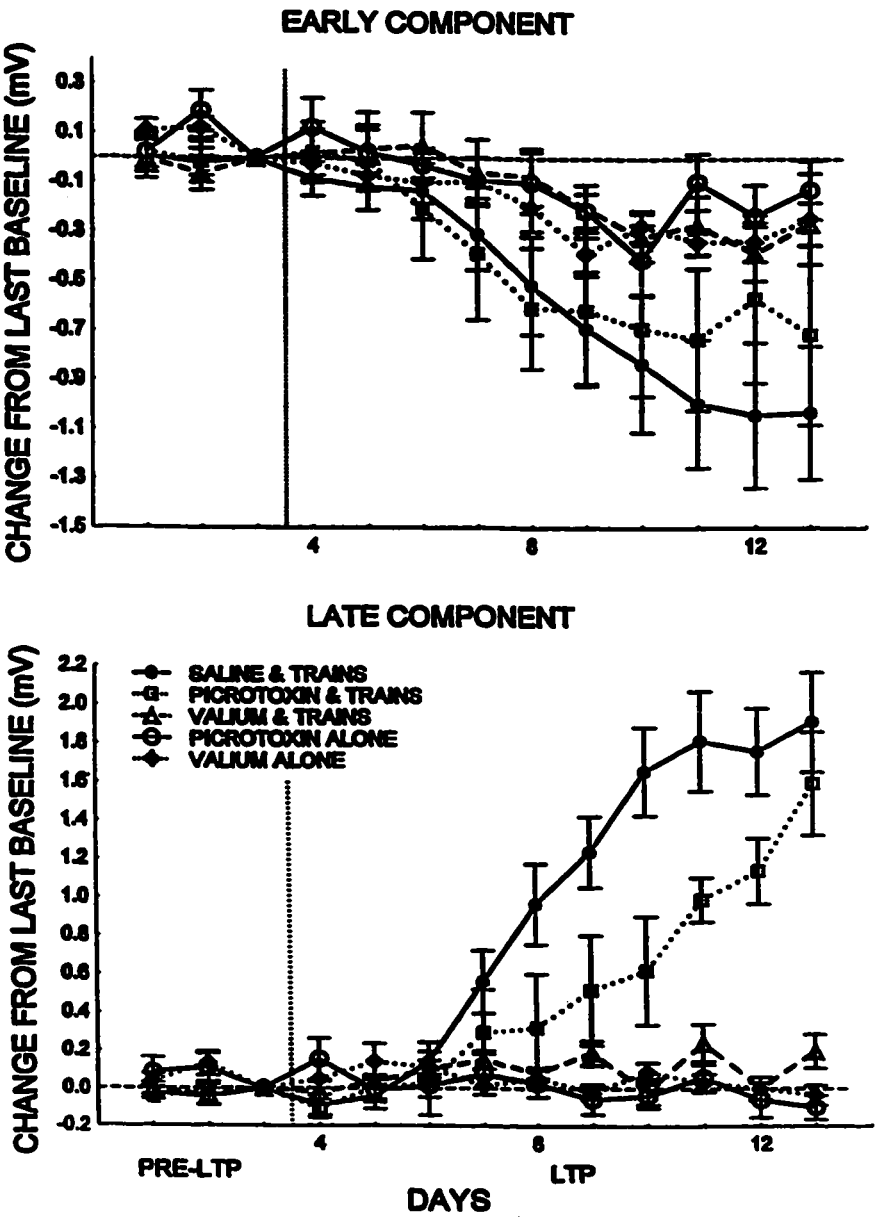

EARLY COMPONENT
CHANGE FROM LAST BASELINE (mV)
0.3
0.1
-0.1
-0.3
-0.5
-0.7
-0.9
-1.1
-1.3
-1.5
4
8
12
LATE COMPONENT
CHANGE FROM LAST BASELINE (mV)
SALINE & TRAINS
PICROTOXIN & TRAINS
VALIUM & TRAINS
PICROTOXIN ALONE
VALIUM ALONE
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
4
8
12
PRE-LTP
LTP
DAYS

Figure 4.2

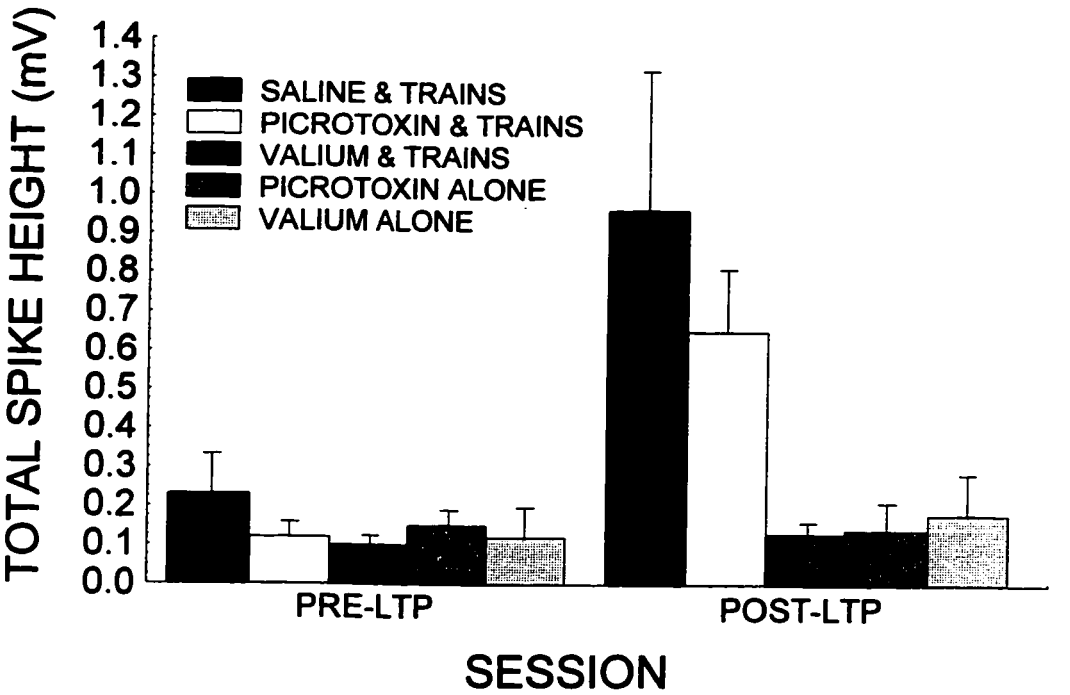

TOTAL SPIKE HEIGHT (mV)
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
SALINE & TRAINS
PICROTOXIN & TRAINS
VALIUM & TRAINS
PICROTOXIN ALONE
VALIUM ALONE
PRE-LTP
POST-LTP
SESSION

Figure 4.3

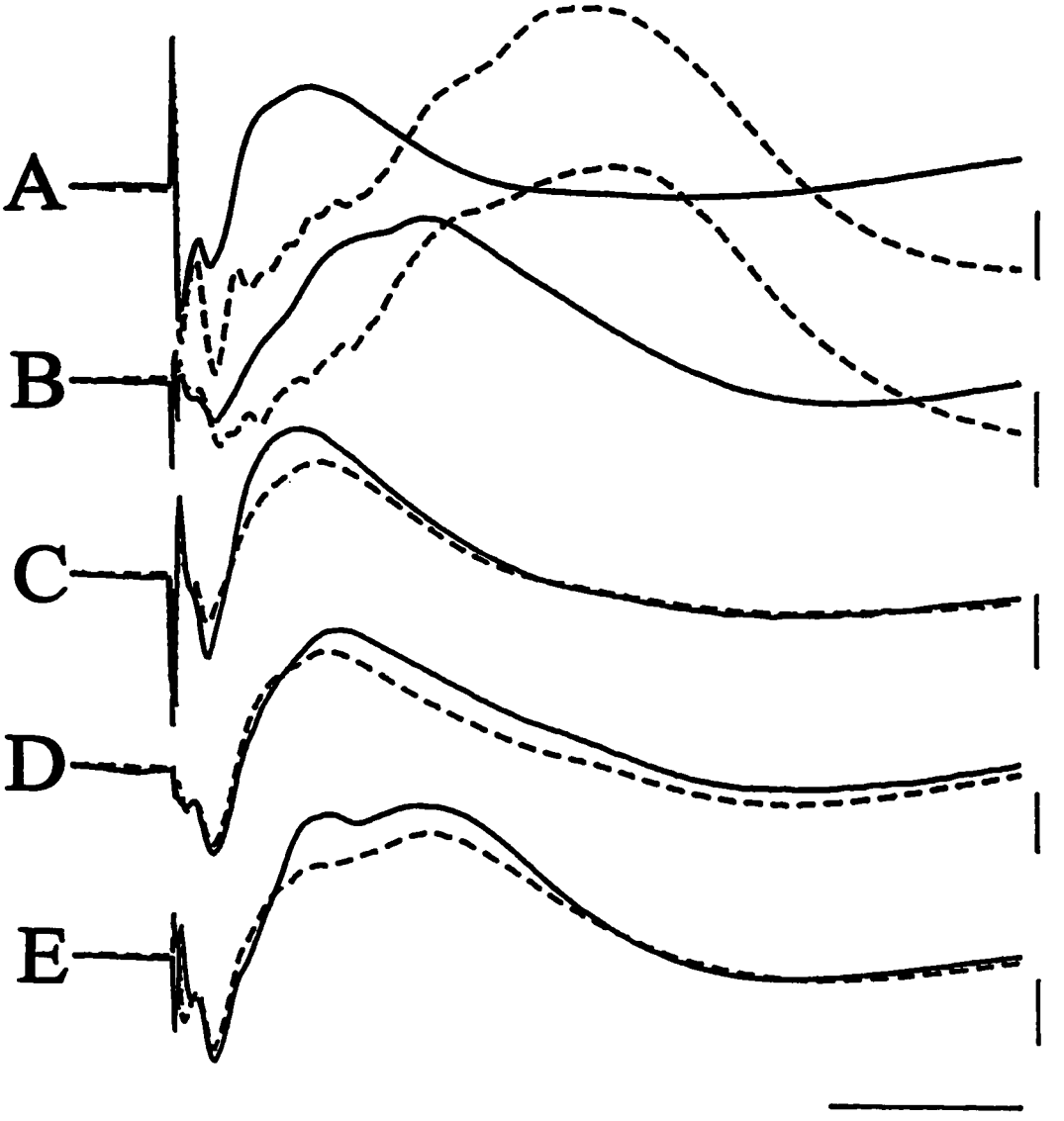


Figure 4.4

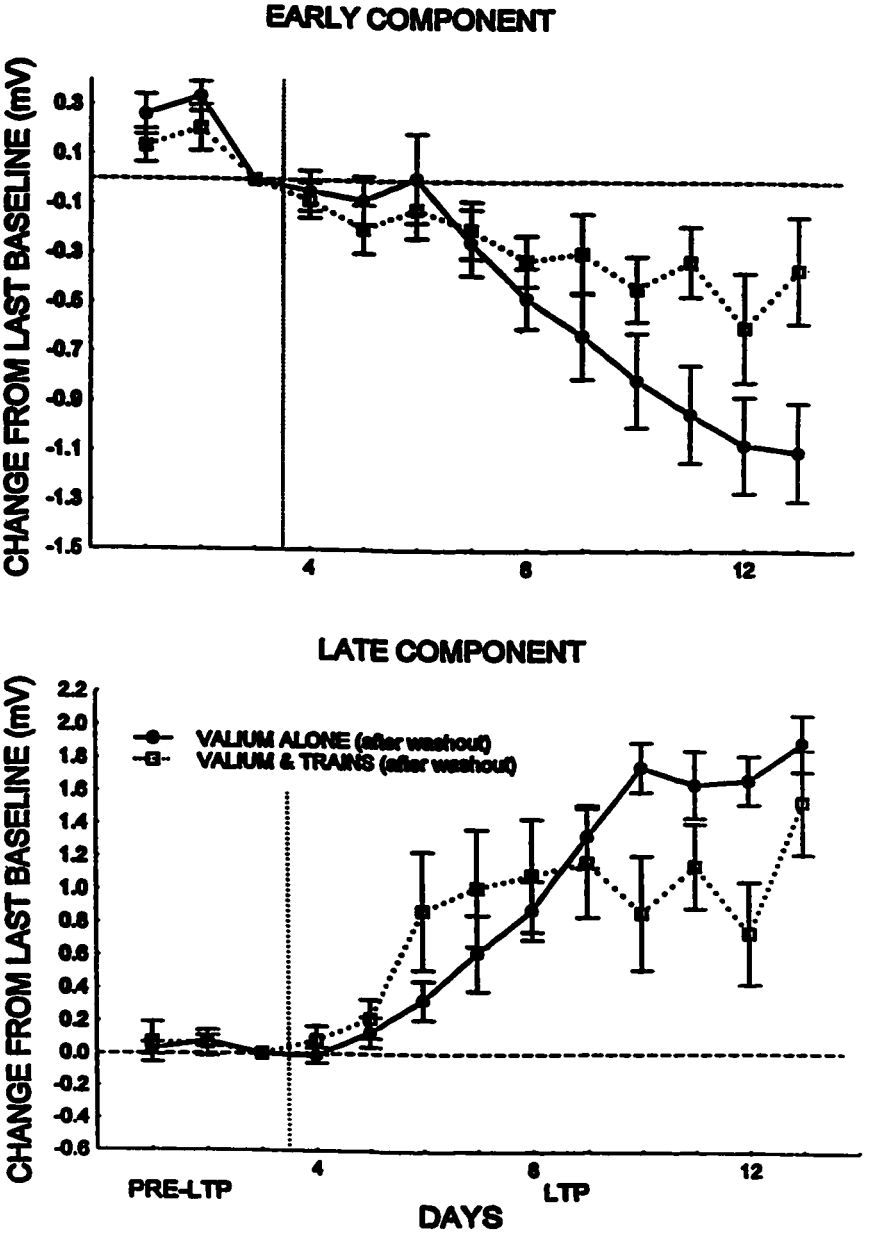

EARLY COMPONENT
CHANGE FROM LAST BASELINE (mV)
0.3
0.1
-0.1
-0.3
-0.5
-0.7
-0.9
-1.1
-1.3
-1.5
4
8
12
LATE COMPONENT
CHANGE FROM LAST BASELINE (mV)
VALIUM ALONE (after washout)
VALIUM & TRAINS (after washout)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
4
8
12
PRE-LTP
LTP
DAYS

