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

By

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ABSTRACT

Correlated firing between adjacent neurons results in increased synaptic efficacy between synapses. This effect is called long-term potentiation (LTP) and is widely regarded as the mechanism underlying learning and memory because it is long-lasting and has been demonstrated in areas of the brain involved in information encoding and storage. Although definitive proof that LTP underlies learning and memory remains elusive, mounting evidence suggests that LTP, learning and memory are related and that LTP is a viable candidate for information storage.

Understanding the nature of the relationship between LTP, learning and memory requires further investigation into the characteristics of LTP in the neocortex of the behaving rat. Neuromodulators have proven integral for successful learning. For this reason, their effect on LTP is of high interest. While neuromodulation of neocortical LTP has been studied *in vitro*, it has never before been investigated in the whole organism. This thesis explored the effects of cholinergic, noradrenergic and dopaminergic modulation on neocortical LTP induction in the behaving rat.

In the cholinergic and noradrenergic experiments, significant group-by-session interactions were obtained in the early-component (monosynaptic) measures of LTP induction: Scopolamine, the cholinergic antagonist, blocked

LTP while clenbuterol, a beta-noradrenergic agonist, enhanced LTP.

The measure of LTP taken from the polysynaptic component resulted in significant group-by-session interactions for three modulatory manipulations. Pilocarpine, the cholinergic agonist, and apomorphine, the dopamine agonist, facilitated LTP. Clenbuterol and the beta-noradrenergic antagonist propranolol attenuated and tended toward attenuating LTP, respectively. Additionally, the neuroleptic haloperidol induced LTD.

Taken together, these results provide more evidence for a relationship between LTP and information processing. However, these results also highlight the complexity of neuromodulatory actions. Further research will aim to determine a more direct link between learning and memory by ascertaining dose-responses curves and applying them to behavioral tasks.

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This thesis is dedicated to my parents, Robbie E. and Stephen J. O'Connor and Ben H. (Jr.) and Donna H. Boyd. Thank you for your love and unwavering encouragement. I am grateful for the sacrifices you have made so that I may have opportunities.

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Tomorrow, and tomorrow, and tomorrow
Creeps in this petty pace from day to day
To the last syllable of recorded time,
And all our yesterdays have lighted fools
The way to dusty death. Out, out brief candle!
Life's but a walking shadow, a poor player
That struts and frets his hour upon the stage
And then is heard no more. It is a tale
Told by an idiot, full of sound and fury,
Signifying nothing.

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LIST OF ABBREVIATIONS

5-HT: Seratonin
a: Amplitude of the response to a single quantum of neurotransmitter
ACh: Acetylcholine
A/D: analog-to-digital converter
ANOVA: Analysis of variance
AMPA: Alpha-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic
Amyg: Amygdala
APO: Apomorphine injection without LTP trains
APOTR: Apomorphine injection with LTP trains
ATP: Adenosine triphosphate
Ca^{2+}: Calcium
CaMKII: Alpha-calcium-calmodulin-dependent protein kinase II
cAMP: Cyclic adenosine monophosphate
cGMP: Cyclic guanine monophosphate
CLENO: Clenbuterol injection without LTP trains
CLENTR: Clenbuterol injection with LTP trains
CR: Conditioned response
CS: Conditioned stimulus
CTA: Conditioned taste aversion
$D_{1\text{-}5}$: Dopamine receptors 1-5
D-APV: D-2-amino-5-phosphonopentanoic acid
DA: Dopamine
DAG: Diacylglycerol
DMSO: Dimethyl sulfoxide
EEG: Electroencephalograph
EPSP: Excitatory postsynaptic potential
ER: Endoplasmic reticulum
fMRI: Functional magnetic resonance imaging
GABA: Gamma-aminobutyric acid
GDP: Guanosine diphosphate
Gpe: Globus pallidus external
Gpi: Globus pallidus internal
GTP: Guanosine triphosphate
h: Hour
H2OTR: Distilled water injection with LTP trains

HAL: Haloperidol injection without LTP trains
HALTR: Haloperidol injection with LTP trains
Hipp: Hippocampus
HMS: Hippocampal memory system
i.c.v.: Intracerebroventricular
i.p.: Intraperitoneal
I_{AHP}: Afterhyperpolarization current
I/O: Input-output measure
I_M: M-current
I_{sADP}: Slow afterdepolarization current
IP_3: inositol-1,4,5-triphosphate
IPSP: Inhibitory postsynaptic potential
K^+: Potassium
LTD: Long-term depression
LTM: Long-term memory
LTP: Long-term potentiation
M_1-M_5: Cholinergic muscarinic receptors 1-5
mGlu: Metabotropic glutamate receptor
mIPSCs: Miniature inhibitory postsynaptic currents
mRNA: Messenger ribonucleic acid
N: Number of release sites of neurotransmitter quanta
NA: Noradrenaline
Na^+: Sodium
NLS: Neocortical learning system
NMDA: *N*-methyl-D-aspartate
p: Probability of the release of a single quantum of neurotransmitter
PET: Positron emission tomography
PKA: Protein kinase A
PILO: Pilocarpine
PIP_2: Phospholipid phosphatidylinositol-4,5-biphosphate
PLC: Phospholipase C
PMTC: Pontomesencephalotegmental
PROPO: Propranolol hydrochloride injection without LTP trains
PROPTR: Propranolol hydrochloride injection with LTP trains
SALTR: Saline injection with LTP trains
SCOP: Scopolamine
sIPSCs: Spontaneous inhibitory postsynaptic currents
SNc: Substantia nigra pars compacta
SNr: Substantia nigra pars reticulata
STM: Short-term memory
Thal:Thalamus

UCR: Unconditioned response
UCS: Unconditioned stimulus
VTA: Ventral tegmental area

PREFACE

Chapter two of this thesis includes modified versions of research and text that have appeared previously in the following journal article:

Boyd TE, Trepel C, Racine RJ (2000) Cholinergic modulation of neocortical long-term potentiation in the awake, freely moving rat. *Brain Research*, 881, 28-36.

Dr. Christopher Trepel was responsible for the low-intensity stimuli experiments while Tiffany Boyd was responsible for the high-intensity stimuli experiments. Because the latter builds upon the former, both manipulations were published together in the aforementioned article.

CHAPTER ONE

GENERAL INTRODUCTION

"You have to begin to lose your memory, if only in bits and pieces, to realize that memory is what makes our lives. Life without memory is no life at all... Our memory is our coherence, our reason, our feeling, even our action. Without it, we are nothing." – Luis Buñuel

1.1 HISTORICAL CONTEXT

Experimental psychologists seek to elucidate processes of the mind and unravel their mechanisms. From Ebbinghaus' work in the late 19th century to present-day cognitive and neuropsychological investigations, a major focus of these efforts has been learning and memory. How do mammalian nervous systems encode and store experience? In the last 50 years, scientists have begun to work across disciplinary boundaries, producing rapid advances in learning and memory research (for thorough historical perspectives, see Polster et al., 1991; Kandel and Squire, 2000).

Although many issues remain unresolved[1], researchers have made substantial progress toward identifying the processes involved in learning and memory and their underlying neural mechanisms.

1.2 MEMORY SYSTEMS

1.2.1 Short- and Long-Term Memory

The distinction between short- (STM) and long-term memory (LTM) remains one of the most widely accepted components of memory theory (for review of this distinction and other concepts discussed in this section, refer to Squire et al., 1993) and it is supported by research on both human and non-human subjects. Brenda Milner's extensive work with the amnesic patient H.M. has yielded classic data supporting the distinction between STM and LTM. H.M. underwent bilateral temporal lobe excision to alleviate epilepsy – surgery that left him with both severe anterograde and graded retrograde amnesia (Scoville and Milner, 1957). H.M.'s digit-span recall and other tests of STM remain normal. However, his LTM deficits illustrate the complexity of this type of memory. H.M.'s graded retrograde amnesia allows for some recall of remote autobiographical information that pre-dates the surgery. In contrast, H.M.'s

[1] Over the last 200 years, learning and memory research has focused upon three main areas: consolidation, or the temporal properties surrounding memory storage; memory representations, or the manner in which the memory is stored; and the existence of multiple memory systems. Debate continues over both the operational definition, and length of time required for consolidation of an experience into memory. Discussions regarding the nature of memory storage have centered upon local or distributed representations within the brain. This debate has concerned both how and where memories are stored. Finally, distinctions between memory systems and the types of

anterograde amnesia prevents him from storing some types of information in LTM (for the last 40 years, Milner and her associates have had to reintroduce themselves to H.M. prior to each testing session!)

Wright et al., (1985) showed a distinction between STM and LTM in pigeons, monkeys and humans using primacy and recency demonstrations. All subjects were shown a series of four colored slides followed by a variable delay and then a test slide. The subjects then indicated if the test item matched one of the four previously presented items. Primacy effects resulted in a large number of correct identifications when the test slide matched the first item, while recency effects led to more accurate identification when the test slide matched the last item. When the test item was presented immediately after the series, all species' answer patterns reflected a recency effect. All subjects demonstrated both primacy and recency effects within delay intervals of 1-2 sec, 1-10 sec and 10-60 sec for pigeons, monkeys and humans respectively. However, when the test item was presented after the delay intervals (e.g., 60+ sec delay for humans), only the primacy effect persisted. The authors assert that an STM accounts for the recency effect while a longer-lasting memory process accounts for the persistence of the primacy effect (Wright et al., 1985).

1.2.2 Declarative vs. Non-Declarative Memory

Within LTM, a distinction between declarative and non-declarative types of memory has emerged (Cohen and Squire, 1980). Declarative memory, also called

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learning and memory they govern remains at the forefront of investigation. The latter two areas

explicit memory, refers to memories for specific facts and events. The declarative memory *system* mediates conscious recall and recollection processes (Cohen and Squire, 1980) as well as material-specific information encoding and recollections (e.g., faces, objects and words) (Squire et al., 1993). The declarative memory system is also hypothesized to include memories tied to a particular place and time, and may include the source of one's own knowledge. Non-declarative memory, also called implicit memory, comprises habits, perceptual learning (Knowlton and Squire, 1993), some forms of conditioning (Zola-Morgan and Squire, 1993), motor skills requiring practice (Cohen and Squire, 1980), priming and some types of habituation. Expression of declarative memory requires conscious recollection, whereas non-declarative memory is expressed through performance (Squire et al., 1993). For example, Milner (reviewed in Milner et al., 1998) demonstrated that H.M. could learn to trace the outline of a figure using a mirror as a guide. H.M.'s ability improved with practice sessions, even though he had no recollection of ever learning the task or of previous practice.

Animal studies cannot be discussed in terms of consciously recollected memories. However, spatial memory tasks are widely thought to tap analogous declarative memory processes in animals. In an experiment using rodents, Kesner and colleagues (1993; DeCouteau and Kesner, 2000) demonstrated a dissociation between declarative and non-declarative memory. Rats with hippocampal, but not caudate, lesions showed impairments on a declarative spatial memory task

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will be briefly reviewed here.

whereas rats with caudate, but not hippocampal, lesions showed memory impairments for a non-declarative motor-response task (Kesner, 1993; DeCouteau and Kesner, 2000). These data suggested not only a dissociation between declarative and non-declarative memory, but also that different brain areas may underlie each.

1.2.3 Episodic vs. Semantic Memory

Declarative memory can be further divided into episodic and semantic categories (Squire et al., 1993). Episodic memory includes memories for specific autobiographical facts and events occupying a spatial and temporal context (e.g., recalling a trip to the zoo as a child), while semantic memory comprises general knowledge about the world (e.g., knowing that Austin is the capital of Texas). Both types of memory involve conscious processing during recollection. A thorough discussion of this distinction is beyond the scope of this thesis. Interested readers are referred to Tulving (1985) for review.

1.3 THE NATURE OF MEMORY STORAGE

1.3.1 Where Declarative Memory Resides

Beginning with Karl Lashley's search for the memory trace, or engram (1950), the quest to identify the absolute location of memory storage has continued at both the macro and micro levels (Polster et al., 1991). At the macro level, researchers have investigated whether memory traces are stored in one or a few discrete, localized areas of the brain, or conversely, whether the traces are

scattered throughout many areas of the brain. At the micro level, researchers have investigated whether memory traces are arranged in a one-to-one neural representation or distributed within a network of neurons (Polster et al., 1991).

Studies employing human amnesics and animal models have identified several structures that are important for declarative memory (Squire et al., 1984; Zola-Morgan et al., 1986; Eichenbaum, 2000; Zola et al., 2000; Clark et al., 2001). Within the temporal lobe, the hippocampus and related cortices (entorhinal, perirhinal, and parahippocampal) have been shown to play a critical role (Squire et al., 1993; Eichenbaum et al., 1996). As well, damage to structures within the diencephalon, such as the anterior thalamic nucleus, mediodorsal nucleus, and connections between the medial thalamus traversing the internal medullary lamina, can disrupt memory (Squire et al., 1993). The establishment of accessible declarative memories requires that both the hippocampal complex and diencephalic system are intact (Zola-Morgan and Squire, 1993; Squire et al., 1993).

Although the hippocampal complex and diencephalon play an important role in declarative memory encoding, these structures do not operate in isolation (Teyler and DiScenna, 1986; Eichenbaum, 2000; Lavenex and Amaral, 2000). Teyler and DiScenna (1986) proposed that the hippocampal complex-diencephalic system interacts with the cortex and other structures during initial encoding. They hypothesized that discrete neocortical loci initially register experiential information. The hippocampus then indexes the spatiotemporal characteristics of

the neocortical loci. Reinstatement of the representation occurs when the neocortical loci are reactivated, and this reactivation depends on the indexing function of the hippocampus. Repeated communication between the neocortex and hippocampus strengthens the memory. If the specific hippocampal to cortical pathways are not reactivated, the memory will fade because the indices within the hippocampus will decay as a function of time (Teyler and DiScenna, 1986).

Experiments using human and non-human subjects support a time-limited role for the hippocampus (and related structures) in the storage of long-term, declarative memory (Squire et al., 1984). Retrograde amnesia studies offer some of the most compelling evidence in favor of this hypothesis. In graded retrograde amnesia, events occurring immediately before trauma onset are completely lost while remote memories remain intact (Zola-Morgan and Squire, 1990; Milner, 1998). The severely amnesic patient H.M. can recall remote episodic memories more reliably than events leading up to the surgery (Sagar et al., 1985). Moreover, monkeys also show graded retrograde amnesia following hippocampal damage. In a controlled experiment, two groups of monkeys were taught 100 object pairs (20 at each of five pre-operative stages) 16, 12, 8, 4, and 2 weeks before one group underwent excision of the hippocampal formation. After surgery, the pairs were presented to the monkeys in a mixed order. Control monkeys demonstrated a recency effect, remembering objects learned in the sessions immediately prior to surgery better than remote sessions. Conversely, monkeys without hippocampi and related structures remembered the more remote

sessions with the same accuracy as the control monkeys but had no memory of the sessions occurring closer to the time of surgery (Zola-Morgan and Squire, 1990). In effect, these monkeys displayed a graded retrograde amnesia similar to that experienced by humans made amnesic as the result of damage to the hippocampus and associated diencephalic areas. Similar studies using fear-conditioning (Kim and Fanselow, 1992) and acquired food preference (Winocur, 1990) in rats, and maze running in mice (Cho et al., 1991), have also demonstrated a temporally graded retrograde amnesia following damage to the hippocampal complex and diencephalic regions. Thus, the temporal dependency on the hippocampal memory system appears to be conserved across species.

The mechanism by which memories become independent from the hippocampal memory system has received less attention. However, most investigators believe they are "consolidated" in the neocortex (for review see Zola-Morgan and Squire, 1993; McClelland et al., 1995). One widely held variation on the indexing theory proposes that the hippocampal and diencephalic memory systems initially bind together the distributed sites within the neocortex that represent the entirety of the event (Zola-Morgan and Squire, 1990). The indexing of the relevant components of the event provides the basis for retrieval of the memory for a limited time (Teyler and DiScenna, 1986). Eventually, through a period of consolidation[2], the neocortex assumes full responsibility for

[2] According to Squire and colleagues (1993), the period of consolidation varies depending upon the strength of the initial learning, the species and the rate of forgetting.

storing and retrieving the memory. Once this has occurred, damage to hippocampal and diencephalic structures will not disrupt the memory.

1.3.2 Where Non-Declarative Memory Resides

Researchers have identified several structures believed to mediate non-declarative memory (habits, motor and perceptual skills, memories expressed as performance). Recent work, for example, suggests that the corticostriatal system, including the neocortex, caudate, putamen and their interconnections, may underlie perceptual skill acquisition (Squire et al., 1993; Teng et al., 2000; DeCouteau and Kesner, 2000).

In a recent study, (Teng et al., 2000) 11 monkeys with hippocampal lesions and seven monkeys with combined lesions of the hippocampus and tail-of-the-caudate nucleus, were tested on two-choice object discrimination, 24-hour concurrent discrimination[3] and pattern-discrimination learning tasks. In the two-choice object discrimination task, monkeys make use of three-dimensional shape, color and size characteristics over several trials to learn which of two objects is always rewarded (e.g., a red vs. a green peanut shell). Only one pair of objects is trained at a time and learning typically occurs quickly within a single session after only a few trials. Concurrent discrimination learning requires that the monkey learn 8 pairs of objects simultaneously. In this study, the objects varied in color, shape and size. In the pattern-discrimination task, monkeys were trained to

[3] In the 24-hour concurrent discrimination task, researchers present monkeys with pairs of objects for one trial each day. One of the objects in the pair is correct. Normal monkeys learn to identify the correct object after repeated exposure over a period of days.

differentiate between choices lacking distinctive color, three-dimensional shape and size cues (e.g., a two-dimensional plus sign vs. a square or an "N" vs. a "W"). It is widely believed that the two-choice object discrimination task requires declarative learning strategies, while the concurrent and pattern-discrimination tasks require non-declarative learning strategies (Squire et al., 1993). Monkeys learn the latter two tasks incrementally over many trials. It has been suggested that monkeys may learn pattern discrimination and concurrent discrimination similar to the way humans learn motor skills or habits (Iversen, 1976). Thus, learning these types of tasks may not require the medial temporal lobe. More specifically, Teng and colleagues wanted to test the possibility that learning the concurrent- and pattern discrimination tasks may require cortico-striatal pathways (2000).

Monkeys with lesions restricted to the hippocampus performed poorly on the object-discrimination task suggesting that the hippocampus is required for this type of learning task and supporting the idea that this task requires declarative learning strategies. These monkeys performed normally on the concurrent- and pattern-discrimination tasks suggesting that the hippocampal formation is not required for these tasks. Monkeys with combined hippocampal and tail-of-the-caudate lesions performed poorly on all three tasks (Teng et al., 2000) supporting the idea that pattern-discrimination and concurrent-discrimination tasks rely on striatal pathways and may require non-declarative learning strategies. Taken together, these results offer further support for the distinction between declarative

and non-declarative memory and that the hippocampus and striatum contribute differently to these types of memory, respectively.

Conditioning, another widely recognized form of implicit memory (Zola-Morgan and Squire, 1993), appears to be mediated by different areas of the brain depending upon the stimuli. Conditioning involves pairing a stimulus that evokes a specific response (the unconditioned stimulus or UCS) with a neutral stimulus (with respect to the response). Eventually, the neutral stimulus (the conditioned stimulus or CS) alone will generate a conditioned response (or CR). The CR reflects the acquisition of an anticipatory response to the UCS. Subjects learn this relationship incrementally over repeated presentations of the paired stimuli. Conditioned taste aversion (CTA) and eye-blink conditioning have proven to be valuable paradigms for investigating this type of non-declarative memory. During CTA, rats learn to associate a specific taste to the malaise induced by a lithium chloride injection. The anterior insular cortex has been implicated in this conditioned response as lesions of this area prevent CTA (Lasiter et al., 1985).

Eye-blink conditioning experiments conducted by Richard Thompson have supported a role for the cerebellum in non-declarative (sensory-motor) learning and memory (reviewed in Kim and Thompson, 1997). In the eye-blink conditioning paradigm, the CS, (usually a tone) is paired with a UCS (an air puff to the eye). Naïve animals blink (UCR) in response to the UCS. However, experiencing the CS and UCS within close temporal proximity causes the animal to develop a conditioned response; it blinks (CR) in response to the tone. Lesions

to the cerebellar cortex or interpositus nucleus prevent acquisition of eye-blink conditioning. Moreover, mutant mice possessing reduced numbers of cerebellar Purkinje cells do not readily acquire the conditioned eye-blink response (Kim and Thompson, 1997).

Priming, another intensively studied form of non-declarative memory (Squire et al., 1993), occurs when the initial exposure to a stimulus facilitates the processing of subsequent exposures. Imaging studies have shown that repeated exposure to stimuli causes reduced activation in the brain. For example, positron emission tomography (PET) has revealed that the initial exposure to words, nonwords, letter strings and letter-like shapes activate an area in the right posterior parietal cortex called the lingual gyrus (Squire et al., 1992). Subsequent exposure to the same stimuli decreased activation in this area (Squire et al., 1992)[4]. Subjects also showed this effect when primed with words written backwards. Using functional magnetic resonance imaging (fMRI), Poldrack and Gabrieli (2001) showed that initial presentation of the stimuli resulted in activity in left inferior temporal, left inferior prefrontal and right cerebellar areas. Upon repeated presentation, the subjects showed reduced activity in these same areas (Poldrack and Gabrieli, 2001).

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[4] It has been argued that the right hemisphere is more adept as processing form-specific components of priming tasks while the left hemisphere may support more abstract perceptual priming (Squire et al., 1993). Squire et al. (1992) presented stimuli that did not change case and remained in the same modality. Their results showed decreased activation upon repetition in the right hemisphere. Poldrack and Gabrieli (2001) claim that their priming generalized to different spatial transformations (inverted text, and normal letters written backwards as well as mirror writing). These results taken together seem to support the speculation of Squire (1993).

The reduced brain activity upon repeated presentation has been interpreted to indicate more efficiency in processing a familiar stimulus; that is, less neural activity is necessary to encode the information making faster recognition possible (Squire et al., 1993). Recent work supports this theory. Using a visual object priming task and fMRI, James and colleagues (2000) showed that brain activity increases and peaks at recognition and then sharply decreases. Recognition occured more quickly for primed stimuli and dissipated rapidly after recognition (i.e., the slope of brain activity is steeper for primed versus non-primed objects). However, the overall amplitude of brain activity for primed and non-primed objects did not differ (James et al., 2000).

1.3.3 Summary: The Location of Memory Storage

In summary, memory research has distinguished several kinds of memory and different neurobiological systems underlying each. The declarative system is believed to rely upon the diencephalic structures and the hippocampal formation (Squire et al., 1984; Zola-Morgan et al., 1986; Squire et al., 1993; Eichenbaum et al., 1996; Zola et al., 2000; Clark et al., 2001). The declarative memory system works in concert with the neocortex to form conscious recollections and consolidate autobiographical details (Cohen and Squire, 1980; Teyler and DiScenna, 1986; McClelland et al., 1995; Eichenbaum, 2000; Lavenex and Amaral, 2000). The non-declarative memory system comprises skill and habit learning (Cohen and Squire, 1980; Kesner, 1993; DeCoteau and Kesner, 2000), some forms of conditioning (Zola-Morgan and Squire, 1993), and priming as well

as the processes through which these memories are formed. These memories do not require participation of the hippocampal formation and diencephalic structures. Rather, non-declarative memories involve interactions between various other subcortical structures (e.g., the neostriatum) and the neocortex.

Why would the brain support two distinct memory systems? Neural network modelers have proposed a comprehensive theory of memory that reconciles the existence of dual memory systems and elucidates their adaptive value to the organism (McClelland et al., 1995; McClelland and Goddard, 1996). McClelland and colleagues, building upon work by others (e.g., Teyler and DiScenna, 1986), suggest that complimentary learning systems exist in the mammalian brain: the Neocortical Learning System (NLS) and the Hippocampal Memory System (HMS) (McClelland et al., 1995; McClelland and Goddard, 1996).

The NLS specializes in gradual extraction of common structure from multiple learning experiences into one representation. Extraction of common structure requires strengthening of connections among neurons that will support generalization (McClelland and Goddard, 1996). The structure of an experience is compared to previous experiences through *interleaved learning* (McClelland et al., 1995). Interleaved learning describes the process whereby the neocortex integrates what it learns in one situation with ongoing learning about previous situations.

The HMS stores compressed representations of new memories so that they can be used to reinstate the fragmented cortical representations and allow

interleaved learning with ongoing experience and other memories (McClelland and Goddard, 1996). Similar to the ideas proposed by Teyler and DiScenna (1986), McClelland and Goddard (1996) postulate that the memories contained within the HMS are not complete copies of cortical activation patterns. Whereas Teyler and DiScenna (1986) proposed that the hippocampal formation binds together elements of memories in various areas of the neocortex, McClelland and Goddard (1996) hypothesize that the HMS serves to actually store abbreviated descriptions that exploit redundancies in cortical activation patterns.

The adaptive value of dual memory systems becomes apparent when underlying characteristics of declarative and non-declarative memory are further analyzed. Declarative memory is characterized as being fast and not always reliable (i.e., prone to retrieval error). Research using neural network models has suggested that because of its flexibility, the declarative memory system is fallible and prone to interference[5] (McClelland et al., 1995; McClelland and Goddard, 1996). However, declarative memory is advantageous because it allows for quick encoding and storage of biologically significant events. Fast learning ensures quick acquisition of new information without repetition. The fast hippocampal system also permits immediate access to the new learning for rapid adaptation. In contrast to the declarative memory system, it is believed that the non-declarative memory system is reliable and consistent, but slow (McClelland et al., 1995;

[5] Interference occurs when the acquisition of new information disrupts the integrity of existing information. Catastrophic interference, a theoretical phenomenon, occurs when the new information acquisition results in the complete loss of the existing information.

McClelland and Goddard, 1996). The interleaved learning strategy is effective for long-term learning and protects against interference. However, this slow process does not afford opportunities for the organism to quickly adapt to a rapidly changing environment.