Figure 4.5

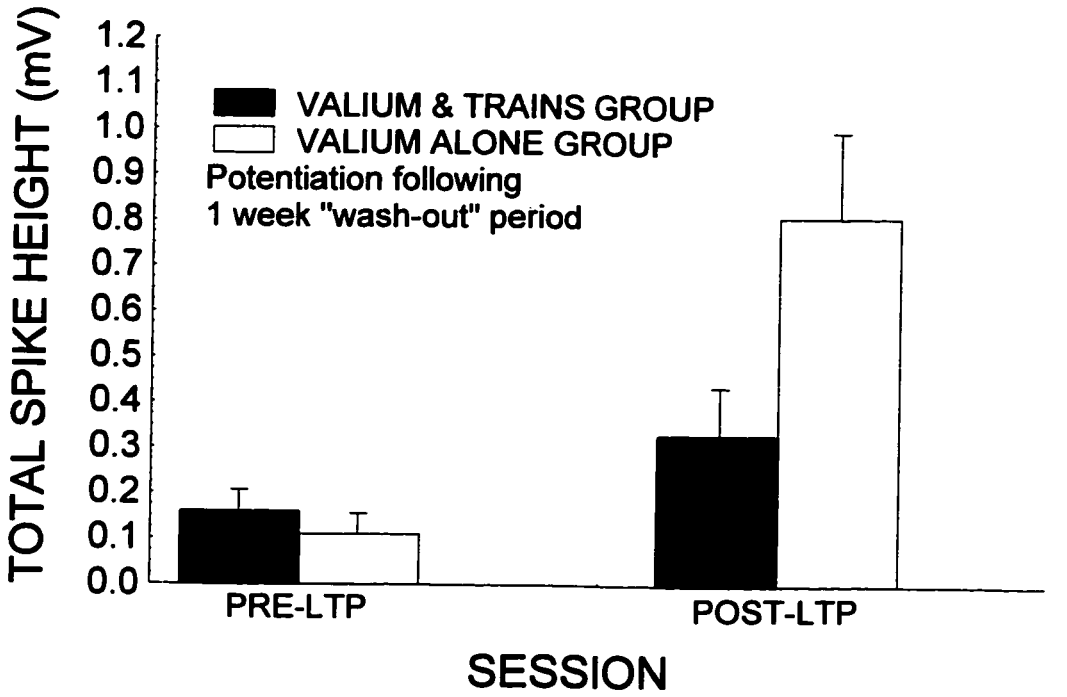
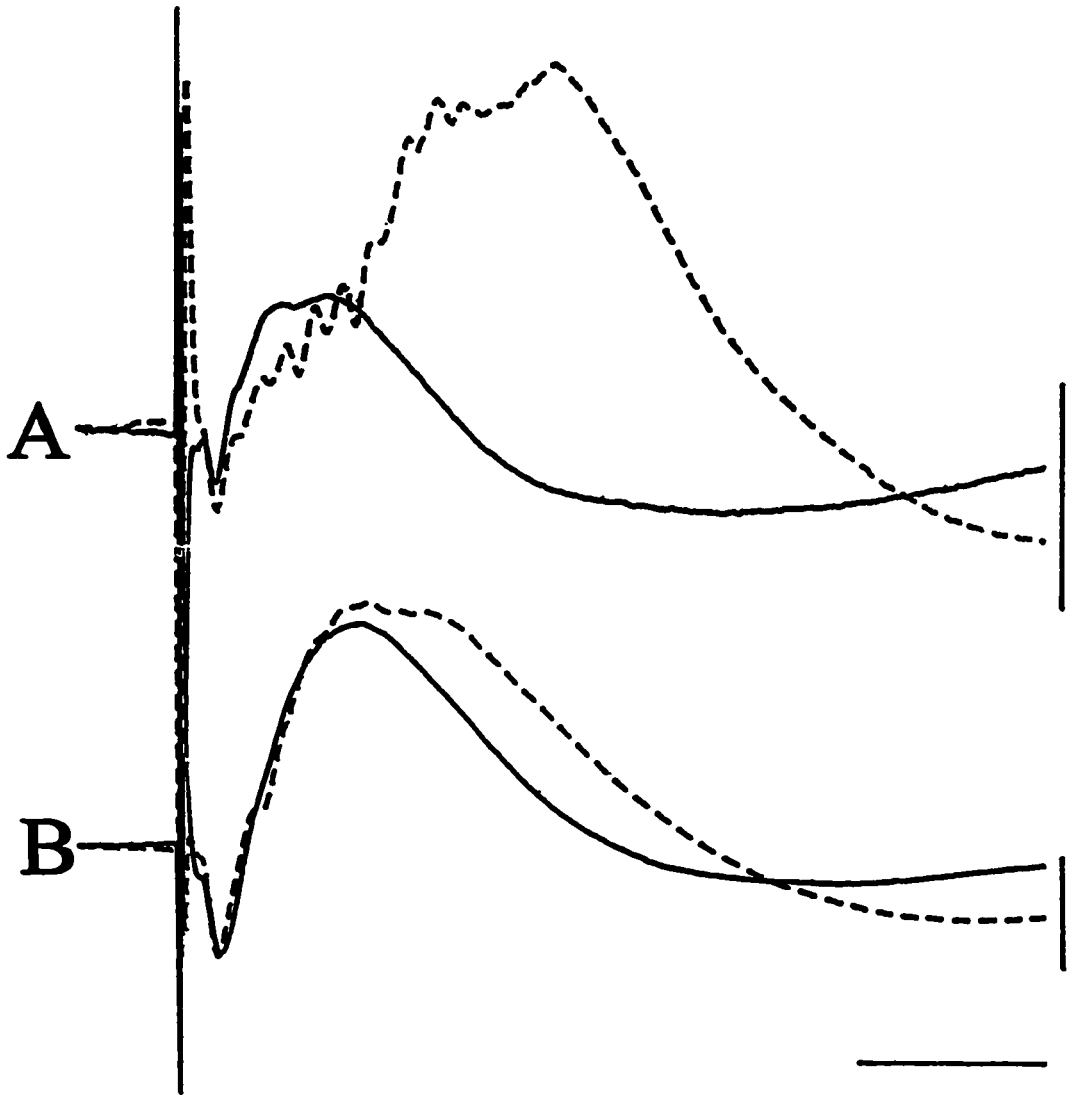

TOTAL SPIKE HEIGHT (mV)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
VALIUM & TRAINS GROUP
VALIUM ALONE GROUP
Potentiation following
1 week "wash-out" period
PRE-LTP
POST-LTP
SESSION

Figure 4.6



CHAPTER FIVE

CHOLINERGIC MODULATION OF NEOCORTICAL LTP

It has been suggested that the slow development of neocortical LTP reflects an adaptive memory mechanism designed to minimize interference between input patterns. Another explanation for the apparent resistance of the neocortex to LTP induction might be a dependence on the co-activation of neuromodulatory systems such as acetylcholine (Brocher et al., 1992; Racine et al., 1994b). Neuromodulators are ligands which do not appear to be involved in the direct transfer of information at synaptic sites but rather influence the likelihood that these sites will respond to other inputs. Neurotransmission, according to Hasselmo (1995), involves activation of receptors on a protein structure directly incorporating an ion channel, while neuromodulation comprises activation of receptors coupled indirectly to ion channels or to the cell nucleus, such as through G-protein or second messenger pathways. Neuromodulators are slower, longer lasting, and more spatially diffuse than neurotransmitters. It is possible that the stimulation sites and stimulation paradigms used in neocortical LTP experiments do not provide an adequate activation of the modulatory systems.

Acetylcholine (ACh) exerts its effects in the CNS through either the muscarinic or nicotinic receptors (Greenamyre and Maragos, 1993). Nicotinic receptors are members of the receptor-gated ion channel superfamily while muscarinic receptors are G-protein coupled receptors (Hosey, 1992). Although both types are found in the cerebral cortex,

the muscarinic receptor predominates (Giacobini, 1990; Greenamyre and Maragos, 1993). The neocortex receives most of its cholinergic input from the basal forebrain (McKinney et al., 1983; Zilles et al., 1991) although there is also a population of cholinergic interneurons present in rat neocortex, concentrated mainly in layers II/III (Eckenstein and Baughman, 1984). There are prominent interlaminar differences in the distributions of the cholinergic receptor subtypes (reviewed in Zilles and Wree, 1995). In motor (Fr1) and sensory (Par1) cortices, for example, the M1 muscarinic receptor subtype shows the highest density in layers II/III while the M2 receptor subtype is densest in layer V of the motor area and layer IV in primary sensory areas. Functionally, M1 and M2 receptors have been shown to be related to K^+ permeability and conductance, respectively.

There is considerable evidence to suggest that the cholinergic system plays an important role in learning and memory processes (for review see Hagan and Morris, 1989). The cognitive deficits associated with Alzheimer's disease (Fibiger, 1991; Greenamyre and Maragos, 1993; Muir, 1997) as well as normal aging (Giacobini, 1990) are both believed to be mediated in large part by reduction of cholinergic function. It has been established in rodents that while cholinergic antagonism with drugs such as scopolamine or atropine blocks discrimination learning (Whitehouse, 1964), inhibitory avoidance learning (Buresova et al., 1964; Meyers, 1965; Berger and Stein, 1969), active avoidance learning (Flood et al., 1981; Flood and Cherkin, 1986) and maze learning (Stevens, 1981; Watts et al., 1981; Lamberty and Gower, 1991), cholinergic agonism with physostigmine and other ligands facilitates both discrimination learning (Whitehouse, 1966) and passive avoidance retention (Haroutunian et al, 1985), and reverses the spatial

learning deficits resulting from administration of scopolamine (Lamberty and Gower, 1991). Studies using human subjects have shown that cholinergic blockade compromises acquisition in declarative memory tasks as measured by recall (Drachman and Leavitt, 1974; Flicker et al., 1990), while non-human primates have been shown to be impaired in several delayed non-matching to sample tasks following the administration of atropine (Penetar and McDonough, 1983) or scopolamine (Bartus and Johnson, 1976; Aigner et al., 1991; Hudzik and Wenger, 1993).

Cholinergic stimulation has been shown *in vitro* to enhance long-term potentiation in both the CA1 field (Blitzer et al., 1990) and dentate gyrus (Burgard and Sarvey, 1990) of the hippocampus as well as in the visual cortex (Brocher et al., 1992). Moreover, cholinergic agents applied *in vitro* to either the hippocampus (Auerbach and Segal, 1994) or the sensorimotor cortex (Lin and Phillis, 1991) have been shown, in the absence of high-frequency or other LTP-inducing stimulation, to produce long-lasting increases in the magnitude of the evoked field responses and to increase the frequency and duration of evoked neuronal discharges. In the hippocampus, this cholinergically-induced LTP has been shown to occlude subsequent LTP induction by electrical stimulation (Auerbach and Segal, 1994), suggesting that these two forms of LTP share a common substrate.

The facilitatory action of ACh on the induction of LTP is most likely the result of enhanced postsynaptic depolarization, permitting greater, or more probable, activation of NMDA receptors. In support of this hypothesis, ACh agonism is known to reduce potassium conductances in both the hippocampus (Bernardo and Prince, 1982; Cole and Nicoll, 1983) and neocortex (McCormick and Prince, 1986), and ACh has been shown to

suppress neuronal adaptation responses (Barkai and Hasselmo, 1994). The strongest evidence, however, has been provided by Markram and Segal (1990a; 1990b) who have demonstrated that iontophoretically-applied ACh can augment NMDA receptor-mediated synaptic responses, possibly by enhancing membrane Ca^{2+} conductances or second messenger systems, such as IP_3, which regulate intracellular Ca^{2+}. Metherate et al. (1987) found in cat somatosensory cortex that iontophoretically administered ACh altered neuronal responsiveness in only 21% (13/61) of the cells recorded from when applied alone. The proportion increased to 75% (66/88), however, when ACh was applied in conjunction with glutamate or tactile stimulation (skin indentation) of the cells' receptive fields. These authors concluded that a primary role of ACh is to enhance the reactivity of previously less effective synaptic connections and, perhaps, to promote the reorganization of somatotopic maps.

It is clear that acetylcholine plays an important role in modulating both learning and memory phenomena as well as long-term potentiation. Cholinergic stimulation has been shown to promote a long-term depression effect in the neocortex of freely-moving animals when standard LTP induction protocols are used (Racine et al., 1994b). The role of cholinergic neuromodulation in the induction and decay of neocortical LTP induced by multiple stimulation sessions, however, is unknown. This chapter examines the contribution of cholinergic mechanisms to potentiation in this preparation. Of particular interest is the extent to which the manipulation of ACh systems might affect the rate of induction of neocortical LTP.

MATERIALS AND METHODS

Surgery, baseline measures and induction of neocortical LTP

Twenty-seven male Long-Evans hooded rats from the McMaster University Breeding Colonies were used in these experiments. At the time of surgery, the animals weighed 300-400 g. All animals were implanted with recording and stimulating electrodes and had baseline measures taken as described previously. LTP-inducing stimulation sessions were delivered daily and consisted of sixty 8-pulse trains (pulse intensity: 159 μA).