1.3.4 How Memories are Stored

The exact processes underlying memory storage remain undetermined. However, one candidate theory was postulated by Karl Lashley's most famous student, Donald Hebb. Hebb first postulated his theory of information storage in the 1940s. He argued that information is stored in distributed neural networks, or cell assemblies, constructed by increasing the strength of the synaptic connections between the participating neurons. In his influential work, *The Organization of Behavior* (1949), he proposed, "When the axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased" (p.62). Hebb's theory continues to guide current memory research.

Bear et al., (1996) summarized the main points of Hebb's cell assembly theory as follows: 1) Reverberation of the neural activity provides short-term storage; 2) Neurons in the cell assembly that are active simultaneously strengthen their connections with each other; 3) Memory of the event is contained within the strengthened connections; 4) Partial reactivation of the assembly leads to the activation of the entire cell assembly and results in recall of the learned event. By

inference, if the initial reverberation is disrupted, any hypothesized structural changes cannot be initiated and the consolidation of the information into long-term memory store fails to take place (Lechner et al., 1999).

1.4 LONG-TERM POTENTIATION: A MODEL OF MEMORY STORAGE

1.4.1 The Discovery of a Plasticity Phenomenon

Since the publication of Hebb's *The Organization of Behavior*, a growing body of evidence has emerged supporting the idea that memories are represented as lasting changes in the functional circuitry of the brain, and that synaptic connections between neurons serve as the pliable substrate for memory traces. In 1966, a graduate student in Per Andersen's lab, Terje Lømø, serendipitously discovered a phenomenon that has provided considerable support for Hebb's hypothesis (Lømø, 1966). Using implanted electrodes, Lømø stimulated the perforant path, a bundle of fibers leading from the entorhinal cortex to the granule cells of the hippocampus. He found that repetitive, high-frequency stimulation caused increases in the granule cells' responses. After a period of rest, Lømø tested the pathway again with a single pulse. To his surprise, the single pulse elicited an enhanced response and subsequent testing revealed that this enhancement lasted for hours. Further investigations (Bliss and Lømø, 1973; Bliss and Gardner-Medwin, 1973) verified this long-term potentiation (LTP) effect and raised questions about the possibility of the new phenomenon being a cellular mechanism for information storage. These initial experiments established

the groundwork for testing the idea that appropriate patterns of activation, and the correlated firing of the pre- and postsynaptic neurons, could result in a long-lasting increase in synaptic efficacy just as Hebb predicted almost two decades earlier.

Although LTP was first demonstrated in the perforant path, most LTP research has been conducted on the synapses between the Schaffer collateral/commissural axons and the apical dendrites of CA1 pyramidal cells (reviewed in Malenka and Nicoll, 1999). However, it has been suggested that LTP at these synapses closely approximates the LTP effects demonstrated in other areas of the mammalian brain, including the neocortex *in vitro* (Bear and Kirkwood, 1993; Kirkwood et al., 1993; Malenka and Nicoll, 1999; but see Trepel and Racine, 1998).

1.4.2 Properties of LTP

LTP remains an attractive model for information storage because of its fundamental properties,[6] including cooperativity (McNaughton et al., 1978) and associativity (Levy & Steward, 1979). Additionally, it is long-lasting (Bliss and Gardner-Medwin, 1973; Reymann et al., 1985; Trepel and Racine, 1998) and has

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[6] Until recent studies demonstrating otherwise (Bonhoeffer et al., 1989; Engert & Bonhoeffer, 1997), LTP was thought to be input-specific (Andersen et al., 1977) Input specificity refers to the idea that when LTP is induced in one set of synapses, the increase in synaptic strength does not generalize to other synapses on the same cell. This property is advantageous because it increases the storage capacity of individual neurons (Malenka and Nicoll, 1999). However, Bonhoeffer and colleagues (Bonhoeffer et al., 1989; Engert & Bonhoeffer, 1997) have shown that potentiation spreads to neighboring synapses within 70 micrometers irrespective of activation history of the synapse. Although this finding has not been replicated, it calls into question one of the fundamental properties of LTP.

been demonstrated in many brain areas believed to support memory (e.g., Bliss and Lømø, 1973; Clugnet and LeDoux, 1990; Trepel and Racine, 1998).

High-frequency stimulation *per se* is not sufficient for LTP induction. The stimulation must be of high enough frequency to cause temporal summation of the excitatory postsynaptic potentials (EPSPs). As well, sufficient numbers of synapses must be simultaneously activated to promote spatial summation of EPSPs. This second condition is refered to as cooperativity – coactive synapses must 'co-operate' to produce enough depolarization to induce LTP (McNaughton et al., 1978).

If strong activation of one set of synapses occurs in close temporal proximity to weak activation of an independent set of synapses on the same postsynaptic cell, the activation of the first set facilitates induction of LTP in the second set. This property is called associativity and is often viewed as the cellular analogue to classical conditioning (Levy & Steward, 1979; Kirkwood and Bear, 1994; Malenka and Nicoll, 1999).

Once induced, LTP can last for hours *in vitro* (Reymann et al., 1985) and days (Bliss and Gardner-Medwin, 1973) and even months *in vivo* (Trepel and Racine, 1998). In the hippocampus, LTP comprises two (LTP1 and LTP2) (Racine et al., 1983) or three (LTP3) (Abraham and Otani, 1991) components. It has been shown that LTP1, LTP2 and LTP3 decay over a period of hours (Racine et al., 1983), days (Barnes, 1979; Racine et al., 1983; Jeffery et al., 1990) or weeks (Barnes and McNaughton, 1985; De Jonge and Racine, 1985; Bliss et al.,

1987) respectively. Moreover, both hippocampal (Maren and Baudry, 1995) and neocortical (Trepel and Racine, 1999) LTP can be disrupted by a variety of manipulations such as electroconvulsive shock[7].

As discussed previously, the hippocampus, together with the neocortex, plays an important role in declarative memory, while other subcortical structures (e.g., the striatum), in conjunction with the neocortex, are critical for many types of non-declarative memory. LTP has been induced in all of these structures (e.g., Bliss and Lømø (1973) for hippocampus; Trepel and Racine (1998) for neocortex; Clugnet and LeDoux (1990) for amygdala). Neocortical LTP induction *in vivo* is similar to most types of non-declarative learning, in that it requires repeated stimulation sessions (Racine et al., 1995), while hippocampal LTP is induced quickly (Racine et al., 1983).

1.4.3 Long-Term Potentiation Induction and Expression

The connection between synapses is strengthened when pre- and postsynaptic neurons fire in a correlated manner. The most widely studied form[8] of LTP begins when an action potential causes the release of the excitatory neurotransmitter glutamate into the synaptic cleft. Glutamate binds to alpha-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic (AMPA) receptors on the postsynaptic cell and increases permeability to Na^+. Influx of Na^+ causes depolarization of the postsynaptic cell. Once the depolarization reaches a

[7] Electroconvulsive shock has been shown to disrupt memory in humans and animals (briefly reviewed in Squire, 1986).

threshold, the Mg^{2+} ion blocking the ion channel associated with another type of glutamate receptor on the postsynaptic cell, the *N*-methyl-D-aspartate (NMDA) receptor, is ejected. The NMDA receptor allows Na^{+} and Ca^{2+} to pass into the cell (Nowak et al., 1984; Mayer et al., 1984). Induction of LTP in the CA1 region of the hippocampus (Coan et al., 1987; Malenka and Nicoll, 1993; Malenka and Nicoll, 1999) and in the neocortex (Artola and Singer, 1987; Artola and Singer, 1990; Kirkwood and Bear, 1994; Trepel and Racine, 1998) has been shown to be NMDA-receptor dependent. The NMDA receptor acts as a coincidence detector, activated when both the pre- and postsynaptic neuron are depolarized (Singer and Artola, 1991). Increased postsynaptic Ca^{2+} is necessary for LTP induction (Malenka and Nicoll, 1999). However, it is unknown whether postsynaptic Ca^{2+} is sufficient for LTP induction. Moreover, it is also unknown whether Ca^{2+} influx alone (e.g., through NMDA receptors or voltage-gated calcium channels) or in combination with Ca^{2+} released from internal stores (Malenka and Nicoll, 1999), triggers LTP induction.

Heightened Ca^{2+} levels initiate biochemical cascades that translate into increased synaptic strength. It is widely accepted that alpha-calcium-calmodulin-dependent protein kinase II (CaMKII) plays an important role in the signal transduction process (Nicoll and Malenka, 1999; Fukunaga and Miyamoto, 2000). High CaMKII concentrations are found within the postsynaptic density where

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[8] Not all forms of LTP are induced with this pattern of events. For example, hippocampal mossy fiber LTP is reportedly independent of both NMDA receptor activity (Harris and Cotman, 1986) and postsynaptic calcium activity (Mellor and Nicoll, 2001).

both AMPA and NMDA receptors are also located. CaMKII antagonism (Malenka et al., 1989) or genetic deletion of the subunit required for proper CaMKII functioning (Silva et al., 1992), also blocks LTP induction. As well, increased concentrations of CaMKII enhance synaptic transmission and occlude LTP induction in area CA1 of the hippocampus (Pettit et al., 1994).

In addition to its calcium-calmodulin dependent functions, CaMKII activity may outlive the Ca^{2+} excess in the postsynaptic cell because of its autophosphorylation capabilities (Lisman, 1994). Autophosphorylation enables CaMKII to become independent of Ca^{2+}-calmodulin and allows CaMKII activity to continue after Ca^{2+} levels have returned to baseline levels (Lisman, 1994). Autophosphorylation has been demonstrated after LTP induction (Fukunaga, et al., 1995). As well, replacement of endogenous CaMKII with a mutated form has been shown to block LTP (Giese et al., 1998). CaMKII may mediate the early phase of synaptic plasticity by phosphorylating the AMPA subunit GluR1 (Barria et al., 1997). Phosphorylation at this site increases existing AMPA receptor single-channel conductances and may recruit new high-conductance-state AMPA receptors (Derbach et al., 1999). In addition to CaMKII, other protein kinases have been suggested to contribute to LTP induction: protein kinase C (Agenstein and Staak, 1997), cAMP-dependent protein kinase (Suzuki, 1994), the tyrosine kinase, Src (Salter, 1998), and mitogen-activated protein kinase (Sweatt, 2001). Ultimately, these pathways can alter protein synthesis and trigger changes in synaptic connectivity that can last for days, weeks or months.

The retrograde messengers are a second class of molecules, after the protein kinases, that are putatively involved in LTP induction and expression. Following activation, it is thought that the postsynaptic cell releases messenger(s) that then drift back across the synaptic cleft to modulate presynaptic function. Putative retrograde messengers include nitric oxide, carbon monoxide, arachidonic acid and platelet activating factor (Malenka and Nicoll, 1999). However, evidence to date does not support a required role for any of these retrograde messengers in LTP induction[9].

NMDA- receptor dependent LTP induction begins in the postsynaptic cell, but it is uncertain whether the expression mechanisms are concentrated on the presynaptic side or the postsynaptic side (Bekkers and Stevens, 1990; Kullman and Nicoll, 1992; Stevens, 1993; Liao et al., 1995). The standard way to experimentally determine a pre- or postsynaptic locus is to conduct a quantal analysis of the release of neurotransmitter during LTP induction (Stevens, 1993). This type of analysis estimates 1) the amplitude (a) of the response to a single quantum of neurotransmitter; 2) the probability (p) of release; and 3) the number (N) of release sites. Increases in a, p, or N indicate more efficient synaptic transmission (Bekkers and Stevens, 1990). Increases in either p or N point toward a presynaptic mechanism while an increase in a (e.g., greater receptor sensitivity)

[9] Malenka and Nicoll (1999) argue that a specific retrograde messenger must meet three criteria before it is recognized as having a required role in LTP induction: 1) it is produced in CA1 pyramidal cell dendrites in response to NMDA receptor activation 2) LTP production is blocked when the activity of the retrograde messenger is inhibited, and 3) application of the retrograde messenger during afferent stimulation in conjunction with an NMDA receptor antagonist causes a

suggests a postsynaptic mechanism. Using isolated hippocampal neurons in cell culture, Bekkers and Stevens (1990) concluded that the mechanism of LTP expression is presynaptic – likely the result of an increase in the probability of quantal release rather than an increase in the number of neurotransmitter release sites. They also suggest that retrograde signals mediate this effect.

In contrast to the results of Bekkers and Stevens (1990), Kullmann and Nicoll (1992) used quantal analysis and determined that LTP expression could be mediated by both a presynaptic and a postsynaptic mechanism. These researchers found that LTP induction caused increases in quantal amplitude (a) in addition to quantal amount (p) (Kullman and Nicoll, 1992). It should be noted that these experiments were conducted in slice preparations, limiting analysis to a relatively short period of time following induction. The mechanism(s) underlying LTP that lasts for weeks or months may be quite different.

Investigations into the expression of LTP have also yielded theories about a postsynaptic locus. One hypothesis focuses upon modification in AMPA receptor function and/or number. It has been shown that synapses lacking functional AMPA receptors, so-called 'silent synapses', convert to functional synapses upon LTP induction (Liao et al., 1995). Moreover, the conversion is a result of AMPA receptors being inserted into the postsynaptic membrane on a relatively rapid time scale of tens of minutes (Lüscher et al., 1999). Further, researchers have shown that upon LTP induction, AMPA receptors move into dendritic spines where they

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synaptic enhancement that occludes LTP. These criteria have not, to date, been satisfied for any

were previously absent, and the number of spines containing AMPA receptors increased. These changes did not occur if NMDA receptor activity was blocked by the antagonist, D-2-amino-5-phophonopentanoic acid (D-APV) suggesting that NMDA receptor activity leads to the translocation of AMPA receptors into dendritic spines (Shi et al., 1999).