EEG activity was monitored during train delivery to ensure that epileptiform discharges were not triggered.

Cholinergic agonism and antagonism

The cholinergic agonist pilocarpine (20.0 mg/kg i.p. in saline, Sigma Chemical Co., USA) and antagonist scopolamine (15.0 mg/kg i.p. in saline, Sigma Chemical Co., USA) were administered in conjunction with high-frequency trains as follows: On the first day of experimentation, animals were injected with pilocarpine (n=5), scopolamine (n=6) or saline (n=6), followed 15 minutes later by the delivery of 60 high-frequency trains. On subsequent days the injection was preceded by an I/O and followed 15 minutes later by 60 high frequency trains. Additionally, a fourth (n=5) and fifth (n=5) group received daily 20.0 mg/kg pilocarpine or 15.0 mg/kg scopolamine injections, respectively, preceded immediately by an I/O, but did not receive trains. These daily LTP

induction regimens were continued for 15 days. Following completion of the LTP induction phase, 4 weekly I/Os were collected to monitor decay.

Analyses

Changes in the field potentials over LTP and decay sessions for both experimental and control animals were measured by subtracting the final baseline responses from all other baseline, potentiated and post-LTP responses. Population spikes were measured from the largest amplitude response in the I/O tests before and after LTP induction and after the decay phase. Repeated measures ANOVAs, comparing all induction and decay days, were calculated for the field measures, while a population spike ANOVA compared the third baseline, final LTP induction, and 4 week decay time-points. The decay rates for both the early and late components were calculated by subtracting the mV value of the final decay measure from the mV value of the final induction measure for each group receiving trains.

Following collection of all electrophysiological measures, animals were perfused and the brains were sliced and stained with Cresyl Violet to verify electrode placements.

RESULTS

Histology

Localization of the stimulation and recording electrode placements was completed in all animals. The ventral tips of the recording electrodes were placed in parietal area 1 or the medial-posterior aspect of frontal area 3, while the stimulating electrode spanned the forceps minor corpus callosum in all animals (Paxinos and Watson, 1986).

Behaviour

Animals injected with pilocarpine appeared agitated for approximately one hour following the injection. Twenty-fours hours after the injections (immediately preceding the I/Os), all animals appeared normal when handled. Animals groomed normally and gained weight throughout the experiment and were completely healthy 24 hours following the cessation of the pilocarpine injection protocol.

The effect of cholinergic agonism and antagonism on LTP induction

Changes in the response amplitude of the early and late components for animals receiving pilocarpine (PILO) or scopolamine (SCOP) alone, or low-intensity trains in conjunction with saline (SAL/T), pilocarpine (PILO/T) or scopolamine (SCOP/T) are shown in figure 5.1. While the early component showed a significant effect of session overall ($F(21,462)=4.50$, $p<0.001$), there were no significant group differences in the pattern or magnitude of the early component changes. The groups receiving trains all

showed a small surface positive shift, that was indistinguishable from the change displayed by animals receiving pilocarpine or scopolamine alone. This apparent lack of an effect was probably due to a confounding of early component EPSP depression in some groups with population spike enhancement in others. Similar surface-positive shifts are induced in both cases. Figure 5.2 illustrates the measurement problem. There are clear population spike enhancements evidenced by the PILO/T group and an absence of population spike potentiation in the PILO group. In fact, the changes shown by the PILO group more closely resemble those expressed by animals that received the NMDA antagonist CPP in the absence of trains (see Fig. 3.1). Further, both the PILO and "CPP alone" animals also showed small amplitude decreases at the *late* component latencies. Although both the PILO and PILO/T groups displayed surface positive shifts of similar magnitude, the mechanisms mediating these shifts are presumably different.

The population spike measures revealed a considerably different pattern (Fig. 5.3), with both session ($F(2,44)=5,26$, $p<0.01$) and group ($F(4, 22)=3.79$, $p<0.01$) showing significant effects. While the SAL/T and PILO animals' population spike measures did not change significantly following the LTP induction regimen, all other groups' did. A surprising result was that both the SCOP and SCOP/T groups showed a considerable increase in spike height following the 15 day drug or drug plus stimulation regimen. Following the 4 week decay period, the 2 scopolamine groups both showed population spike decay, though both were still enhanced relative to baseline, while the pilocarpine groups showed small *increases* in population spike height at the end of the 4 week decay period. The SAL/T group population spike measures were slightly reduced following the

4 week follow-up period.

The late component showed a significant interaction between group and session ($F(84,441)=2.83$, $p<0.001$). All three groups receiving trains showed late component potentiation (Fig. 5.1), with the PILO/T group showing considerably more potentiation than any other group, followed by the SAL/T which showed only slightly more late component potentiation than the SCOP/T group. Neither the PILO nor SCOP groups showed any potentiation effects. The late component increased over the course of the stimulation regimen, with both the PILO/T and SAL/T groups reaching asymptotic levels of potentiation after 7 days of trains, and the SCOP/T group reaching asymptote by the ninth day of stimulation.

The decay rates of both the early and late components varied according to group assignment. The percent decay 4 weeks from the last day of the high-frequency stimulation/drug regimen for the SAL/T, PILO/T and SCOP/T groups are shown in Table 5.1. The early component of both the SAL/T and SCOP/T had decayed fully 2 weeks following the cessation of trains. The PILO/T group, however, was still potentiated at the end of the follow-up period. Conversely, while the PILO/T and SAL/T both showed some degree of decay 4 weeks following the stimulation protocol, the SCOP/T group showed no evidence of late component decay by the end of the follow-up period. Moreover, while the parametric data collected in chapter 2 indicated that the early component effects were longer-lasting than the late component effects, the late component effects in this experiment out-lasted the early component effects.

DISCUSSION

The present data indicate that while cholinergic mechanisms play a clear role in the induction and decay of neocortical LTP in the freely moving animal, the nature of that role is complex. To begin, no significant differences were found in our early component measures. This result was probably the product of the stimulus intensity used to induce LTP rather than an inability of the drugs to influence the induction of LTP in this preparation. A low stimulus intensity was chosen for this experiment because a pilot study indicated that high-intensity trains caused a late component potentiation ceiling to be reached such that the effects of cholinergic *agonism* were obscured. The low-intensity trains, however, caused the opposite problem: The smaller surface-positive shifts attributable to the population spike potentiation fell within the range of early component depression, or downward drift, seen in control groups. It is only by looking at the response morphology, and measuring population spike amplitudes, that the differences can be seen. It is also important to note that, in contrast to the data presented in chapter 2, where 24 daily sessions of 159 μA trains reliably induced an early component potentiation effect, the present experiment used only 15 days of trains in order to avoid tolerance or other deleterious effects resulting from the repeated application of the drugs. The shorter induction regimen likely contributed to the small potentiation effects in the early component. Moreover, in addition to the drug manipulations and injection regimen, the stimulation intensity used in these manipulations is considerably smaller than that used in experiment 1 of chapter 2 (159 μA vs. 1259 μA). If the recording electrode is not

optimally located for maximizing the monosynaptic component, it is not surprising that the polysynaptic component might be more robust. Theoretically, it should be possible to find recording sites that show *only* polysynaptic components.

Although there were no statistically significant early component differences between the groups, it is interesting that the PILO group showed a strong surface positive shift (Figure 5.1). This may represent a depression effect induced by ACh activation alone. Although not evident in this experiment, we previously found that pilocarpine combined with *single* stimulation sessions could induce a depression effect (Racine et al., 1994b).

Clear differences were apparent in the late component changes, with the PILO/T group showing a much stronger late component potentiation than either the SAL/T or SCOP/T groups. Moreover, the SAL/T group showed a stronger effect than the SCOP/T group. These data indicate that while cholinergic agonism enhances the induction of LTP of the novel late component, antagonism slows the development of potentiation. These data are consistent with demonstrations that scopolamine blocks LTP in the hippocampus (Hirotsu et al., 1989) and that cholinergic agonists facilitate neocortical LTP induction *in vitro* (Lin and Phillis, 1991; Brocher et al., 1992). Concurrently, while the SCOP/T group showed the greatest amount of population spike *potentiation*, the PILO/T group showed the least amount of population spike *decay*. While these apparently contradictory results are puzzling in the context of data showing that scopolamine blocks the induction of hippocampal CA1 population spike potentiation (Hirotsu et al., 1989), it has also been demonstrated that acetylcholine suppresses synaptic potentials in both the dentate gyrus

(Yamamoto and Kawai, 1967) and CA1 region (Valentino and Dingledine, 1981) of the hippocampus, as well as in primary visual cortex (Brocher et al., 1992). One possibility is that scopolamine facilitates population spike potentiation in the chronic preparation by antagonizing the normally suppressive effects of endogenous ACh, but only does so in laminae which generate the monosynaptic, early component (Kahle and Cotman, 1989; Hasselmo and Schnell, 1994). In this case, both pilocarpine and scopolamine could enhance LTP by differentially influencing neuronal circuits underlying the separate components of the neocortical response. The SCOP/T group also showed both continued population spike enhancements as well as no sign of late component decay over the 4 week follow-up period. As it is likely that the monosynaptic events are driving the later polysynaptic events, it seems reasonable that scopolamine should have the same effect on both components of the evoked potential.

As was discussed in chapter 4, the GABAergic and cholinergic systems are believed to engage in some degree of co-regulation. For example, GABA agonism has been shown to reduce ACh turnover in both the hippocampus and neocortex (Wood, 1986), while septal ACh decreases the release of GABA (Hasuo et al., 1988). Moreover, pharmacological blockade of both the cholinergic and GABAergic systems has been shown to produce similar effects. Both interfere with new learning but leave the recall of previously learned information untouched (Ghoneim et al., 1975; 1977), and both have been shown to disrupt declarative, but not non-declarative, memory (Nissen et al, 1987; Fang et al., 1987). One substantial difference between the intact and *in vitro* preparations is the degree to which these neuromodulatory systems are damaged by the slicing

procedure (e.g.: Buckmaster and Schwartzkroin, 1995). Although the data resulting from the GABAergic and cholinergic manipulations presented in this thesis are less straight-forward than what has been previously reported for the *in vitro* slice, these data may be the result of interactions between multiple, undamaged modulatory systems. Further research will be required before this possibility can be assessed.

The role of the cholinergic system in memory has received considerable attention over the last 20 years, primarily because of evidence suggesting that the performance deficits associated with both senescence and Alzheimer's disease are correlated with a loss of central cholinergic function (Coyle et al., 1983; Giacobini, 1990; Decker and McGaugh, 1991; Muir, 1997). The discovery of LTP in the freely-moving animal provides a new preparation for the study of chemical messenger systems in the LTP model of memory and may provide a novel means of behaviourally assessing the changes associated with diseases such as Alzheimer's as they relate, for example, to a loss of cholinergic tone in the basal forebrain.

FIGURE CAPTIONS

Figure 5.1. The effects of cholinergic agonism and antagonism on the induction of long-term potentiation. The mV differences between the last baseline and all other sweeps are plotted in this figure for the baseline (pre-LTP), LTP induction (LTP) and 4 week decay (post-LTP) periods. **Top:** Changes in the early component for groups receiving either saline, pilocarpine or scopolamine and trains, or pilocarpine or scopolamine alone. Regardless of the treatment conditions, all animals showed a similar surface positive shift (indicated as a negative mV shift from baseline). **Bottom:** Changes in the late component over days. While the groups that received either pilocarpine or scopolamine alone did not show any late component changes, the groups that received trains all showed late component potentiation. The PILO/T groups showed the greatest amount of late component potentiation, followed by the SAL/T and SCOP/T groups respectively.

Figure 5.2. Representative sweeps taken pre- and post-LTP induction for groups that received pilocarpine and trains (**A**) or pilocarpine alone (**B**). It is clear that while the early component of both groups showed a surface positive shift, the mechanisms mediating these effects were different as indicated, for instance, by the lack of population spike enhancement displayed by the PILO animal. Pilocarpine administration alone had no effect on the late component. Vertical calibration: 1.0 mV; horizontal calibration: 10 ms.

Figure 5.3. Total population spike height measures are shown for time-points prior to LTP induction (pre-LTP), 24 h following the induction protocols (post-LTP) and after a 4 week decay period (decay). Although SAL/T animals did not show population spike potentiation, the PILO/T and SCOP/T groups showed population spike enhancements following the induction regimen. Interestingly, the SCOP group also showed population spike enhancement despite the fact that this group did not receive high-frequency stimulation. Additionally, the PILO/T group showed no evidence of population spike decay 4 weeks following the induction regimen and, in fact, showed a small increase at this time point.

TABLE LEGENDS

Table 5.1. Percent decay of the early (Early Component % Decay) and late (Late Component % Decay) field potential components for groups receiving saline (SAL/T), pilocarpine (PILO/T) or scopolamine (SCOP/T) in conjunction with high-frequency stimulation. In contrast to the data presented in chapter 2, the late component showed less decay (0-41%) than the early component (61-100%) by the end of the decay period. The early component of both the SAL/T and SCOP/T groups had decayed fully after 14 days of decay (a), while the late component of the SCOP/T group had not decayed 4 weeks following the induction regimen (**).