Lüscher et al. (2000) have proposed a model of sequentially occurring expression mechanisms. In agreement with previously described data, the initial step is the phosphorylation by CaMKII of the AMPA subunit GluR1 and an increase in sin le-channel conductance. Through a process similar to exocytosis, AMPA receptors migrate to the postsynaptic densities of both functional and silent synapses. This migration increases the size, and alters the shape, of the postsynaptic density, creating perforations in the postsynaptic density (resulting in the so-called "perforated synapse"). Some dendritic spines, through mechanisms that are still poorly understood, cleave to form multispine synapses that contact the same presynaptic bouton. Retrograde communication, possibly through cell-adhesion molecules, affects presynaptic structural changes that result in presynaptic multiplication.

1.5 CONNECTING LTP TO LEARNING

Since its discovery, LTP has generated speculation that it could be the cellular mechanism underlying memory storage. Countless studies have been

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of the putative retrograde messengers.

undertaken attempting to support this idea. These studies have proceeded along three lines: 1) Demonstrations that learning produces LTP-like changes in synaptic strength; 2) Demonstrations that saturation of learning occludes LTP or vice versa and; 3) Demonstrations that pharmacological/genetic manipulations blocking LTP also block learning.

A recent study produced compelling evidence that learning can produce LTP-like changes that occlude subsequent LTP induction. After teaching rats to acquire a reaching skill for food reward, Rioult-Pedotti and colleagues (1998) examined evoked field potentials in forelimb motor cortex slices. The authors found that the amplitude of layer II/III field potentials in the hemisphere contralateral to the forelimb that performed the task (the 'trained' hemisphere) was significantly increased relative to field potentials taken from the untrained hemisphere. In addition, subsequent LTP induction was reduced in the trained hemisphere versus the untrained hemisphere suggesting that learning the skilled reaching task was partly due to LTP-like mechanisms (Rioult-Pedotti et al., 1998).

Occlusion experiments have typically focused upon the hippocampus and its mediation of spatial memory.[10] One common test used to assess spatial learning is the water maze. In this task, a rodent is forced to swim in a circular pool of

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[10] One of the key types of declarative memory that is believed to require the hippocampus is spatial memory. Evidence supporting this assertion stems from two discoveries 1) the hippocampus contains complex cells that fire when an animal is in a specific location within the environment (O'Keefe and Dostrovsky, 1971) and 2) hippocampal lesions disrupt spatial navigation tasks (O'Keefe et al., 1975). However, McClelland and Goddard (1996) argue that the seeming spatial role of the hippocampus is actually a manifestation of a more general capacity to associate arbitrary fragments of an episode experienced in temporal proximity (i.e., the importance

opaque water until it finds and mounts a platform hidden just beneath the surface. This skill requires spatial learning because the rodent learns to swim to the platform using visual cues in the room. Although normal rats learn this task in a few trials, rats with hippocampal lesions perform poorly at this task (Morris et al., 1982). Recently, it was shown that LTP-induced synaptic saturation impaired learning in the water maze (Moser et al., 1998). Using an electrode array that straddled the perforant path and a stimulation protocol comprising cathodal stimulation at different cross-sectional sites, LTP was saturated to varying levels. Spatial learning was disrupted in animals with less than 10 percent residual LTP but not in animals that were capable of more potentiation (Moser et al., 1998).

Results showing a direct link between LTP and learning and memory through pharmacological or genetic manipulations have been inconsistent. Morris (1989) showed that pharmacological blockade of NMDA receptors, in a dose sufficient to block LTP in the hippocampus, also blocked successful navigation of the water maze. This result suggested that NMDA receptors might be involved in some types of learning, and further supports the idea that LTP and learning might operate through similar mechanisms. However, these results have subsequently been criticized (reviewed in Cain, 1998) along several lines. Cain and colleagues have shown that familiarity with the individual learning components that the rodent must synthesize for successful navigation of the water maze promotes successful acquisition of the water maze task even if NMDA, cholinergic, or

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of the hippocampus for rodents reflects a more general function in memory formation than a

noradrenergic antagonists have been administered (Saucier et al., 1996). However, it is possible that the "non-spatial" pretraining administered by Saucier et al. (1996) actually engaged spatial processing systems making subsequent acquisition more difficult to disrupt. If the hippocampus acts more generally to associate temporally correlated experiences as suggested by McClelland and Goddard (1996), it is reasonable to suspect that a similar, but not exact, set of experiences (the non-spatial pretraining) might activate the same systems required to navigate the water maze.

Mayford et al. (1996) have shown that both LTP and spatial learning are impaired in mice with controlled expression of CaMKII. Genetically engineered mice with suppressed expression of CaMKII in the hippocampus showed impaired LTP and spatial learning, while mice with suppressed expression of CaMKII in the amygdala and neostriatum showed impaired fear conditioning and no LTP. When CaMKII was expressed, animals showed LTP in the hippocampus and amygdala as well as spatial learning and fear conditioning respectively. Similarly, Wilson and Tonagawa (1997) engineered mice with targeted deletions of the gene responsible for the functional subunit within NMDA receptors in the CA1 field of the hippocampus. These mice showed impaired performance on the water maze spatial learning task, blocked LTP induction under standard electrophysiology induction regimens, and a failure to form normal place fields.

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specialization for space).

In summary, the evidence supporting LTP as a mechanism underlying some types of learning and memory continues to grow. The majority of studies have focused upon the hippocampus. Indeed, data collected from humans with retrograde amnesia supports the idea that memories become independent of the hippocampus and eventually reside in other parts of the brain – presumably the neocortex (Squire et al., 1993; McClelland et al., 1995). If LTP represents a mechanism for information storage, it should be readily demonstrated in structures hypothesized to store memory. Neocortical LTP has been demonstrated, but it differs from hippocampal LTP in important ways.

1.6 NEOCORTICAL LTP

Scientists have amassed considerable data supporting an important role for the neocortex in information storage (Squire et al., 1993). However, investigations into neocortical LTP have not progressed as rapidly. Tsumoto (1992) suggests that the complexity of neocortical circuitry, coupled with the relatively delayed implementation of the neocortical slice procedure, have contributed to this lag.

Although it has been proposed that neocortical LTP shares many features in common with hippocampal LTP (Bear and Kirkwood, 1993; Malenka and Nicoll, 1999), neocortical LTP also differs in fundamental ways. Most demonstrations of neocortical LTP have been made in the anaesthetized animal (acute, *in vivo*) (e.g., Voronin, 1984; Keller et al., 1991; Kimura et al., 1994) and *in vitro* slice

preparations (e.g., Perkins and Teyler, 1988; Kimura et al., 1989; Artola and Singer, 1990; Kato et al., 1991; Kirkwood and Bear, 1994). Neocortical LTP has been difficult to reliably elicit *in vitro* – often requiring reductions in GABAergic inhibition through pharmacological manipulations or the use of young animals (Komatsu et al., 1981; Artola and Singer, 1987; Kimura et al., 1989; Ito and Hicks, 1994; Crair and Malenka, 1995). Even though these manipulations facilitated LTP induction, consistency proved elusive and raised the concern that neocortical LTP could only be reliably induced during a critical period of development (Crair and Malenka, 1995; Kirkwood et al., 1995). The difficulty and unreliability of neocortical LTP challenged the notion that LTP-like mechanisms could underlie information storage.

Recognizing this challenge, Racine and colleagues embarked upon a series of studies (Racine et al., 1994a; Racine et al., 1994b) attempting to induce LTP in the neocortex. Initially, they demonstrated neocortical LTP in anaesthetized rats lasting two hours (Racine et al., 1994a). Subsequent investigation focused upon LTP induction in the adult, freely moving rat. Racine et al. (1994b), attempted LTP induction using a variety of protocols, including the application of stimulus trains effective in hippocampal LTP induction, and manipulations of the electrical stimulus parameters. They also tried co-activation of multiple cortical and/or thalamic inputs, pharmacological reduction of GABAergic inhibition, housing animals in enriched environments and using the delivery of stimulation trains as a cue for a learning task. Finally, they attempted to facilitate induction by injecting

the cholinergic agonist pilocarpine in the hope that it would co-activate cholinergic[11] inputs and result in LTP. None of these manipulations produced LTP although the final manipulation produced a long-term depression (LTD) (Racine et al., 1994b).

1.7 NEOCORTICAL LTP IN THE FREELY MOVING RAT

It has been proposed that the hippocampal and neocortical learning systems compliment each other and work together to store memories (Teyler and DiScenna, 1986; McNaughton and Morris, 1987; McClelland et al., 1995; McClelland and Goddard, 1996). The hippocampal memory system learns quickly via synaptic changes within the structure. These changes within the hippocampus reinstate recent memories within the neocortex causing synapses within the neocortex to change. The learning in the neocortex is slow and requires repeated reinstatements of information that are interleaved with existing information (McClelland et al., 1995). Remote memories are based upon these gradual changes and are less vulnerable to catastrophic interference (McClelland et al., 1995).

Since the neocortical learning system is slow and requires repeated exposure so that the information can be interleaved with existing information, Racine and colleagues attempted LTP induction using a spaced-trains protocol. For the first time, they showed that the adult neocortex in the freely moving rat could support

[11] Acetylcholine and stimulation to cholinergic projections have been shown to facilitate

LTP (Racine et al., 1995). Using daily stimulation sessions comprising high-frequency trains delivered to the white matter and recording evoked potentials in sensorimotor cortex, they showed that the LTP developed slowly over a period of five days, reached asymptote by 15 days and lasted 4 weeks (Racine et al., 1995). Since that time, studies have been conducted to further characterize the nature of the LTP generated in the freely moving adult rat (Chapman et al., 1998; Trepel and Racine, 1998; Trepel and Racine, 1999; Ivanco et al., 2000; Trepel and Racine, 2000).

The strength of the LTP effect depends upon the number of trains delivered per session, the number of daily sessions, and the train intensity. A thorough analysis of the induction and decay kinetics showed that LTP is optimally induced with repeated, high-frequency, high-intensity stimulation trains over a period of at least 6 days (Trepel and Racine, 1998). In addition, the neocortical LTP effect is NMDA-dependent and input-specific (Trepel and Racine, 1998). Induction of LTP using the spaced and repeated trains protocol results in increased dendritic arborization and spine density of pyramidal neurons in layer III (Ivanco et al., 2000). These changes are similar to those seen following motor skill learning (e.g., a reaching task) (Withers and Greenough, 1989). The kinetics underlying the induction of LTP in the freely moving, adult rat represent a logical first step in the characterization of this phenomenon. However, given the growing evidence that LTP and memory are related in some way, it is also important to elucidate the

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neuronal excitability. This topic is discussed at length in the introduction to Chaper 2.

ways in which endogenous modulators affect the phenomenon. Doing so makes progress toward the goal shared by experimental psychologists - understanding processes of mind and their underlying mechanisms.

1.8 WHAT IS NEUROMODULATION?

In the cortex, acetylcholine, norepinephrine, seratonin, dopamine and several types of peptides primarily have a modulatory influence. That is, they do not appear to participate in the direct transfer of information to cortical structures (see figure 1.1). Instead, they alter cortical processing characteristics by influencing synaptic transmission and cellular firing patterns (e.g., pyramidal cell adaptation to repeated stimulation). Neuromodulators project diffusely to many areas of the brain (see figures 1.2, 1.3 and 1.4). The diffuse projections allow for spatially diffuse effects. For all neuromodulatory systems discussed within this thesis, projections stem from subcortical, primarily brainstem, nuclei and broadly innervate cortical regions (reviewed in Cooper et al., 1996). Innervation of the three neuromodulatory systems discussed within this thesis (acetylcholine (ACh), noradrenaline (NA) and dopamine (DA)) arises mainly from the basal forebrain, locus coeruleus and ventral tegmental areas respectively. Brief anatomical reviews of neuromodulatory projections are contained within each subsequent chapter of the thesis.

ACh, NA and DA have all been implicated in learning and memory. Evidence supporting their involvement stems from their effects on learning and

memory tasks as well as their roles in memory-damaging disease states. The literature supporting the involvement of each neuromodulator, in both humans and non-human animals, will be discussed more thoroughly in each subsequent chapter. As well, the final chapter will discuss ways in which investigations into disease states, such as Alzheimer's, drug addiction, and schizophrenia, have promoted further understanding of learning and memory.

This thesis focuses upon neuromodulatory actions on long-term potentiation. These mechanisms are largely transduced by intracellular second messenger systems (see figure 1.5). Second messenger systems engage subsequent biochemical pathways that are typically tied to protein kinase activation. Second messenger system activation can produce coordinated changes in multiple ion channels within the membrane as well as influencing other cellular processes not directly linked to ion fluxes (Kaczmarek and Levitan, 1987). The specific second messengers activated by ACh, NA and DA as well as their specific modulatory actions on cellular excitability will be discussed within subsequent chapters of the thesis.