Figure 5.1

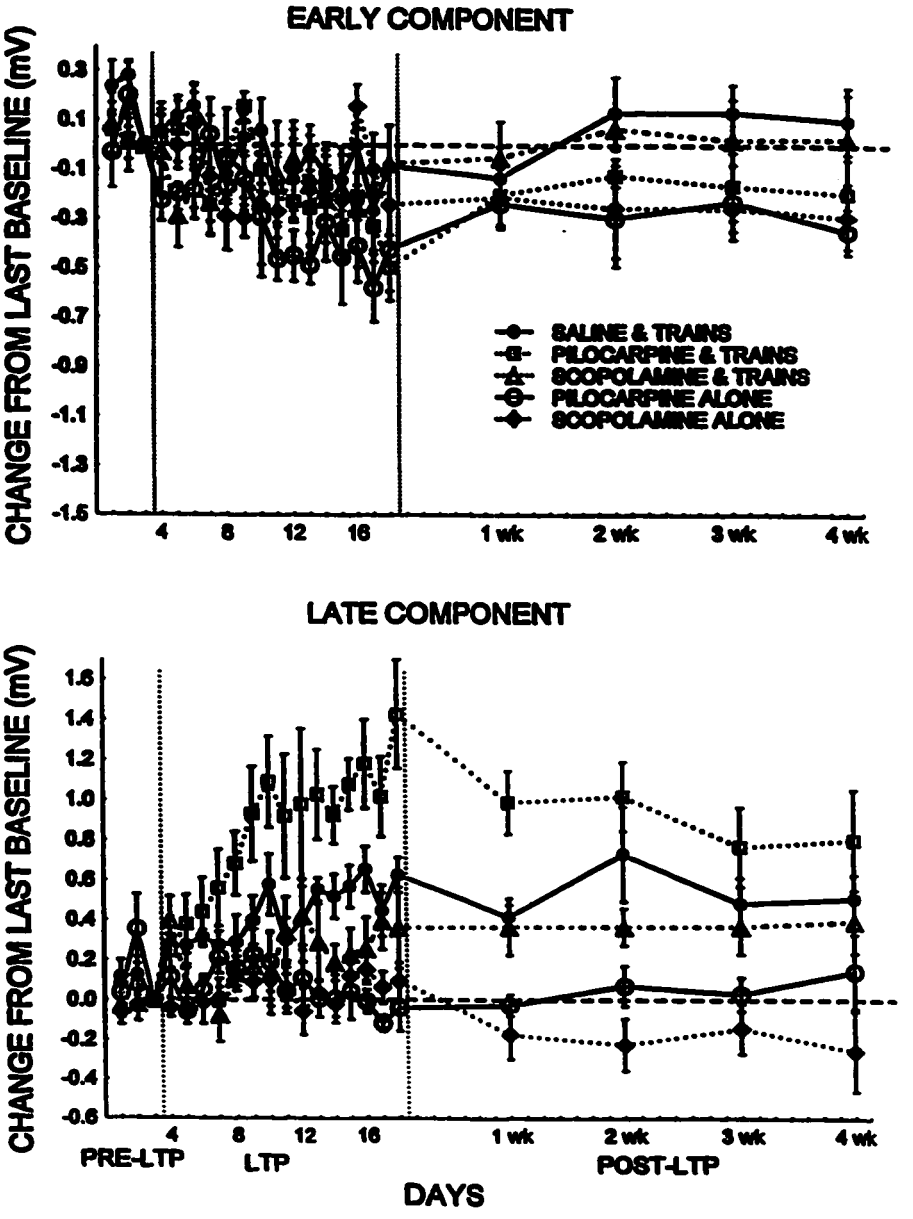

EARLY COMPONENT
CHANGE FROM LAST BASELINE (mV)
0.3
0.1
-0.1
-0.3
-0.5
-0.7
-0.9
-1.1
-1.3
-1.5
4
8
12
16
1 wk
2 wk
3 wk
4 wk
SALINE & TRAINS
PILOCARPINE & TRAINS
SCOPOLAMINE & TRAINS
PILOCARPINE ALONE
SCOPOLAMINE ALONE
LATE COMPONENT
CHANGE FROM LAST BASELINE (mV)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
4
8
12
16
1 wk
2 wk
3 wk
4 wk
PRE-LTP
LTP
POST-LTP
DAYS

Figure 5.2

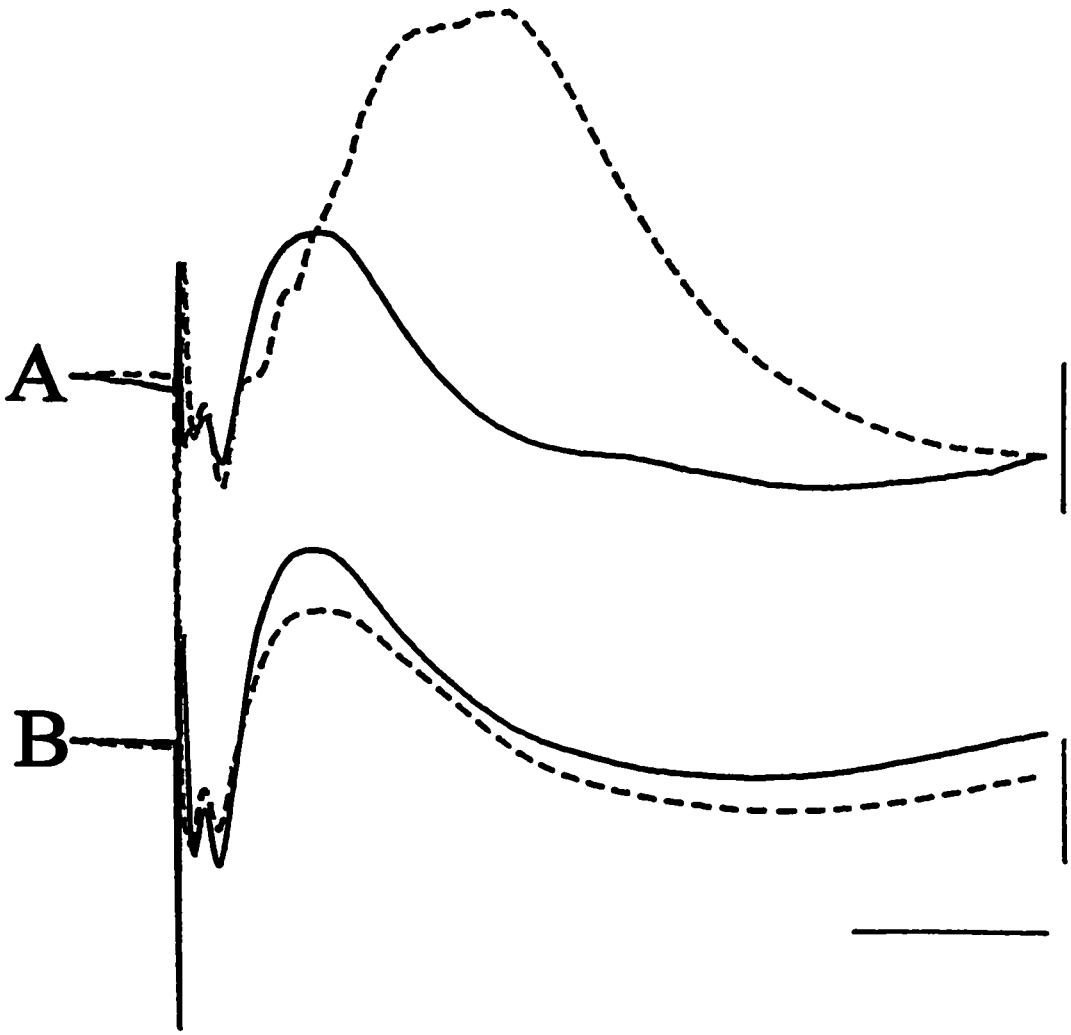


Figure 5.3

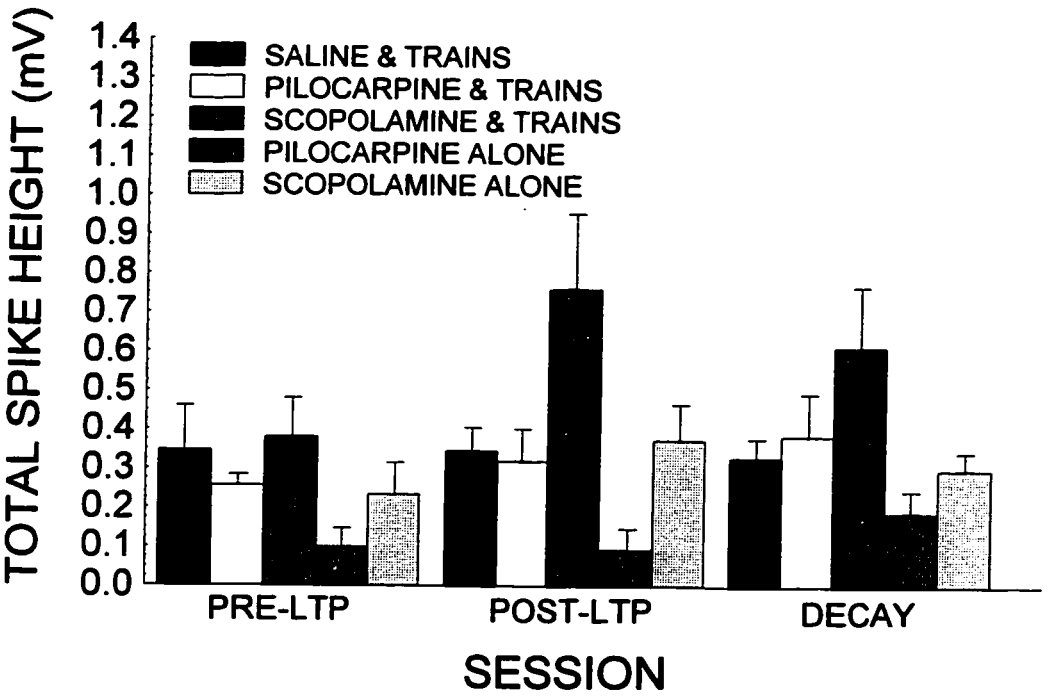

TOTAL SPIKE HEIGHT (mV)
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
SALINE & TRAINS
PILOCARPINE & TRAINS
SCOPOLAMINE & TRAINS
PILOCARPINE ALONE
SCOPOLAMINE ALONE
PRE-LTP
POST-LTP
DECAY
SESSION

Table 5.1

Group	Early Component % Decay	Late Component % Decay
SAL/T	100%[a]	17%
PILO/T	61%	41%
SCOP/T	100%[a]	**

CHAPTER SIX

BLOCKADE AND DISRUPTION OF NEOCORTICAL LTP FOLLOWING ELECTROCONVULSIVE SHOCK

This chapter describes the effects of maximal electroconvulsive shock (MES) on neocortical LTP induction. MES has been shown to disrupt memory formation when delivered post-trial in learning tasks (Zubin and Barrera, 1941; Brody, 1944; Quartermain et al., 1965; McGaugh and Alpern, 1966; Barnes et al., 1994; Bohbot et al., 1996). MES is also considered an animal model of electroconvulsive therapy (ECT), which is used to treat depression in human populations. It was used extensively in attempts to define consolidation gradients, but these experiments were abandoned when the "gradients" were found to be dependent upon many additional variables, including the presence or absence of anaesthetics, the placement of the stimulating electrodes, the amplitude and frequency of the electrical stimulus and the duration of the induced seizures (Polster et al., 1991; see Abrams, 1992 for review). One of the most striking and cited effects of human ECT is the memory dysfunction accompanying treatment (Squire, 1977; Weeks et al., 1980; Krueger et al., 1992), although the locus of this effect is unknown. Nevertheless, the memory disrupting effects of MES are widely accepted.

The first animal study designed to examine the effects of MES on memory consolidation used a classical conditioning paradigm in which rats were trained to

associate the presence of a light with foot-shock (Duncan, 1949). MES was then administrated to the animals at varying intervals, from 20 sec to 14 h, following the learning trials. This early study demonstrated that the interval between the task learning and the administration of MES was important for retention. The closer the MES followed the training, the less the rat appeared to be able to associate the light and the shock. Disruptions were found with training-MES intervals up to 1 hour. Subsequent to this, Cronholm and Lagergren (1959) reported that human patients also showed a temporally graded retrograde amnesia following ECT. In their study, subjects were presented with a set of numbers 5, 15 and 60 sec prior to an ECT session. The patients' recall of these numbers was significantly better the longer the interval between learning and ECT.

The generally disruptive effects of ECT on memory were explored by Cronholm and Molander (1961) using a variety of paired associate tasks, including word, figure and letter-symbol pairs. On the day before the first ECT treatment, a baseline was established by testing patients on both initial learning performance and recall 6 hours after learning. On the next day, the procedure was repeated except that an ECT session occurred 1 hour after the learning phase. Recall 6 hours after learning was significantly poorer when ECT followed acquisition.

The most disturbing instances of memory loss associated with ECT, however, are those where *distant* biographical information is lost following treatment. Janis (1950) obtained extensive personal histories from both control subjects and patients scheduled to undergo ECT. In contrast to controls, who rarely displayed any deficits, every patient tested displayed marked deficits for the previously reported memories 4 weeks following

the course of their ECT regimen. Moreover, patients followed 10-14 weeks later continued to show memory deficits. Squire et al (1981) obtained similar results when patients were tested on recall of autobiographical information several months following a course of ECT. In addition, ECT patients were less likely than controls to recognize omitted facts when reminded of them. A number of other studies have reported similar findings for both autobiographical and non-autobiographical information (e.g.: Squire et al., 1975; Weiner et al., 1986). It therefore appears that ECT is capable of compromising remote memories in addition to recent learning. These data are not without controversy, however. Weeks et al. (1980) conducted a comprehensive set of experiments on cognitive impairment and found that 1 week after ECT treatment, patients showed neither memory deficits relative to their pre-ECT performance, nor relative to matched non-ECT control subjects.