As observed by Hasselmo (1995), neuromodulatory effects have historically been grouped into two classes based upon the results of electrophysiological techniques (e.g., iontophoresis): excitation or inhibition of cortical cell firing. Increases in cellular firing rate led to an "excitatory" label while decreases in firing rate led to an "inhibitory" label for the neuromodulator under investigation. However, Hasselmo (1995) argues that the ambiguous results of many

experiments within this field suggest that this classification scheme is too simplistic. For example, categorizing dopamine as an "inhibitory" neuromodulator ignores the possibility that it can have both inhibitory and excitatory effects on cellular functioning through a variety of mechanisms.[12] Hasselmo (1995) suggests that analysis of neuromodulatory effects on evoked potentials and EEG in conjunction with single-unit recordings, affords understanding of the complex dynamics by which neuromodulators exert their influence in the brain. Doing so allows one to understand the full spectrum of modulatory effects ranging from an influence on single cells to changes affecting the entire organism.

Subsequent reviews of the "excitatory" and "inhibitory" effects of ACh, NA and DA follow in each chapter. The mixed results within each review support Hasselmo's assertion that these neuromodulators can promote seemingly contradictory effects on cellular functioning.

1.9 NEUROMODULATION OF NEOCORTICAL LTP IN THE FREELY MOVING RAT

Neuromodulation, by definition, refers to an alteration in the electrical response properties of a neuron as a result of intracellular biochemical changes following synaptic or hormonal stimulation (Kaczmarek and Levitan, 1987). A

[12] Inhibition could occur through a variety of mechanisms such as direct hyperpolarization, enhancement of adaptation currents, reduction of excitatory synaptic transmission, or excitation of inhibitory interneurons. On the other hand, excitation could occur via direct depolarization,

full understanding of neocortical LTP requires an exploration of the molecular modulators that contribute to the effect. It has previously been shown that LTP in the chronic preparation is modulated by gamma-aminobutyric acid (GABA), the primary mediator of inhibition in the brain (Trepel and Racine, 2000). The $GABA_A$ agonist diazepam blocked LTP induction and surprisingly, the antagonist picrotoxin slowed the development of LTP. While the blockade of LTP agrees with previous *in vitro* LTP (Del Cerro et al., 1992) and memory (Izquierdo et al., 1990) studies, the antagonist data are less congruent with *in vitro* LTP studies (Wigstrom and Gustafsson, 1985; Abraham et al., 1987) and the broader memory literature (Izquierdo and Medina, 1990). Neocortical LTP in the freely moving rat appears to be governed by different rules than the LTP effects seen in the hippocampus *in vitro* and *in vivo*, as well as the neocortex *in vitro*. The following experiments are designed to explore the role played by endogenous neurotransmitter systems – the same systems that have been shown to modulate memory and LTP in other preparations – on the kinetics of neocortical LTP in the adult freely moving rat. Each agonist was hypothesized to increase LTP whereas each antagonist was hypothesized to attenuate LTP. Literature reviews of each neuromodulator's effect on memory and LTP are included in each chapter.

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suppression of adaptation currents, reduction of inhibitory synaptic transmission, or direct inhibition of inhibitory interneurons.

FIGURE CAPTIONS

Figure 1.1 Neurotransmitter and neuromodulatory influences on cortical pyramidal cells. I. Neurotransmission **A.** Afferent fibers release glutamate (or aspartate) that promotes EPSPs in the distal dendrite via activation of AMPA or NMDA receptors. **B.** Afferent or intrinsic fibers activate feedforward or feedback inhibitory interneurons. Feedforward inhibition is inhibition activated by afferent input or input from other cortical regions whereas feedback inhibition is inhibition activated by excitatory output of cortical neurons. Interneurons release GABA. GABA acts at $GABA_A$ receptors causing fast IPSPs if the membrane potential is depolarized above the chloride reversal potential. **C.** Intrinsic fiber synapses from other pyramidal cells release glutamate (or aspartate), which elicits an EPSP in the proximal dendrite. **D.** Current injection into pyramidal cells initially causes high-frequency generation of action potentials. However, neuronal firing eventually slows due to activation of Ca^{2+} and voltage-dependent K^+ channels (spike-frequency adaptation). **II. Neuromodulation 1.** Glutamate (acting through metabotropic glutamate receptors) and GABA (acting through $GABA_B$ receptors) exert neuromodulatory influences as a result of local activity. **2.** Neuromodulatory innervation from subcortical structures influence cellular function via volume transmission of substances like ACh, NA, DA, 5-HT, and other peptides. **A.** Activation of presynaptic metabotropic glutamate receptors at afferent fiber synapses causes decreased release of glutamate and suppression of EPSPs. This effect is weaker at intrinsic fiber synapses. **B.** Activation of

presynaptic $GABA_B$ receptors and muscarinic ACh receptors blocks GABA release and reduces IPSPs. **C.** Presynaptic ACh, $GABA_B$, and NA activity, results in decreased glutamate release and causes decreased EPSPs evoked by intrinsic fibers. Activation of these receptors has little influence on afferent fiber synaptic potentials. **D.** Infusion of ACh and NA suppresses K^+ currents that underlie spike-frequency adaptation. Consequently, increased spiking is seen following current injection. Text and figure adapted from Hasselmo, 1995.

Figure 1.2 Cholinergic pathways in the rat brain. This sagittal view illustrates nuclei that contain neurons that release ACh. The cortex primarily receives cholinergic input from the nucleus basalis (red) and medial septal and diagonal band nuclei (navy). Together, these two nuclei comprise the basal forebrain. The axonal projections are shown as arrows. Adapted from Rosenzweig et al., 1999.

Figure 1.3 Noradrenergic pathways in the rat brain. This sagittal view illustrates the diffuse noradrenergic projections within the rat brain. Axonal projections are shown as arrows. Adapted from Rosenzweig et al., 1999.

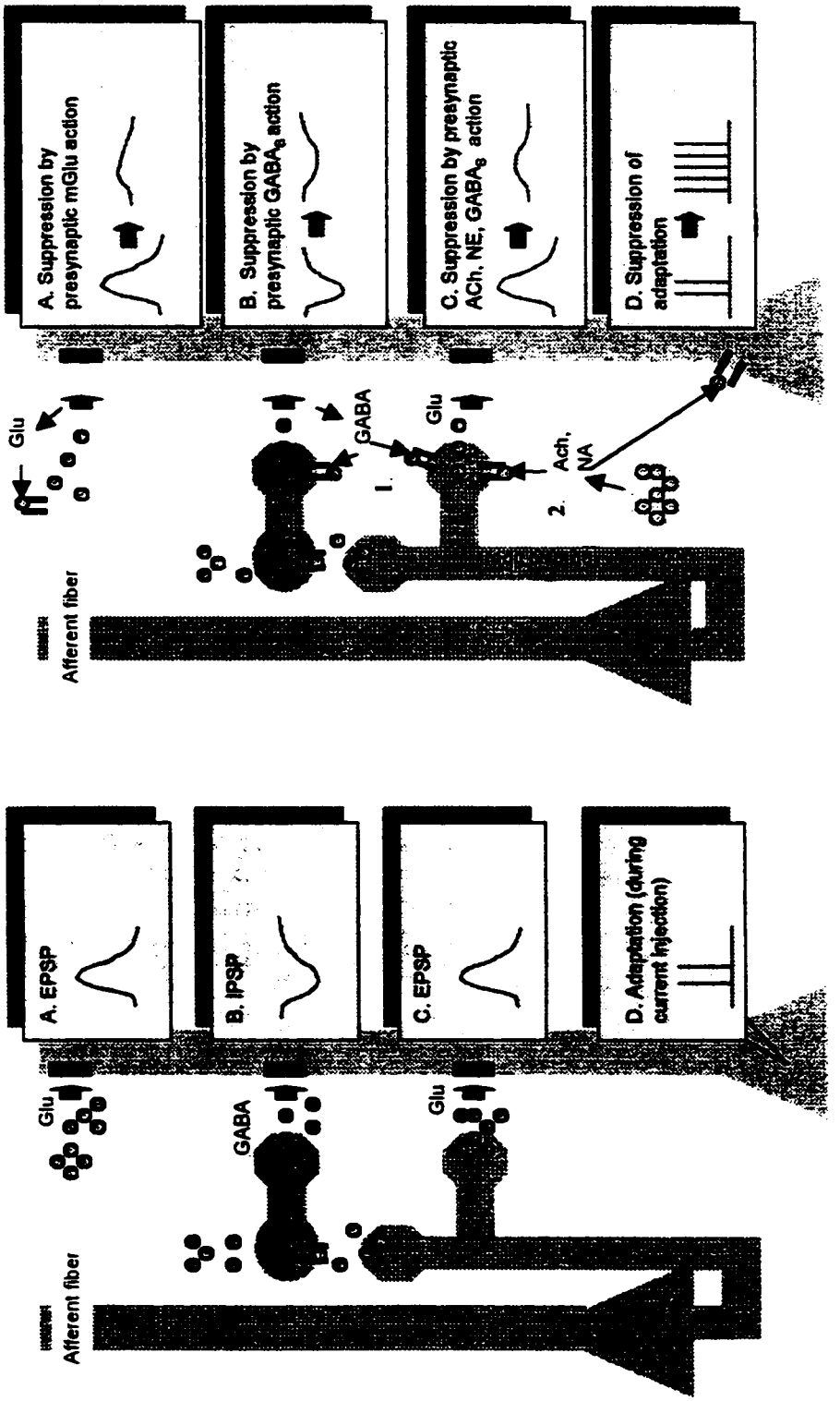
Figure 1.4 Dopaminergic pathways in the rat brain. I. Dorsal mesostriatal (nigrostriatal; yellow) and ventral mesostriatal (blue) dopamine systems. From the substantia nigra, dopaminergic pathways project to the olfactory tubercule, nucleus accumbens and caudate putamen. **II. Mesolimbocortical and**

mesothalamic dopamine systems. The axonal projections are shown as arrows. Adapted from Rosenzweig et al., 1999.

Figure 1.5 Four second- messenger systems. I. The adenlylate cyclase/cyclic adenosine monophosphate (cAMP)-dependent protein kinase system. Neurotransmitter binds to a stimulatory G-protein-coupled receptor activating the alpha subunit of the G-protein. The alpha unit of the G-protein exchanges the nascent GDP (guanosine diphosphate) for GTP (guanosine triphosphate). Then, the alpha subunit detaches from the beta and gamma subunits and activates the effector protein adenylate cyclase. The alpha subunit slowly converts GTP back to GDP by removing phosphate. This action terminates the activity of the G-protein (not shown). Adenylate cyclase converts adenosine triphosphate (ATP) to cAMP. Increased cAMP in the cytosol activates the down-stream enzyme cAMP-dependent protein kinase (PKA). cAMP is broken down by a phosphodiesterase (not shown). PKA transfers phosphate from ATP within the cytosol to other cellular proteins (e.g., ion channels in the membrane; phosphorylation, not shown). Phosphorylation changes the protein conformation and directly influences subsequent functioning of the protein. Protein phosphatases transfer phosphate from the phosphorylated proteins and terminate the phosphorylation sequence (not shown). **II.** The guanylate cyclase/cyclic guanine monophosphate (cGMP)-dependent protein kinase system. The coupling mechanisms for the cGMP second messenger system are similar to those of the cAMP, but less well

understood (Kaczmarek and Levitan, 1987; Levitan and Kaczmarek, 1997). The enzyme guanylate cyclase produces cGMP from GTP. **III.** The inositol triphosphate/diacylglycerol-protein kinase C system. Neurotransmitter initially binds to a G-protein-coupled receptor as described before. The alpha subunit detaches from the G-protein and binds to the effector protein phospholipase C (PLC). PLC in turn acts on the membrane phospholipid phosphatidylinositol-4,5-biphosphate (PIP_2) cleaving it into two second messengers, diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3). DAG causes the activation of protein kinase C (PKC) while IP_3 diffuses within the cytosol and binds to IP_3-gated Ca^{2+} channels on the smooth endoplasmic reticulum (ER) stimulating the release of Ca^{2+} from internal stores. Adapted from Bear et al., 1996. **IV.** Intracellular Ca^{2+} ions as second messengers. Calcium influx through membrane channels, or elevation via second messenger pathways described in III lead to a variety of effects on cellular proteins. Ca^{2+} ions can interact directly with cytoplasmic proteins such as calmodulin, adenlylate cyclase, cyclic nucleoside phosphodiesterase and calcineurin. Interaction with these proteins regulates the activity of down-stream enzymes and proteins. Adapted from Levitan and Kaczmarek, 1997.

Figure 1.1 Neurotransmitter And Neuromodulatory Influences On Cortical Pyramidal Cells.