ECT has also been shown to produce temporally graded *anterograde* amnesia. Squire et al. (1976) tested subjects' ability to recognize material (pictures of common objects, yearbook photos, line drawings and common words) learned 40 min or 3 1/2 hours after ECT. Under these conditions marked anterograde amnesia was evidenced by all subjects. Moreover, subjects' memories were better the longer the interval between the ECT and subsequent learning. This effect has been shown to hold in verbal learning tasks (Halliday et al., 1968), picture recognition tasks (Zinkin and Birtchnell, 1968), and non-verbal (Sackheim et al., 1986) and verbal (Calev et al., 1989) paired associate learning paradigms.

Several other factors have been shown to influence the ability of ECT to impair

recall. Subjects show better recall following right unilateral, as opposed to bilateral, ECT (Lancaster, 1958; Cannicott and Waggonner, 1967). Additionally, sine-wave stimulation has been reported to produce greater impairment than brief pulse stimulation (Daniel et al., 1983). Factors such as these are often used to explain the variability seen in studies of human ECT.

Considerable attention has been directed at the effects of MES stimulation on hippocampal function, primarily because of the important role believed to be played by this structure in both memory (Andersen and Trommald, 1995; McClelland et al., 1995) and long-term potentiation (for review see Bliss and Collingridge, 1993). In rats, MES stimulation has been shown to reversibly disrupt hippocampal LTP (Hesse and Teyler, 1976) and the induction of LTP in slices taken from rats that have undergone repeated MES stimulation has been shown to be impaired (Anwyl et al., 1987).

On the other hand, MES itself has been shown to produce potentiation effects in the hippocampus (Stewart et al., 1994) lasting several months (Burnham et al., 1995) and these data have raised the possibility that the prevention of potentiation associated with MES is itself the result of an LTP-like synaptic saturation (Stewart and Reid, 1993; Barnes et al., 1994).

The experiment presented here demonstrates that MES stimulation (i) induces a temporally-graded blockade of neocortical LTP induction when paired with high-frequency stimulation; (ii) transiently disrupts established LTP in the neocortex and; (iii) does not result in the LTP-like potentiation effects normally recorded in the hippocampus following the repeated application of MES.

MATERIALS AND METHODS

Surgery, baseline measures and induction of neocortical LTP

Thirty male Long-Evans hooded rats from the McMaster University Breeding Colonies were used in these experiments. At the time of surgery, the animals weighed 300-400 g. All animals were implanted with recording and stimulating electrodes and had baseline measures taken as described previously. LTP-inducing stimulation sessions were delivered once every 48h according to the schedule described below and consisted of sixty 8-pulse trains as described in chapter 2 (pulse intensity of 1259 μA). EEG activity was monitored during train delivery to ensure that epileptiform discharges were not triggered.

Maximal electroconvulsive shock administration

Beginning 24 hours after the third baseline I/O test, animals received MES stimulation at 2-day intervals according to group designation. The MES stimulus consisted of 150 mA, 60 Hz sine-wave pulses delivered for 200 ms via corneal electrodes. The first group was an MES control (MES only, n=4) which was included to verify that MES by itself had no effect on the baseline response. Animals in this group received 5 MES sessions spaced 48h apart over a total of 10 days. The second, third and fourth groups received 60 high frequency trains once every 48h, followed 5 minutes (MES immediately, n= 6), 1 h (MES 1h after trains, n=5) or 6 h (MES 6h after trains, n=5) later

by MES stimulation. A fifth (trains only, n=5) group received high-frequency trains alone every 48 h to provide a baseline level of LTP. I/O measures were collected every day. On the days when high-frequency trains and MES stimulation were delivered, the I/O measures were collected immediately preceding the administration of high-frequency trains. These regimens were followed for 10 days. One week following the trains/MES protocol, animals that received MES alone, MES immediately or MES 1h after trains were given 60 high-frequency trains every 48 h in the absence of MES stimulation for 10 days to confirm that LTP could be induced in these animals. I/Os were again collected daily throughout the LTP induction procedure, and were subsequently collected for 2 weeks following the delivery of trains.

A separate group of animals (n=5) received 60 high-frequency trains every 48h for 10 days and then received a single MES stimulation to determine the effects of MES on an already established LTP. This was done to test for depotentiation effects of MES. I/Os were collected each day throughout the LTP induction procedure and were then collected 5 minutes, 30 minutes, 1h, 6h, 12h and 24h following the MES stimulation.

Analyses

Changes in the field potentials over LTP and decay sessions for both experimental and control animals were measured by subtracting the final baseline responses from all other baseline and potentiated responses. Population spikes were measured from the largest amplitude response in the I/O tests before and after LTP induction and after the decay phase. Repeated measures ANOVAs were calculated over the induction period for

the MES blockade study. For animals that had previously established LTP disrupted with MES, all induction and post-MES data points were included in the ANOVA. Following collection of all electrophysiological measures, animals were perfused and the brains were sliced and stained with Cresyl Violet to verify electrode placements.

RESULTS

Histology

Localization of the stimulation and recording electrode placements was completed in all animals. The ventral tips of the recording electrodes were placed in parietal area 1 or the medial-posterior aspect of frontal area 3, while the stimulating electrode spanned the forceps minor corpus callosum, in all animals (Paxinos and Watson, 1986).

Behaviour

Animals that received MES stimulation reliably displayed a tonic-clonic convulsion which lasted between 10-25 sec. The severity of the seizures appeared to increase slightly as the experiment progressed.

Experiment 1: Temporally graded blockade of neocortical LTP with MES stimulation

The repeated application of electroconvulsive shock blocked the induction of neocortical LTP in a temporally-graded fashion. Figure 6.1 shows changes in the early and late components for control animals and animals receiving MES 5 minutes (immediately), 1h or 6h following high-frequency stimulation. There was a significant interaction effect between session and group for the early component ($F(48,240)=2.85$, $p<0.001$). The animals receiving trains alone potentiated normally, showing a large surface-positive shift and an increase in population spike amplitude. There was a nearly complete blockade of LTP of the early component in the animals receiving MES

immediately after the trains. A substantial suppression of LTP was also seen in the groups receiving MES at 1h and 6h after the trains. MES alone had no effect.

The population spike measures showed a significant interaction effect between session and group ($F(4,20)=2.87$, $p<0.04$). The control group and the group that received MES 6h after trains both showed population spike enhancements, while the animals that received MES alone, 5 minutes after trains, or 1h after trains showed *reductions* in population spike amplitude (Fig. 6.2).

There was also a significant interaction between group and session for the late component ($F(48,240)=6.06$, $p<0.001$). For this component, only the animals that received 48h spaced trains alone, and those animals that received MES 6h following trains showed potentiation of the late components, with the latter showing approximately half as much potentiation as the former. When the groups that had potentiation blocked by MES were left for 1 week and then repotentiated, all animals showed potentiation of all components. This indicates that the application of MES stimulation did not cause long-lasting deleterious effects.

Experiment 2: MES disruption of previously established LTP

The effects of MES stimulation on the early and late components of fully potentiated neocortical responses is shown in Figure 6.3. There was an overall effect of session for both the early ($F(18,72)=2.30$, $p<0.01$) and late ($F(18,72)=16.63$, $p<0.001$) components. The application of MES caused some increase in variability, but the most striking effect was a shift in amplitude back towards baseline levels. This effect,

however, lasted for less than 12h, and was most pronounced in the late component. The population spike amplitudes, which showed a significant effect of session ($F(7,28)=6.21$, $p<0.001$), were also depressed by MES (Figure 6.4). These amplitudes were reduced to baseline levels when measured 5 minutes following MES, and then recovered over the next 24h. The group measures were not significantly different from potentiated levels after 24h (Tukey's HSD test: $p>0.36$). Figure 6.5 shows a set of representative sweeps taken before and after LTP induction, and following the 6 post-MES time-points (5 min, 30 min, 1h, 6h, 12h and 24h).

DISCUSSION

This study was designed to investigate the effects of maximal electroconvulsive shock stimulation on the induction and expression of neocortical LTP in the awake, freely moving rat. The results demonstrated that the induction of neocortical LTP was interrupted or blocked by the application of MES stimulation. However, while animals receiving MES stimulation immediately following, or within 1 hour of, high-frequency trains failed to develop LTP, a temporal window appears to exist between 1 and 6 hours during which time a reduced level of LTP can be induced. These data are in agreement with previous hippocampal studies in which the repeated application of MES stimulation served to suppress the induction of hippocampal LTP by as much as 83.5% (Hesse and Teyler, 1976; Anwyl et al., 1987). A recent study, however, showed that hippocampal LTP could be *enhanced* by pairing high-frequency stimulation with MES (Barnes et al., 1994). Although we did not follow our high-frequency trains immediately with MES, a delay of only 5 minutes between administration of high-frequency stimulation and MES completely blocked LTP induction in our neocortical recording site. We were surprised to find that MES *alone* produced no potentiation effects in the neocortical response. Such effects have been found in the hippocampus (Stewart et al., 1994; Burnham et al., 1995). One possibility is that neocortical GABAergic systems are being more strongly engaged in response to the convulsive stimuli, perhaps even kindled or potentiated. An enhancement of inhibition in this way would act against the development of potentiation at excitatory synapses and thereby prevent the induction of neocortical LTP.

Additionally, the interaction between the high-frequency and MES stimulation may be producing a heterosynaptic long-term *depression* effect which is countermanding the development of potentiation. Such an effect is consistent with the reduction in amplitude of both the early and late components in response to the combined stimulation. Further research into the characteristics of LTD in the neocortex is required before this possibility may be accurately assessed.

In agreement with hippocampal data (Hesse and Teyler, 1976), we have also shown that MES can partially or completely reverse existent LTP, but only temporarily. Our responses returned to their fully potentiated levels within 12 hours post-MES and this rapid recovery confirms that the blockade of LTP *induction* by MES was not due to some non-specific decrease in excitability of the tissue.

There have been a number of short and long-term changes reported following repeated MES tonic-extensor seizures including the down-regulation of β-adrenergic receptors (Pandey et al., 1979) and increases in serotonin-2 receptors (Biegon and Isreali, 1987) and α_2-adrenoceptor binding sites (Stockmeier et al., 1987). One of the most interesting changes, however, is the increase observed in neuropeptide-Y (NPY) levels following MES stimulation. NPY immunoreactivity (Wahlestedt et al., 1990; Stenfors et al., 1992; Kragh et al., 1994) has been shown to increase in both the hippocampus and neocortex in response to MES. There is also considerable evidence that NPY may modulate memory. Intraventricular administration of NPY enhances memory retention for T-maze footshock avoidance and step-down passive avoidance training in mice (Flood et al., 1987; Flood et al., 1989). NPY administration post-training, however,

produces an inverted U-shaped dose-response curve for task retention (Flood et al., 1987; for review of effects of NPY on memory see Morley and Flood, 1990).

It is possible that MES stimulation, following high-frequency stimulation, is causing a sudden increase in NPY levels, resulting in compromised potentiation. Bath application of NPY causes a long-lasting, reversible reduction of the orthodromic EPSP recorded intracellularly from CA1 pyramidal neurons in *in vitro* preparations (Colmers et al., 1987). This work has led to the assertion that NPY acts presynaptically in the CA1 region to reduce excitatory input to pyramidal neurons (Colmers et al., 1987) a hypothesis supported by the subsequent observation that NPY directly suppresses synaptic transmission in CA1 (Klapstein and Colmers, 1992). This reduction in excitation may serve to suppress events that normally follow high-frequency stimulation, thereby weakening or preventing the induction of LTP.

Electroconvulsive stimulation (ECS) can produce a retention deficit if it is administered shortly following a learning task in both rats (eg: Quartermain et al., 1965) and humans (Squire et al., 1981; for review see Abrams, 1992). The severity of this deficit declines with the interval between the learning task and ECS administration (McGaugh and Herz, 1972; Maki, 1985; Bohbot et al., 1996). This so-called retrograde amnesia gradient is believed to reflect the time course of memory consolidation. Bohbot et al. (1996) trained rats in the Morris water maze and then applied ECS at varying intervals following a single escape to the new platform location. This study of spatial memory disruption showed that ECS applied between 0-15 seconds after a learning trial caused clear retrograde amnesia for that learning, while intervals 30 seconds and longer

did not have any effect. Moreover, Gold et al. (1973) paired inhibitory avoidance training in rats with ECS delivered through direct cortical stimulation and demonstrated retrograde amnesia gradients between 5 seconds and 240 minutes, depending on the location and intensity of the stimulation. The longest of these gradients fall between our 1h and 6h time-points, indicating some compatibility with our data. These authors also found that stimulation of frontal regions had higher retrograde amnesia thresholds than stimulation of posterior regions, suggesting that the frontal regions may be less vulnerable to memory failures. Although it is difficult to comment on the relative contributions of the neocortex in these tasks, or on the rate at which different features of task learning are transferred from subcortical to cortical areas, the present study is in general agreement with the behavioural data in demonstrating that, just as a consolidation gradient exists for learning tasks, a similar gradient may exist for the consolidation of neocortical LTP.

FIGURE LEGENDS

Figure 6.1. The effects of MES applied at different intervals on the induction of long-term potentiation. The mV differences between the last baseline and all other sweeps are plotted in these figures for the baseline (pre-LTP) and LTP induction (LTP-induction) periods. **Top:** Changes in the early component over days. While animals that received high-frequency train sets every 48h showed a strong shift in the surface-positive direction (indicated as a negative mV shift from baseline), animals that received similar high-frequency train sets followed immediately, 1h later or 6h later by MES stimulation showed a smaller early component potentiation. Animals that received MES alone showed a negligible early component change. **Bottom:** Changes in the late component over days. Only those animals receiving LTP-inducing trains alone or trains followed 6h later by MES showed a significant enhancement of the late component. All other groups showed no change from baseline. Note that, contrary to the effects seen in the hippocampus, MES administered alone does not cause potentiation of the late components.