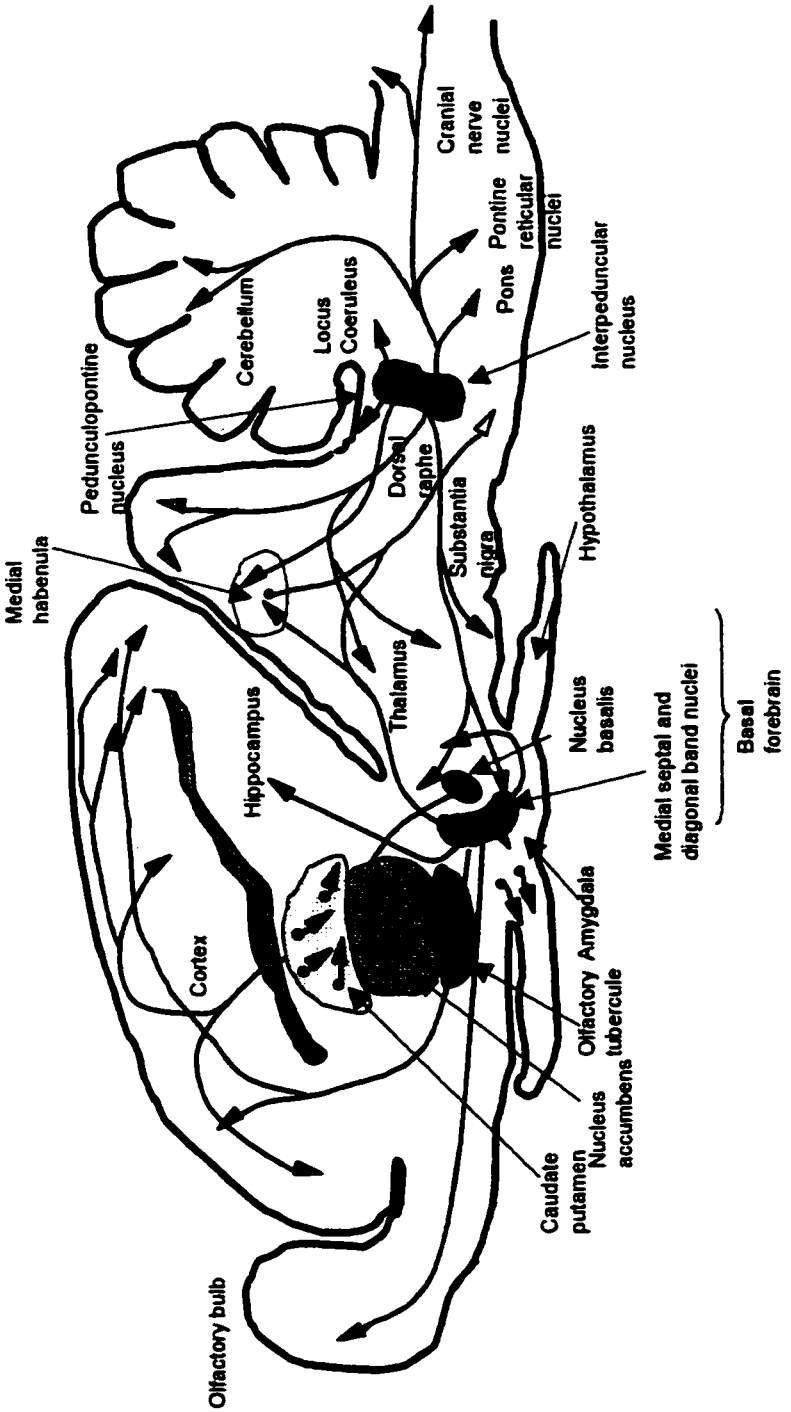


Figure 1.2 Cholinergic Pathways in the Rat Brain

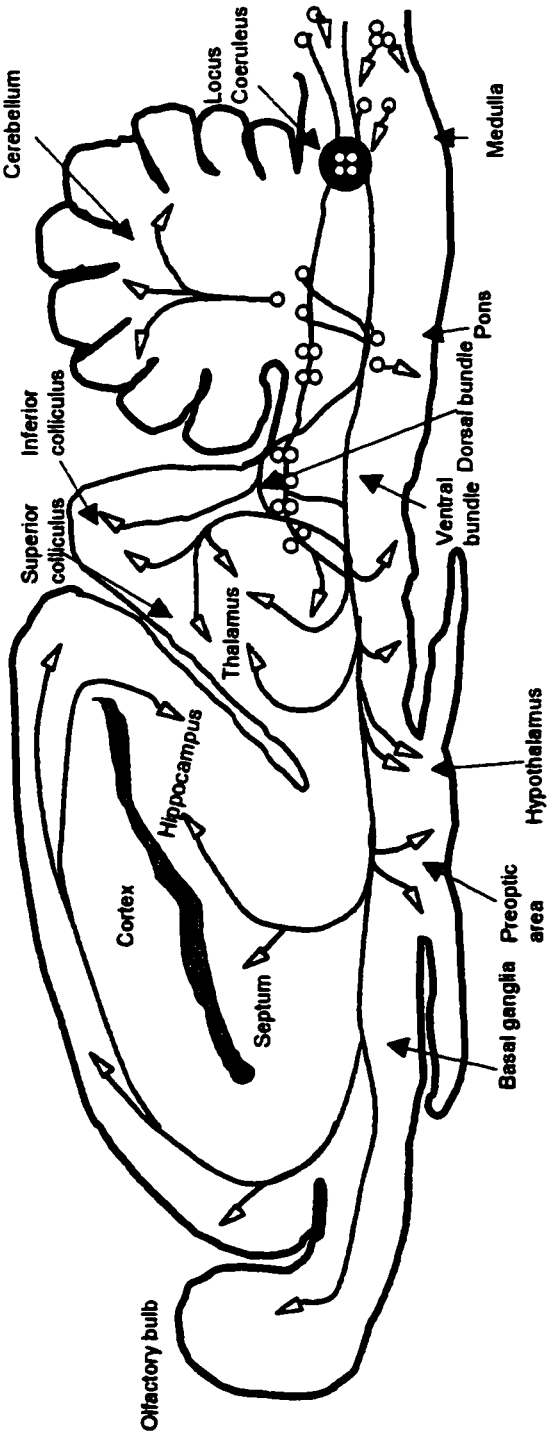
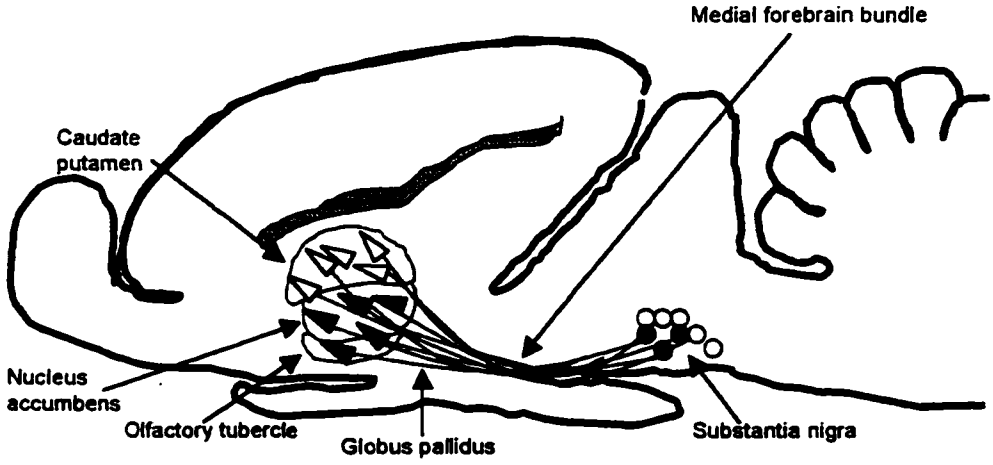


Figure 1.3 Noradrenergic Pathways in the Rat Brain

Figure 1.4 Dopaminergic Pathways in the Rat Brain

I. Dorsal mesostriatal (nigrostriatal; yellow) and ventral mesostriatal (blue) systems



II. Mesolimbocortical and mesothalamic systems

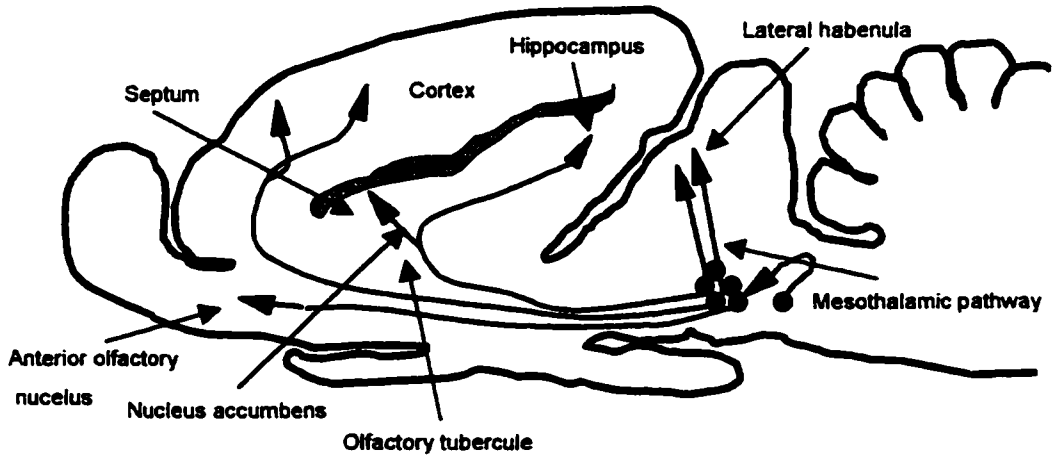
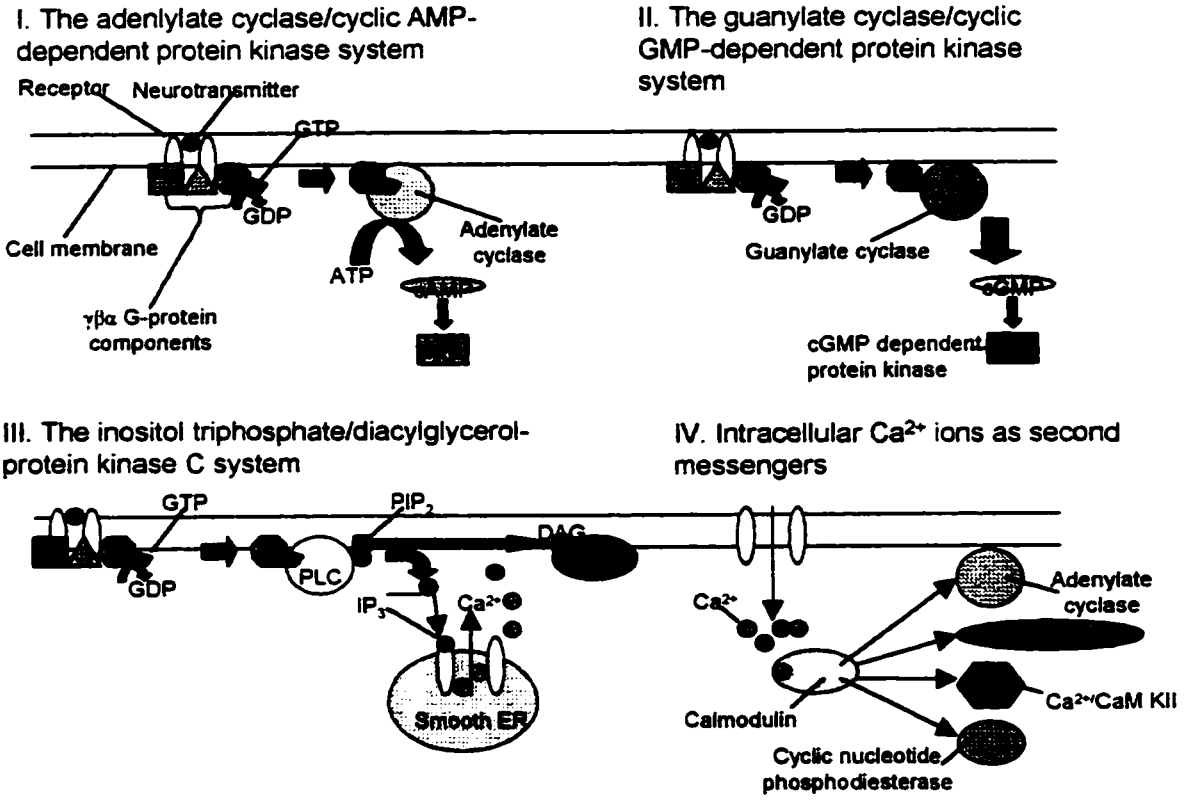


Figure 1.5 Four Second- Messenger Systems



CHAPTER TWO

CHOLINERGIC MODULATION OF NEOCORTICAL LONG-TERM POTENTIATION IN THE FREELY MOVING RAT

2.1 INTRODUCTION

2.1.1 Cholinergic Projections to the Neocortex

There are two major diffuse cholinergic modulatory systems within the brain, the basal forebrain complex and the pontomesencephalotegmental cholinergic (PMTC) complex (Woolf et al., 1984; see figure 1.2). Within the basal forebrain complex lie two concentrations of cholinergic nuclei. The medial septal nuclei primarily innervate the hippocampus via the fimbria fornix, while the basal nucleus of Meynert provides 70-80 percent of the cholinergic innervation to the primate cortex (Nabeshima, 1993). The neurons within the PMTC lie within the pons and midbrain tegmentum. The PMTC acts together with other systems to regulate the excitability of the dorsal thalamus. These cells also project to the basal forebrain complex.