Figure 6.2. Total population spike height measures are shown for time-points prior to LTP induction (pre-LTP) and 24 h following the induction protocols (post-LTP). Animals that received LTP-inducing trains alone and those that underwent MES 6h following trains showed population spike enhancements, while animals in all other groups showed no enhancement. Note that animals in the MES only and 1h MES

conditions appeared to show small *decreases* in population spike height.

Figure 6.3. The effects of a single MES seizure on a fully potentiated neocortical evoked potential. The mV differences between the last baseline and all other sweeps are plotted in these figures for the baseline (pre-LTP), LTP induction (LTP-induction) and post-MES (post-MES) periods. **Top:** Changes in the early component over days. The application of MES appeared to cause a small loss of the surface-positive amplitude shift normally associated with potentiation of this component, though it was still well within the range of a potentiated response. **Bottom:** Changes in the late component over days. Along with the population spikes, this component showed the largest effect overall, being reduced to a near-baseline state for a period of approximately 1h. Thereafter, the late component recovered back to its fully potentiated level within 12 hours.

Figure 6.4. Total population spike height measures are shown for time-points prior to LTP induction (pre-LTP), following the induction of LTP (LTP) and at various times following a single session of MES (5 min, 30 min, 1 hour, 6 hours, 12 hours and 24 hours following MES). Five minutes following the application of MES the population spikes were significantly depressed, however, by 30 minutes post MES they had recovered sufficiently to a point where they were no longer statistically different from before the application of MES. It is clear, however, that even 24 hours following MES, the population spikes do not reach the same mean level of potentiation shown prior to MES.

Figure 6.5. Representative sweeps recorded during the course of recovery from the effects of a single session of MES. The first set of sweeps (A) shows a baseline (solid) response compared to a fully potentiated response (dashed). The next 6 sets of sweeps compare a fully potentiated response (solid in all cases) to a response recorded 5 min. post-MES (B), 30 min. post-MES (C), 1 h post-MES (D), 6 h post-MES (E), 12 h post-MES (F) and 24 h post-MES (G). The late component showed complete recovery in this animal while the early component remained altered at the end of the recovery period. Vertical calibration: 1.0 mV; horizontal calibration: 10 ms.

Figure 6.1

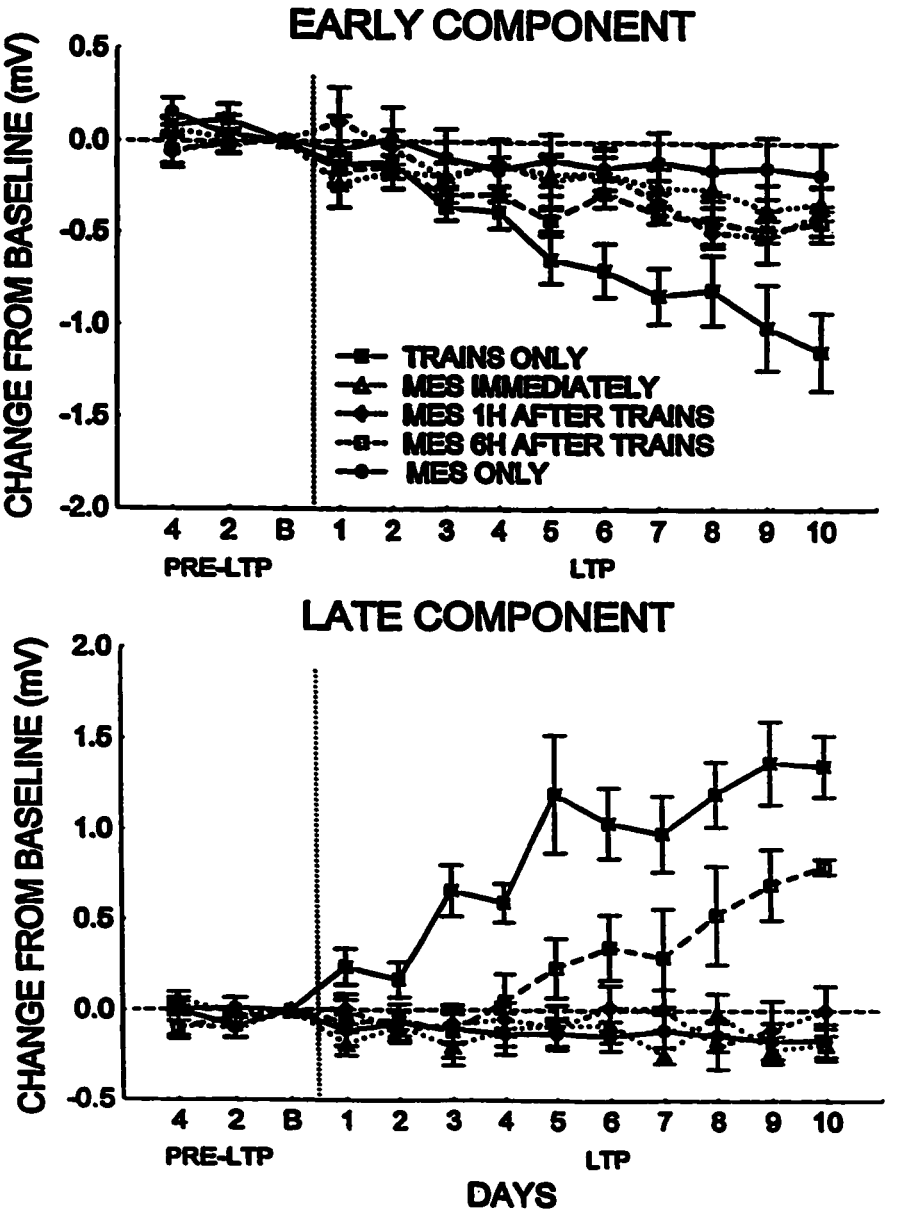

EARLY COMPONENT
CHANGE FROM BASELINE (mV)
0.5
0.0
-0.5
-1.0
-1.5
-2.0
TRAINS ONLY
MES IMMEDIATELY
MES 1H AFTER TRAINS
MES 6H AFTER TRAINS
MES ONLY
4 2 B 1 2 3 4 5 6 7 8 9 10
PRE-LTP
LTP
LATE COMPONENT
CHANGE FROM BASELINE (mV)
2.0
1.5
1.0
0.5
0.0
-0.5
4 2 B 1 2 3 4 5 6 7 8 9 10
PRE-LTP
LTP
DAYS

Figure 6.2

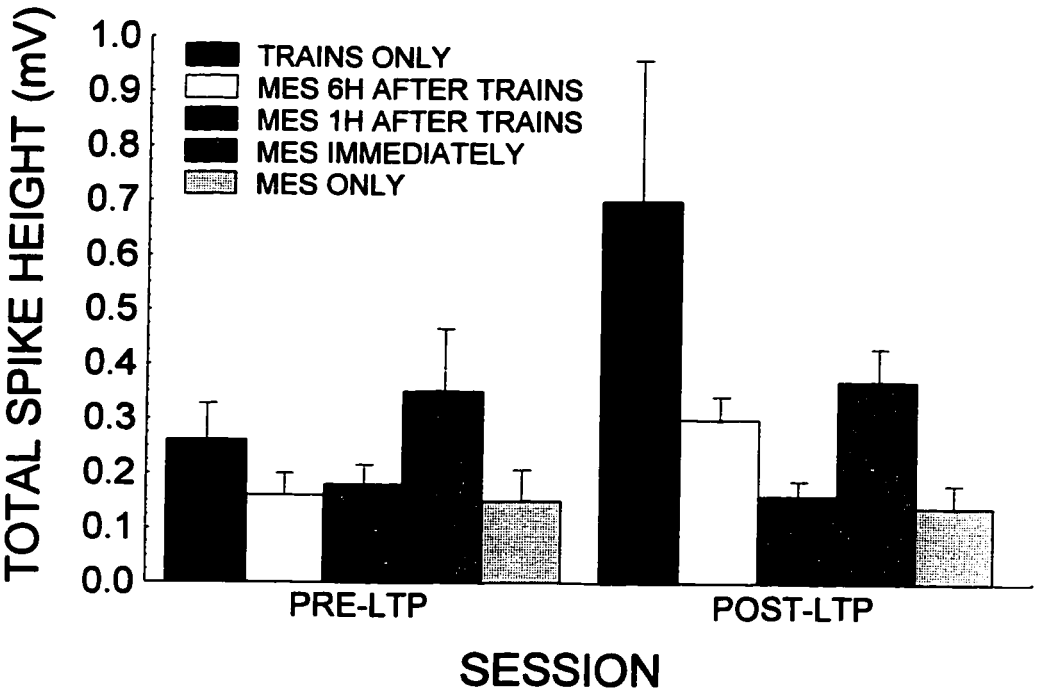

TOTAL SPIKE HEIGHT (mV)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
TRAINS ONLY
MES 6H AFTER TRAINS
MES 1H AFTER TRAINS
MES IMMEDIATELY
MES ONLY
PRE-LTP
POST-LTP
SESSION

Figure 6.3

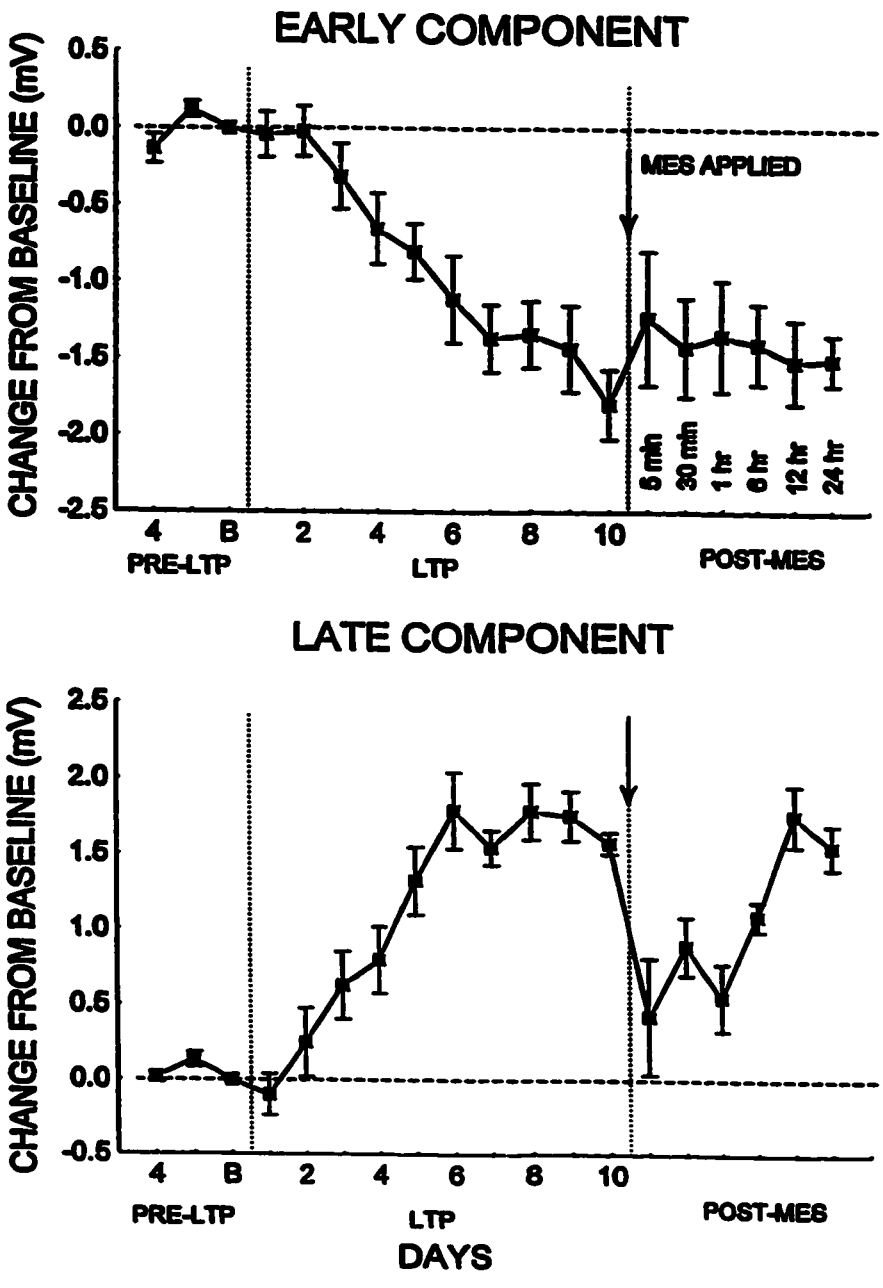

EARLY COMPONENT
CHANGE FROM BASELINE (mV)
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
MES APPLIED
6 min
30 min
1 hr
6 hr
12 hr
24 hr
4
B
2
4
6
8
10
PRE-LTP
LTP
POST-MES
LATE COMPONENT
CHANGE FROM BASELINE (mV)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
4
B
2
4
6
8
10
PRE-LTP
LTP
POST-MES
DAYS

Figure 6.4

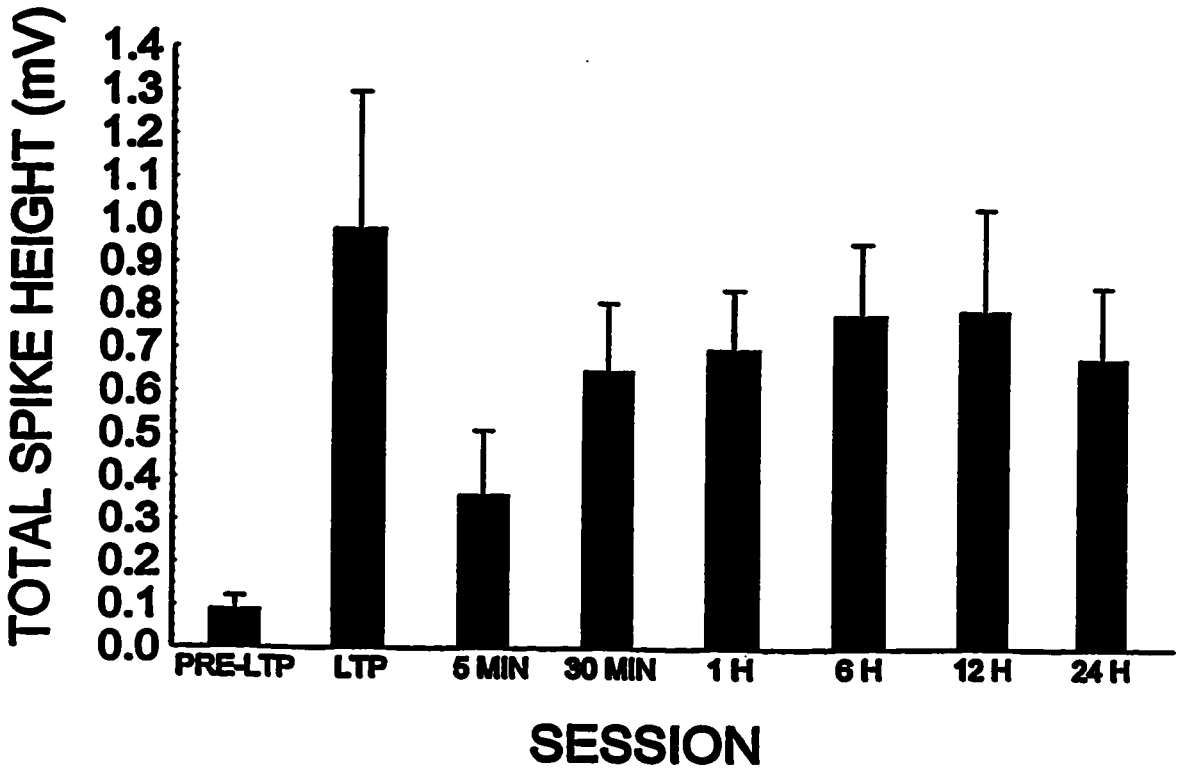
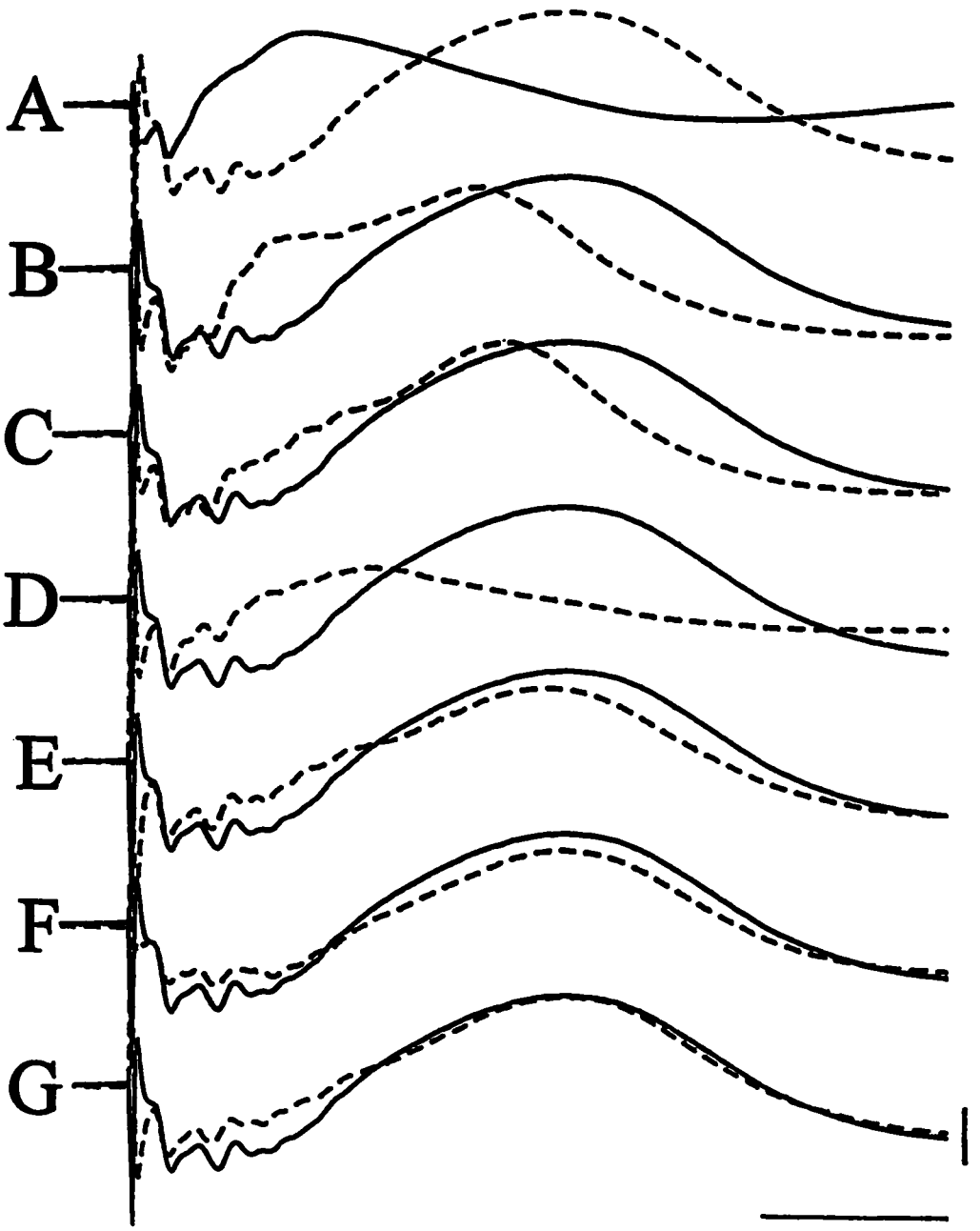

TOTAL SPIKE HEIGHT (mV)
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
PRE-LTP
LTP
5 MIN
30 MIN
1 H
6 H
12 H
24 H
SESSION

Figure 6.5



CHAPTER SEVEN

GENERAL DISCUSSION

While it is currently believed that the neocortex plays a crucial role in the storage of long-term memory (eg: McClelland et al., 1995), it has shown remarkable resistance to the induction of long-term potentiation, the leading model of synaptic, use-dependent plasticity (Kirkwood and Bear, 1994; Racine et al., 1995a; for review, see Bear and Kirkwood, 1993). This resistance has been most pronounced in the intact animal (Racine et al., 1995a), although even *in vitro* demonstrations of neocortical LTP have usually required the use of young animals and/or GABAergic blockade (Artola and Singer, 1987; Perkins and Teyler, 1988; Hess et al., 1996). If long-term potentiation is to be taken seriously as a physiological model of memory, it is critical that both its kinetics and mechanisms be described in the chronic preparation.

This thesis describes a novel form of neocortical long-term potentiation, one that is expressed and maintained in the freely-moving animal (rat). Before summarizing the findings of this thesis, however, it is important that several issues be discussed, albeit briefly.

Defining LTP

Racine et al. (1983) investigated LTP in a variety of limbic forebrain pathways, including several hippocampal pathways, and determined that there were at least two

forms of LTP in these sites, possessing time constants of 1.5 hours and 5 days, respectively. Subsequently, Abraham and Otani (1991) reviewed the hippocampal LTP data and determined that, depending upon the induction regimen, 3 forms of LTP could be induced with time constants of 2 hours (LTP1), 4 days (LTP2) and 20 days (LTP3), respectively. Discovering these LTPs has required the use of chronically implanted, freely-moving animals so that response amplitudes could be monitored for the requisite period of time.

Most of the neocortical LTP work, however, has been done in the *in vitro* slice preparation. As a result, a complete analysis of the kinetics of LTP as it is expressed in this structure has not been possible until recently (Racine et al., 1995b). Rather than searching for a viable *in vivo* preparation, most researchers have been content to adapt their definition of LTP to accommodate the preparation being used. Castro-Alamancos et al. (1995), for example, refer to LTP as a, "sustained (*more than 30 min*) increase in synaptic responsiveness that follows conditioning stimuli [my italics]". This definition is clearly satisfied by the shortest duration LTP effect (LTP1) which has a time constant of about 2 hours (Racine et al., 1983; Abraham and Otani, 1991). Researchers using the slice preparation have good reason to apply a liberal definition of LTP — slices only tend to survive 6 to 12 hours following dissection. But such definitions run into trouble when considering the theoretical framework within which these data are presented (eg: McClelland et al., 1995). In short, while the *in vitro* preparation is clearly very powerful in its ability to permit control of the experimental conditions, when used in isolation it may preclude the meaningful study of those components of the learning and memory

process that are of greatest interest.

Contrasting the in vitro and in vivo preparations

The slice has a number of other inherent shortcomings which make comparisons with intact animals risky, and may account for the discrepancies in the LTP effects reported. The most obvious of these is that the slicing procedure damages the tissue. Additionally, slices lack the inputs and outputs normally found *in situ*, eliminating most of the normal neuromodulatory processes. Even the artificial cerebrospinal fluid may lack critical elements.

One of the circuit alterations in the slice preparation is a shift in balance between excitation and inhibition. Hypoxia kills inhibitory interneurons, and various stages of the tissue preparation may cause epileptiform afterdischarges to occur in the slice. Moreover, anatomical and electrophysiological data have confirmed that the preparation of slices compromises the inhibitory networks in the hippocampus (Han et al., 1993; Buckmaster and Schwartzkroin, 1995; Sloviter and Brisman, 1995). This loss of inhibitory tone is important in the context of potentiation, because inhibitory processes are believed to play a critical role in the induction of LTP (for review, see Bliss and Collingridge, 1993). Inhibition is often further reduced by the use of young animals and the addition of bicuculline or picrotoxin to the ACSF. Taking these factors into account, it is remarkable that the neocortex shows any resistance whatever to the induction of LTP. For many reasons, one might expect the tissue to be on the verge of a seizure throughout the recording procedure. Consequently, it is surprising that few researchers using the *in vitro*

preparation have controlled for the effects of afterdischarges, or have even provided for the monitoring of these events.

The *in vivo* preparation, by contrast, possesses its full complement of inhibitory, neuromodulatory and anatomical inputs. As a result, one should probably *expect* the features of LTP to be expressed differently between the 2 preparations. Indeed, several differences have already been noted. For example, potentiation of excitatory inputs to inhibitory interneurons has been demonstrated *in vivo* (Buzsaki and Eidelberg, 1982; Kairiss et al., 1987) but has not been shown *in vitro* (Abraham et al., 1987).

LTP-induced response morphology changes in the intact and in vitro preparations

In vitro studies of motor, somatosensory (e.g.: Castro-Alamancos et al., 1995) and visual (eg: Kirkwood and Bear, 1994) neocortical LTP have reported the effects of potentiation on the fast, primarily monosynaptic component of the evoked potentials. In these experiments, LTP results in the enhancement of components present in the baseline response. By contrast, neocortical LTP in the chronically prepared animal results in a strong surface positive shift of the early component (avg. latency-to-peak: ~8.0 msec), an increase in both the amplitude and number of population spikes superimposed on this early component, and an increase in the amplitude of the later, polysynaptic components (avg. latency-to-peak of the dominant late component: ~20.0 msec) (see Figure 2.2). The most likely explanation for these differences between *in vitro* and *in vivo* preparations is that deafferentation, resulting from the slicing procedure, destroys many of the inputs that contribute to the evoked response. Intact neuromodulatory networks, for example, may

contribute to the LTP-induction in the chronic preparation.

Taking these differences between the *in vitro* and *in vivo* preparations into account, the main findings of this thesis are reviewed below.

Neocortical LTP in the freely-moving animal follows rules compatible with theories of neocortical learning and memory

In vitro, both hippocampal CA1 and the visual cortex have been shown to express a similar form of LTP following similar induction procedures (Kirkwood et al., 1993). This observation, however, appears to be inconsistent with the proposed role of the neocortex in memory storage. The neocortical system is believed to learn through many repetitions of similar acts of information processing, producing gradual, incremental changes in the synaptic connections between neocortical neurons (McClelland et al., 1995). One might expect, then, that neocortical LTP would develop over several stimulation sessions.

Chapter 2 describes the kinetics of neocortical LTP in the adult, freely-moving animal. These data underscore the importance of input repetition for LTP induction, including variations on the number of trains given within a stimulation session, the number of stimulation sessions used, and the inter-stimulus interval. In almost all cases, greater numbers of stimulus repetitions resulted in larger, and longer-lasting, LTP effects. For example, 25 days of stimulation produced longer-lasting LTP than did 10 days of stimulation, while the delivery of 60 trains/session induced a larger amplitude LTP compared to that resulting from 5 trains/session (Figure 2.3). In addition, the stimulus

interval data indicate that the largest LTP effects are expressed by animals receiving trains with a 24h inter-session interval (ISI), a result consistent with the idea that the temporal spacing of the inputs is critical for the prevention of interference. Once again, the *decay* of LTP appeared to be dependent upon the total amount of stimulation received. Animals that received stimulation every 6h (receiving the greatest amount of total stimulation) showed the slowest rate of decay, while animals that received trains every 48h decayed most quickly (Figure 2.6).