2.1.2 Muscarinic Receptors and Signal Transduction

Acetylcholine binds to two classes of receptors in the central nervous system: nicotinic and muscarinic. Nicotinic receptors belong to the receptor-gated ion channel family while muscarinic receptors belong to the superfamily of G-

protein-coupled receptors (Hosey, 1992). The discussion within this thesis will focus on the muscarinic class. To date, five muscarinic receptor subtypes have been identified (M_1-M_5) (Bonner et al., 1987). In the hippocampus and cortex, the prominent receptor subtypes include M_1, M_3 and M_4. In the striatum, the M_4 receptor predominates. The M_2 subtype is expressed at low levels within the basal forebrain and is expressed at higher levels in the brain stem. The M_5 subtype is expressed at low levels generally, with limited distribution within the brain (Ablordeppey et al., 1998).

Activation of M_1, M_3 or M_5 initiates the phospholipase C second-messenger cascade (PLC) (Peralta et al., 1988; see figure 1.5).[1] The M_2 and M_4 receptors activate the G_i class of G-proteins that inhibit adenylate cyclase (Hosey, 1992).

2.1.3 Muscarinic Modulatory Actions

Application of ACh to pyramidal cortical neurons maintained *in vitro* can produce both inhibitory and excitatory effects (McCormick and Prince, 1985; Krnjevi□, 1993; Murakoshi, 1995). It is thought that inhibition occurs as a result of the excitation of GABAergic interneurons (McCormick and Prince, 1985), depression of excitatory synaptic transmission, or depression of Ca^{2+} currents (Krnjevic, 1993).

ACh potentiates glutamate-evoked responses in slices of somatosensory cortex (Metherate, et al., 1987), sensorimotor cortex (Lin and Phillis, 1991a)

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[1] PLC in turn acts on the membrane phospholipid phosphatidylinositol-4,5-biphosphate (PIP_2) cleaving it into two second messengers, diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3). DAG causes the activation of protein kinase C (PKC) while IP_3 diffuses within

auditory cortex (Cox et al., 1994), visual cortex (Murakoshi, 1995) and CA1 (Markram and Segal, 1990).

Cholinergic effects in the brain are predominantly excitatory and occur primarily as a result of K^+ current suppression (Krnjevic, 1993) (see figure 2.1). The principle features of cholinergic excitatory action involve reduction in resting potassium conductance and prolonged slowing of repolarization following spikes (Krnjevic, 1993). These actions have been demonstrated in both the hippocampus (Dodd et al., 1981) and neocortex (McCormick and Prince, 1986). ACh is believed to suppress at least three types of K^+ currents: the leak current, the voltage-dependent, slow afterhyperpolarization (I_{AHP}) current and the non-inactivating M-current (I_M). Blockade of these K^+ currents enhances the excitability of neurons and, in the case of the I_{AHP} and I_M, leads to repetitive firing.

The 'leak' K^+ current helps maintain the resting membrane potential. Suppressing this current depolarizes the cell, enhances excitatory postsynaptic potentials and facilitates cell firing (McCormick and Williamson, 1989).

The I_{AHP} prevents repetitive firing by holding the cell below the action potential threshold (Kaczmarek and Levitan, 1987). By suppressing this current, ACh facilitates subsequent firing over a period of seconds (Krnjevic, 1993; Cox et al., 1994). In the presence of ACh, a slow after depolarization (I_{sADP}) replaces the I_{AHP} promoting further firing. The I_{sADP} results from a rise in intracellular Ca^{2+}

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the cytosol and binds to IP3-gated Ca^{2+} channels on the smooth endoplasmic reticulum stimulating

secondary to Ca^{2+} influx into the cell via non-selective cation channels (Haj-Dahmane and Andrade, 1998). Repeated depolarization initially causes burst firing. However, the neuron quickly accommodates and firing decreases. Suppression of the I_{AHP} prevents spike-frequency adaptation to depolarization (McCormick and Williamson, 1989, Cox et al., 1994) and facilitates a more sustained burst response (Cox et al., 1994).

The I_M is a slowly developing (100 msec) outward K^+ current active at membrane potentials between rest and the threshold for an action potential (Kaczmarek and Levitan, 1987). The I_M is a non-inactivating K^+ current inhibited by acetylcholine. Increased depolarization opens these channels allowing K^+ efflux and repolarization. Increased membrane hyperpolarization closes these channels (Kaczmarek and Levitan, 1987).

2.1.4 Cholinergic Modulation of Memory

There is considerable evidence 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) and normal aging (Giacobini, 1990), may be mediated by a reduction in cholinergic functioning. It has also been established in rodents that cholinergic antagonism (Flood et al., 1981; Lamberty and Gower 1991; Bymaster et al., 1993; Whitehouse, 1996) and agonism (Haroutunian et al., 1985; Smith et al., 1996;

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the release of Ca^{2+} from internal stores.

Nagahara and Handa, 1999) attenuate and facilitate, respectively, learning for a variety of tasks[2]. Specifically, Sen and Bhattacharya (1990) showed that the effect of muscarinic agonism and antagonism may depend upon the muscarinic receptor stimulated. They implemented an active-avoidance paradigm to teach rats two different responses. Acquisition of the first response, jumping to an adjacent non-electrified chamber during a buzzer to avoid foot shock, was termed conditioned response 1 (CR1). Jumping to the adjacent non-electrified chamber in the 15-sec adaptation period prior to the buzzer was termed conditioned response 2 (CR2). Rats typically learned CR1 in fewer trials than CR2. The M_1 receptor agonists arecholine and McN-A-343 facilitated acquisition of both CR1 and CR2 (Sen and Bhattacharya, 1990). The broad-spectrum antagonist scopolamine attenuated acquisition of CR1 and CR2. The M_2 agonists carbachol and physostigmine inhibited acquisition at low doses (1μg/rat icv; 0.1mg/kg i.p., respectively) and facilitated acquisition at high doses (10μg/rat icv; 0.5mg/kg i.p.). The M_2 antagonists gallamine and AF-DX 116 facilitated learning. As well, hemicholinium, an ACh synthesis blocker, impaired learning for CR1 and CR2 (Sen and Bhattacharya 1990). Taken together, these results suggest that M_1 receptor actions oppose those initiated by M_2 action. These results also imply that scopolamine may have more binding affinity for M_1 receptors than M_2 receptors (Sen and Bhattacharya, 1990).

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[2] Lesions of cholinergic projections have produced learning deficits in the following tasks: habituation, taste avoidance, discrimination, passive avoidance, active avoidance, spatial

Studies using human and non-human primates have also shown that cholinergic agonism and antagonism facilitates and attenuates memory respectively (Drachman and Leavitt, 1974; Aigner et al., 1991; Hudzik and Wenger,1993). For example, Flicker et al. (1990) tested healthy subjects aged 18-30 on recent memory following subcutaneous scopolamine injection. When compared to saline injection, subjects receiving medium (0.43mg/70kg) or high doses (0.65mg/70 kg) of scopolamine recalled fewer details of a paragraph read to them as well as fewer items on a shopping list. Subjects who received a low dose (0.22mg/70kg) of scopolamine had no memory impairments. In the same study, subjects receiving medium and high doses of scopolamine also performed significantly worse on a delayed spatial recall test than their placebo counterparts (Flicker et al., 1990).

2.1.5 Cholinergic Modulation of LTP

LTP, like memory, is influenced by cholinergic manipulations, but most experiments to date have been conducted in *in vitro* preparations. Cholinergic agonists, for example, enhance LTP induction in both the CA1 (Blitzer et al., 1990) and dentate gyrus (Burgard and Sarvey, 1990) regions of the hippocampus as well as in the visual cortex (Bröcher et al., 1992). Even cholinergic agents by themselves have been shown to produce a long-term enhancement of responses in the hippocampus (Auerbach and Segal, 1994) and sensorimotor cortex (Lin and Phillis, 1991b). In the hippocampus, this cholinergically-induced LTP has been

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alternation, delayed match to sample, T-maze alternation, cross maze, stone maze, radial arm

shown to occlude subsequent LTP induction by electrical stimulation (Auerbach and Segal, 1994), suggesting that these two forms of LTP share a common substrate.

Cholinergic stimulation has been shown to promote a long-term depression effect in the neocortex of freely moving animals following single-session stimulation protocols (Racine et al., 1995). The following experiments were undertaken to characterize the role of cholinergic neuromodulation in the induction of the long-lasting neocortical LTP induced by the spaced and repeated stimulation protocol. Based on the LTP slice literature and behavioural data, it was predicted that the cholinergic agonist pilocarpine would enhance LTP induction, while the antagonist scopolamine would attenuate it.

2.2 MATERIALS AND METHODS

2.2.1 Animals and Surgery

Fifty-five 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. 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 vertical tip separation for the

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maze, hole-board food search, water maze, and timed conditioning responding (Dekker, 1991).

recording electrode was 1.0 mm, to span the pyramidal cell dipole. The tip separation was 0.5 mm for the stimulating electrode, to span the corpus callosum. Following anaesthesia with sodium pentobarbital (65 mg/kg), a bipolar recording electrode was implanted into the primary motor cortex (2.0 mm anterior to Bregma and 4.0 mm lateral to the midline), and a stimulating electrode was implanted into white matter (the forceps minor corpus callosum, 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 white matter 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 then inserted into a 9-pin miniature connector plug that 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 10-14 days following surgery. All experimental procedures were conducted in accordance with the Guidelines of the Canadian Council on Animal Care and approved by the McMaster University Animal Research Ethics Board.

2.2.2 Baseline Measures and Induction of LTP

Electrical stimuli were produced by a Grass S88 stimulator, coupled to photoelectric stimulus isolation units. Recorded signals were fed into Grass Model 12 EEG amplifiers, high-pass filtered at 0.3Hz, low-pass filtered at 3Hz, and digitized (10KHz) with a 12-bit A/D converter.

Three sets of field potential input/output (I/O) measures, spaced at 48 h, were taken to establish baseline. Pulses of increasing intensity were delivered to white matter at a frequency of 0.1 Hz. Six 50 ms responses were evoked, amplified, digitized and averaged at each of ten intensities (16, 32, 64, 100, 160, 250, 500, 795, 1000, 1260 μA). The evoked field potentials comprised 2 main components: an early, monosynaptic, surface-negative response, and a larger, polysynaptic late response. The polysynaptic component was often most evident following LTP induction. Also evident at latencies coinciding with the early component were at least one, and usually several, population spikes that increased in amplitude and number following potentiation. Further characterization of these response components has been published elsewhere (Chapman et al., 1998; Trepel and Racine, 1998).

Beginning 24 hours after the third baseline I/O test, 60 high-frequency trains (ITI: 10 sec) were delivered daily to white matter. Trains (24 msec duration) consisted of 8-pulses, with a pulse frequency of 300 Hz. Intra-train interval was 0.1 msec. Pilot work indicated that differential effects might be obtained depending on whether low- or high-intensity trains were used to induce LTP. Consequently, two different tetanic pulse intensities were used in these experiments: 160 μA ("low-intensity") and 1260 μA ("high-intensity"). EEG was monitored with either an Explorer IIIA digital oscilloscope (Nicollet Instrument Corp., Madison, WI) or a Grass Model 7D polygraph (Grass Instrument Co., Quincy, MA) to ensure that epileptiform discharges were not triggered.

2.2.3 Cholinergic Agonism and Antagonism

The cholinergic agonist pilocarpine (10-20 mg/kg i.p. in saline, Sigma Chemical Co., USA) and antagonist scopolamine (15 mg/kg i.p. in saline, Sigma Chemical Co., USA) were administered in conjunction with high-frequency trains. These drugs were chosen specifically because they are widely used in behavioural tests of memory (e.g., Decker and McGaugh, 1991), and because they have broad-spectrum effects on ACh muscarinic receptors. Pilocarpine binds to all muscarinic receptors, but with more affinity to M_1. Scopolamine binds to all five muscarinic receptors with equal affinity (but see Sen and Bhattacharya, 1990).

On the first day of experimentation, animals were injected with pilocarpine (low-intensity group, n=5; high-intensity group, n=7), scopolamine (low-intensity group, n=6; high-intensity group, n=10) or saline (low-intensity group, n=6; high-intensity group, n=8), followed 15 minutes later by the delivery of 60 high-frequency trains. On subsequent days, the injection was preceded by an I/O test and followed 15 minutes later by 60 high-frequency trains. Additionally, a seventh (n=7) and eighth (n=6) group received daily pilocarpine or scopolamine injections preceded immediately by an I/O test, but did not receive trains (these groups provided drug-only controls). These daily LTP induction regimens were continued for 10 and 15 days for the high- and low-intensity groups, respectively. Following completion of the LTP induction phase, 2 weekly I/O measures were collected to monitor decay.