While higher intensity stimulation induced a faster, larger LTP effect relative to low intensity stimulation (Figure 2.5), LTP was also induced following high-frequency stimulation with intensities as low as 16 μA. LTP following low intensity trains appeared to be restricted to the polysynaptic component(s) (Figure 2.5). This is likely due to the recording electrodes not being optimally placed to maximize the monosynaptic component. As only 3/7 of the animals in the 16 μA group potentiated, we have demonstrated that a threshold for LTP induction appears to exist around 16 μA.

One of the most important results reported in this thesis was that the delivery of only 1 train/day, or the use of trains with pulse intensities as low as 16 or 32 μA, reliably induced neocortical LTP. These results demonstrate that, rather than being resistant to the induction of LTP, the neocortex is at least as reactive as the hippocampus so long as the stimulation trains are *spaced and repeated.* This conclusion is important in light of the difficulties reported by researchers using the *in vitro* preparation (for reviews, see Tsumoto, 1992; Bear and Kirkwood, 1993), and stresses the need to search for stimulation protocols that follow rules appropriate to the system under investigation.

Neocortical LTP in the freely-moving animal is NMDA receptor dependent

The experiment presented in chapter 3 demonstrated that the selective blockade of NMDA receptors with the antagonist CPP prevents the induction of LTP in a dose-dependent manner. These data show for the first time that an NMDA-based mechanism contributes to neocortical LTP in the freely moving animal and so are in agreement with *in vitro* demonstrations (Artola and Singer, 1987; Sutor and Hablitz, 1989; Artola and Singer, 1990; Kirkwood et al., 1993; but see Komatsu et al., 1991). Additionally, NMDA blockade appeared to unmask a depression of both the early and late components, an effect that has also been demonstrated *in vitro* (Hirsch and Crepel, 1991). These data provide support for the "dual threshold" model proposed by Artola et al. (1990) in which long-term depression is obtained if the level of postsynaptic depolarization reaches a certain level, but stays below that required for NMDA receptor activation, while LTP is induced if a second, higher level of depolarization is reached permitting NMDA receptor activation. It will be of interest to determine if this model continues to hold in the intact preparation, where the stimulation must be spaced and repeated.

Neocortical LTP in the freely-moving animal may be modulated both GABAergically and cholinergically

Administration of either diazepam (Figure 4.1) or scopolamine (Figure 5.1) prior to the delivery of high-frequency stimulation slows the development of neocortical LTP. These data are in agreement with previous electrophysiological results showing that these drugs prevent the induction of hippocampal and pyriform cortex LTP (Del Cerro et al.,

1992; Hirotsu et al., 1989) as well as behavioural results indicating that GABAergic (Tomaz et al., 1982; Lister, 1985) and cholinergic (Whitehouse, 1964; Meyers, 1965; Berger and Stein, 1969; Watts et al., 1981; Flood and Cherkin, 1986; Decker et al., 1990; Lamberty and Gower, 1991) antagonism interfere with learning and memory task performance. As well, it was shown that the administration of pilocarpine enhances LTP of the polysynaptic components (Figure 5.1) which is consistent with previous reports on the effects of cholinergic agonism on the induction of hippocampal and visual cortex LTP (Blitzer et al., 1990; Burgard and Sarvey, 1990; Brocher et al., 1992) as well as learning and memory (Whitehouse, 1966; Haroutunian et al, 1985; Lamberty and Gower, 1991).

In addition, a number of new results were presented that differed from those reported previously. In the freely-moving rat, the administration of picrotoxin prior to the delivery of trains appears to *slow* the development of neocortical LTP (Figure 4.1 and 4.3) . This is in contrast to *in vitro* data which has shown the opposite effect (Wigstrom and Gustafsson, 1983, 1985; Abraham et al., 1987), but is in agreement with behavioural data showing that picrotoxin may impair some memory tasks (Nabeshima et al., 1988; Chavez et al., 1995). Moreover, it was shown that prior exposure to diazepam coupled with high-frequency stimulation impairs the development of subsequent potentiation.

Electroconvulsive stimulation can induce a temporally-graded blockade, and disruption, of neocortical LTP in the freely-moving animal

ECT is known to produce substantial memory loss in humans, and so it was of interest to know what the effects of such stimulation would be on the induction and

expression of long-term potentiation. MES stimulation applied 5 minutes, or 1 hour after, LTP-inducing trains prevented the induction of LTP. However, MES stimulation applied 6h after LTP-inducing trains did not prevent the induction of LTP. There thus appears to be a window within which MES can interrupt the induction of LTP. This is theoretically interesting in the context of data indicating that retrograde amnesia gradients reflect the time course of memory consolidation (eg: McGaugh and Herz, 1972; Maki, 1985; Bohbot et al., 1996) and suggests that the consolidation gradients characteristic of both learning tasks and neocortical LTP are mediated by a common mechanism.

Although MES stimulation was found to attenuate an already potentiated response, it recovered back to its potentiated level within 24 hours. This result agrees with *in vitro* data from Hesse and Teyler (1976), and confirms that the prevention of LTP by MES was not the product of some non-specific decrease in tissue excitability.

What is the significance of neocortical LTP in vitro?

If neocortical LTP *in vivo* is related to learning and memory processes and appears substantially different from neocortical LTP induced *in vitro*, then an obvious question needs to be addressed; namely, what is the role of the LTP recorded in the neocortical slice preparation? One possibility is that the surgical alteration of the circuitry leaves the tissue more reactive. Another possibility is that the electrode placements are more readily optimized in the slice so that the small increments of potentiation induced by a single session of stimulation are more readily seen.

We must also consider the possibility that the phenomenon induced in the slice is mechanistically different from that induced in the chronic preparation. It is possible, for example, that the LTP induced in the slice is more closely related to developmental plasticity than to learning and memory. This would be consistent with its age dependency. During a certain period of early postnatal development, neocortical neurons are vulnerable to experience-dependent modifications of their response properties. In rat visual cortex, for example, the critical period extends from approximately postnatal day (P) 18, when the optics clear, to P45 (Kirkwood et al., 1995). The induction of visual cortical LTP *in vitro* also appears to possess a critical period, showing the highest probability of induction between P11-20 (Perkins and Teyler, 1988; Kato et al., 1991).

Recently, critical period plasticity of the types expressed in primary sensory cortices have been correlated directly with the induction of neocortical LTP. In both S1 and V1, the mature response features of cortical cells are believed to result in large part from the activity-dependent refinement of topographical projections from thalamic nuclei to the cortex (Goodman and Shatz, 1993). In rat primary somatosensory cortex, Crair and Malenka (1995) reported that the thalamocortical synapses comprising whisker barrels support LTP during a period that closely matches the time frame (from P1-4) in which barrels are maximally susceptible to modification by sensory perturbation. From P3-7, stimulation of the ventrobasal nucleus of the thalamus paired with intracellular depolarization of layer IV cells resulted in LTP that lasted at least 40 minutes. From P8-14, however, this protocol did not induce potentiation. This LTP could be blocked by either intracellular BAPTA injection or APV.

In rat primary visual cortex, Kirkwood et al. (1995) induced LTP in slices taken at different ages from light- and dark-reared rats. They found that LTP induced by white matter stimulation followed the critical period for visual development, being maximally induced from P14-21, thereafter declining to adult levels by P35. Moreover, the decline in the ability to support LTP shown by adult rats could be delayed by dark-rearing. Slices taken from 5-6 week old rats that had been raised in complete darkness showed equivalent LTP to slices taken from juvenile rats. These data provide powerful evidence for the hypothesis that LTP may mediate the patterning of cortical circuits.

The effects reported by both Crair and Malenka (1995) and Kirkwood et al. (1995) may be mediated by maturational changes in the responsiveness of the NMDA receptor. In the former study, the demonstration that both BAPTA and APV blocked LTP clearly suggest a role for the NMDA receptor, while evidence from previous studies indicate that the NMDA receptor may play an important role in developmental plasticity of the visual cortex. Tsumoto et al. (1987) found that APV blocked visual cortex neuronal responses more effectively in critical period-aged kittens than in adults, suggesting that NMDA receptors may be preferentially involved in plastic change during that time. Further, Fox et al. (1991) demonstrated that the loss of NMDA receptor function normally associated with maturation can be delayed if animals are dark-reared. This observation is important as dark-rearing was originally shown to extend the critical period for geniculocortical axon segregation in visual cortex (Cynader and Mitchell, 1980), a process that was later shown to be NMDA-dependent (Bear et al., 1990). Moreover, as was discussed in chapter 3, a variety of manipulations interfering with the

normal functioning of the NMDA receptor have been shown to alter the development of the visual cortex (e.g.: Kleinschmidt et al., 1987; Gu et al., 1989; Bear et al., 1990).

There is good evidence for the notion that many developmental and use-dependent plasticity processes are related to, or perhaps mediated by, a mechanism like long-term potentiation. An important question that remains to be answered is whether the effects reported *in vivo* are, in fact, related to those seen *in vitro*. Although it is tempting to speculate that LTP *in vitro* is related to critical period plasticity while LTP *in vivo* mediates learning and memory, the technical difficulties associated with performing a developmental study using the *in vivo* preparation described in this thesis preclude a direct comparison of the two preparations, at least on this question. LTP in the adult, however, can be more directly compared between *in vitro* and *in vivo* preparations by taking slices from pre-potentiated animals. This work is underway.

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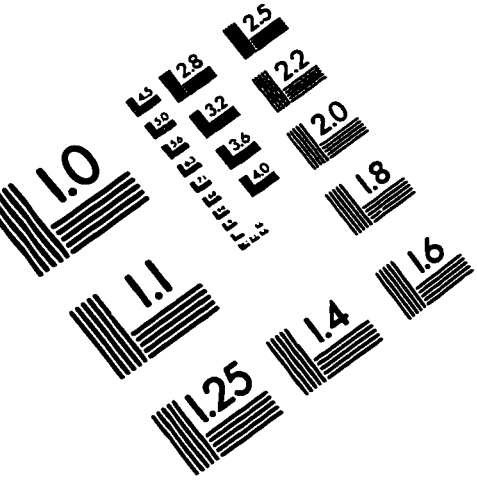
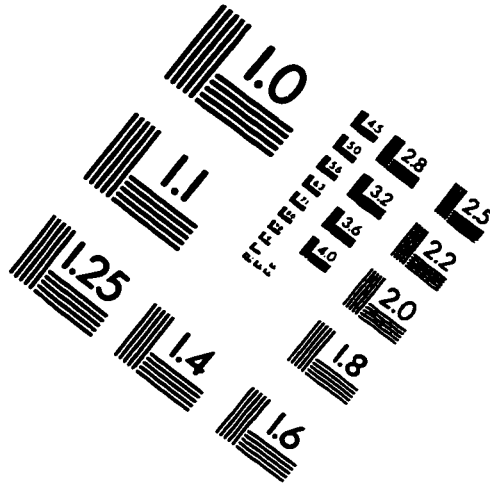
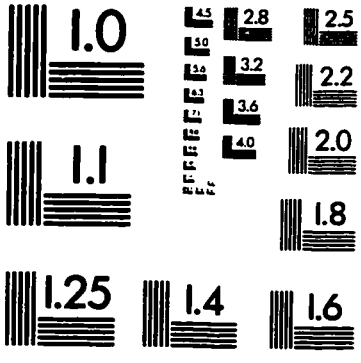
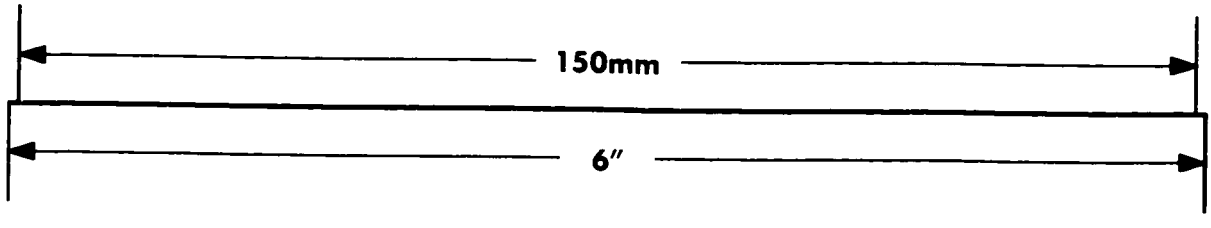
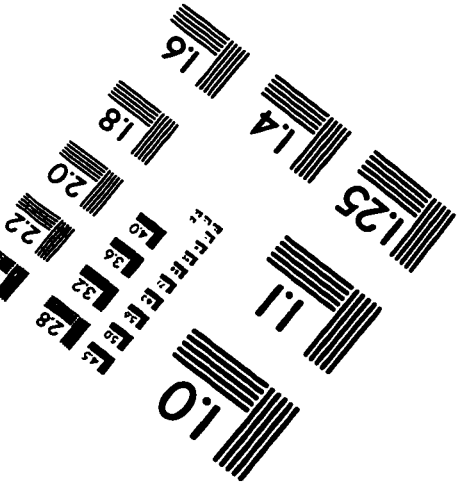
IMAGE EVALUATION TEST TARGET (QA-3)

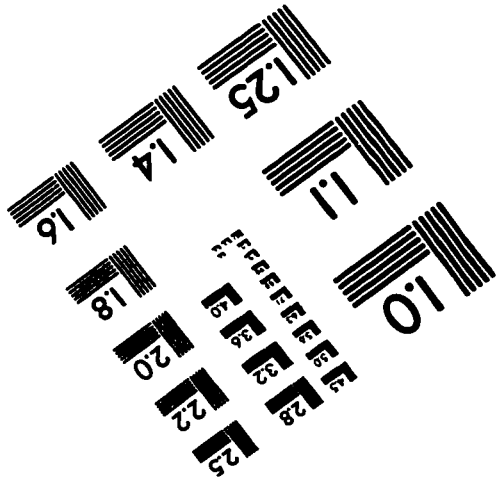












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989