2.2.4 Analyses

Changes in the field potentials over LTP-induction and decay sessions were measured by subtracting the final baseline responses from all potentiated responses at a single mid-range I/O test intensity that best reflected potentiation. The dominant early and late components were measured separately at fixed latencies corresponding to the peak response of the components being analyzed (see figure 2.2). Control animals were then analyzed 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. For amplitude measures, repeated measures ANOVAs were calculated over all induction and decay days. Statistical analyses were conducted using STATISTICA software. Population spikes were measured from the largest amplitude response in the I/O measures before and after LTP induction and after the decay phase. The heights (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 test and the I/O test following completion of trains. One animal each from the SAL/High, PILO/High and SCOP/High groups was discarded from the population spike measures because of artifactual contamination. Once all recordings were complete, animals were perfused and the brains were sliced and stained with cresyl violet to verify electrode placements. In all animals, stimulating and

recording electrodes were positioned within their target structures (Paxinos and Watson, 1997)

2.2.5 Behaviour

Animals injected with pilocarpine showed walking and crouching behaviour, and diarrhea five to seven minutes after the injection. These side effects diminished one to two hours post injection. When handled 24 hours later, the animals appeared normal. Scopolamine animals displayed no visible adverse effects during the experiment. All animals groomed themselves normally and gained weight throughout the experiment. Electrophysiological measures were recorded, and behaviour observed, as animals moved freely inside a dimly lit testing box. The box measured 30.5 cm x 28 cm x 40.5 cm (w x l x h), with a clear Plexiglass door and a mirror mounted on the back wall to facilitate behavioural observation.

2.3 RESULTS

2.3.1 Response Morphology

The responses were similar to those previously reported (Trepel and Racine, 1998; Chapman et al., 1998). There was a very short-latency surface-positive spike-like response, which previous experiments have shown to be a mix of antidromic and orthodromic effects (Chapman et al., 1998). This is followed by an early response component with a mean latency to peak of 7.8 msec. There is usually one strong late peak in the post-LTP record representing polysynaptic

activity. The average latency to peak for these components was 19.3 msec. The effect of scopolamine and pilocarpine on the *train-evoked* response in a representative animal can be seen in figure 2.3. Both scopolamine and pilocarpine depressed the train-evoked response.

2.3.2 Cholinergic Manipulations Combined with Low-Intensity Trains Preferentially Modulate Late-Component Potentiation

Changes in the response amplitude of the early and late components for animals receiving pilocarpine (PILO) or scopolamine (SCOP) alone, or in conjunction with low-intensity trains, are shown in figure 2.4. While the early component showed a significant effect of session overall ($F_{(19,475)} = 5.24$, $p < 0.001$), there were no significant group differences in the pattern or magnitude of the early-component changes (figure 2.4 top). 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 (which can have a similar effect on field potential morphology) in others.

Figure 2.5 illustrates this measurement problem using representative examples from the groups treated with pilocarpine. There were population spike enhancements in a few of the animals from the PILO/Low group and an absence

of population spike potentiation in the PILO group. In the present bipolar recordings, the population spikes have a polarity opposite to that of the population EPSP. As a result, population-spike potentiation results in an initial apparent decrease in amplitude often followed by a reversal in the polarity of the early component (Chapman et al., 1998; Trepel and Racine, 1998). By contrast, the changes shown by the PILO group more closely resemble the depression resulting from the application of the NMDA antagonist CPP (Trepel and Racine, 1998), a similarity further supported by the small amplitude decreases at the *late*-component latencies. Therefore, although both the PILO and PILO/Low groups displayed surface-positive shifts of similar magnitude, the mechanisms mediating these shifts are presumably different.

Although the population-spike measures tended toward enhancement following high-frequency stimulation, and a few animals showed clear changes, there was considerable variability, and the differences were not significant (data not shown).

The late component, on the other hand, showed a significant interaction between group and session ($F_{(76,475)} = 3.91$, $p < 0.001$). All three groups receiving trains showed late-component potentiation (figure 2.4 bottom), though further repeated measures ANOVA analysis revealed that the PILO/Low group showed significantly greater potentiation than the SAL/Low group ($F_{(19, 171)} = 2.60$, $p < 0.001$). The SAL/Low and SCOP/Low groups did not differ from each other.

2.3.3 Cholinergic Agents Combined with High-Intensity Trains Preferentially Modulate Early-Component Potentiation

In contrast to the effects of low-intensity stimulation, the application of high-intensity trains in combination with the administration of cholinergic agents produced robust changes in the early component of the evoked responses. Figure 2.6 shows changes in the early and late components of the neocortical evoked potentials for animals receiving high-intensity trains in conjunction with saline (SAL/High), pilocarpine (PILO/High) or scopolamine (SCOP/High), or pilocarpine (PILO) or scopolamine (SCOP) alone. The early component showed a significant group-by-session interaction ($F_{(56, 462)} = 2.52$, $p < 0.001$). The SCOP/High group differed from the SAL/High group ($F_{(14, 224)} = 3.15$, $p < 0.001$) indicating that scopolamine blocked LTP induction. The SAL/High and PILO/High groups both showed substantial (and equivalent) early-component potentiation.

Despite this depression of early-component LTP, there was some residual enhancement of the population spikes in the SCOP/High group. This point is made clearly by a comparison of representative sweeps from a SCOP and SCOP/High animal (figure 2.7).

The population-spike measures showed a significant interaction effect between session and group ($F_{(8,60)} = 2.30$, $p < 0.05$). All groups that received trains in conjunction with a drug or saline injection showed population spike

enhancements, while the animals that received pilocarpine or scopolamine alone showed no significant change from baseline (figure 2.8).

A significant group-by-session interaction was also found for the late component ($F_{(56,462)} = 9.29$, $p < 0.001$). As figure 2.6 (bottom) reveals, all three groups that received high-intensity stimulation showed robust late-component enhancements. The PILO/High group showed slightly more late-component potentiation, but this result did not differ significantly from the SAL/High or SCOP/High groups. Neither the PILO nor SCOP groups showed any late-component potentiation effects.

2.3.4 Decay

All groups that showed potentiation effects remained at least partially potentiated by the end of the 2-week decay period. The groups that showed the most potentiation generally showed the greatest rate of decay (e.g., PILO/High early component, SAL/High and PILO/High late components).

2.4 DISCUSSION

Previous attempts to induce neocortical long-term potentiation by pairing tetanic stimulation with cholinergic activation in freely moving rats produced depression effects that lasted several weeks (Racine et al., 1995). These studies, however, did not use multiple, spaced stimulation sessions to induce LTP. In contrast to the depressed field potentials obtained previously, the experiments reported here yielded component-specific LTP effects.

2.4.1 Early-Component Changes

The prediction that cholinergic antagonist administration would attenuate LTP was realized in the early-component response changes occurring after repeated application of high-intensity tetani. The application of high-intensity trains resulted in strong potentiation effects in both the SAL/High and PILO/High groups. Scopolamine, on the other hand, suppressed LTP in this condition. These data are consistent with reports that cholinergic antagonists block hippocampal LTP (Hirotsu et al., 1989; Markevich et al., 1997) and behavioural tasks requiring memory. No significant differences were found in the early-component or population-spike measures following the application of low-intensity trains.

2.4.2 Late-Component Changes

The prediction that cholinergic agonist application would enhance LTP induction was realized most clearly in the late components of responses in animals receiving low-intensity tetani. The groups that received pilocarpine and trains showed stronger late-component potentiation effects than either their saline or scopolamine counterparts in both the low- and high-intensity groups, although the high-intensity effect was not significant. These data are consistent with demonstrations that cholinergic agonists facilitate neocortical LTP induction *in vitro* (Lin and Phillis, 1991b; Bröcher et al., 1992).

2.4.3 Contrasting the High- and Low-Intensity Induction Regimens

A reliable LTP effect in the early component of the low-intensity groups was not seen. This precluded an assessment of cholinergic manipulation. The

late component, however, showed a strong potentiation effect at the low intensity. As discussed previously (Trepel and Racine, 1998), such early and late-component dissociations may be due to a non-optimal placement of the recording electrode. The polysynaptic field of activation might be expected to cover more area than the monosynaptic field of activation. However, both the early and late components were well represented in the evoked responses, so the dissociation may be due to an independent LTP induction within horizontal pathways (which presumably carry much of the polysynaptic input) (Chapman et al., 1998).

By day seven of the induction regimen, the average late-component response amplitudes of the stimulated experimental groups are arranged as follows: SCOP/Low < SAL/Low < PILO/Low < SCOP/High < SAL/High < PILO/High. This is exactly what one would predict, assuming that (1) low-intensity trains result in less activation than high-intensity trains (Kobayashi et al., 1997) and; (2) cholinergic neurotransmission can act to increase post-synaptic depolarization (Metherate et al., 1987; Markram and Segal, 1990b; Burgard and Sarvey, 1990; Keller et al., 1991; Markevich et al., 1997;). It must be kept in mind, however, that these results are based upon systemic drug administration. Further work is required to confirm that these results are due to the modulation of cholinergic function at the cortical site itself. This work is underway.

2.4.4 Monosynaptic vs. Polysynaptic Effects

The cholinergic agents showed somewhat different efficacies depending upon the response components. The effect of scopolamine on LTP induction was

much greater in the monosynaptic component than the polysynaptic component. Potentiation of the early component was completely blocked by scopolamine. Pilocarpine, on the other hand, enhanced LTP of the late component, but had no effect on the early component.

The fact that the monosynaptic and polysynaptic LTP effects are showing somewhat independent responses to these cholinergic agents indicates that the LTP effects may be induced within separate targets (as opposed to the polysynaptic targets simply responding passively to an increased volley from the monosynaptic source). Possible potentiation sites for the polysynaptic responses are the synaptic connections of horizontal fibers (Markram and Segal, 1990b; Lee et al., 1991; Aroniadou and Keller, 1995; Hess et al., 1996; Rioult-Pedotti et al., 1998). In addition, targets could be determined by the local application of drugs specific to the M_1 acetylcholine muscarinic receptor (most dense in layers II/III) or the M_2 receptor (most dense in layer V) (for review see Zilles and Wree, 1995).

FIGURE CAPTIONS

Figure 2.1 ACh suppresses various potassium currents I. ACh suppresses the 'leak' current. At rest, the neuronal membrane is permeable to K^+ ions. These ions 'leak' across the membrane down both electrical and concentration gradients through open K^+ channels. These channels are largely responsible for maintaining the resting membrane potential at around –65mV. The Na^+ - K^+ pump (depicted in red) moves K^+ ions back into the cell and Na^+ ions out of the cell against their concentration gradients. When ACh binds to muscarinic receptors, the open K^+ channels close. This action prevents leakage and causes an intracellular positive charge build-up facilitating subsequent firing. **II.** ACh suppresses the afterhyperpolarization current. During an action potential, rapid influx of Na^+ through voltage-gated channels causes depolarization and a reversal in polarity. Eventually, Na^+ channels become inactive and K^+ channels open promoting efflux of positive ions and repolarization. Typically, a hyperpolarization occurs whereby the membrane becomes more negative than the resting membrane potential (the undershoot). During this time, the neuron is relatively refractory and subsequent firing becomes more difficult. However, ACh bound to muscarinic receptors suppresses the K^+ currents responsible for repolarizing the neuron. Ca^{2+} influx through non-specific cation channels replaces the afterhyperpolarization with a slow afterdepolarization that promotes subsequent firing. **III.** ACh suppresses the M-current. The I_M is a slowly

developing (100msec) outward K^+ current active at membrane potentials between rest and the threshold for an action potential (Kaczamerek and Levitan, 1987). Blockade of I_M by ACh leads to a net accumulation of K^+ and increased excitability of the cell. **IV.** ACh suppresses spike-frequency adaptation. One functional effect of reduced I_{AHP} and I_M is the suppression of spike-frequency adaptation. Depolarizing current injected into a pyramidal cell causes repetitive firing of that cell. Eventually, the cell adapts to the sudden depolarization and firing stops. Blockade of I_{AHP} and I_M disrupts adaptation and cells continue to fire in response to the depolarizing stimulation.

Figure 2.2 Representative examples of neocortical field potentials evoked by white matter stimulation. The solid line represents an unpotentiated evoked field potential, while the dashed line represents an evoked field potential following spaced and repeated tetani. Peaks of early and late components are identified with arrows. As well, examples of the cursor positions used to set the latencies for the peak amplitude measurements are drawn. Vertical calibration 1.0 mV; horizontal calibration 10 msec.

Figure 2.3 The effect of pilocarpine (top) and scopolamine (bottom) on the train-evoked response in a representative animal. Scopolamine produced a marked depression while pilocarpine produced a slight depression in the train-evoked response. Vertical calibration 1.0 mV; horizontal calibration 10 msec.

Figure 2.4 The effects of cholinergic agonism and antagonism on the induction of long-term potentiation by application of low-intensity stimulation. 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 2 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 small 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 statistically significant late-component potentiation. Pilocarpine enhanced late-component LTP when given in conjunction with low-intensity, high-frequency trains.

Figure 2.5 Representative sweeps taken pre- and post-LTP induction for groups that received pilocarpine and low-intensity trains (Top) or pilocarpine alone (Bottom). While the early component of both groups showed an amplitude shift in the same direction, the mechanisms mediating these effects were different (as indicated by the lack of population spike enhancement

displayed by the pilocarpine-alone animal). Moreover, pilocarpine administration alone had no effect on the late component.

Figure 2.6 The effects of cholinergic agonism and antagonism on the induction of long-term potentiation by application of high-intensity stimulation. 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 2-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. Animals that received pilocarpine or saline in conjunction with high-intensity stimulation showed a similar and large deep negative shift indicative of population spike potentiation. Scopolamine blocked early-component LTP when given in conjunction with high-intensity, high-frequency trains. **Bottom:** Changes in the late component over days. The effects of cholinergic agonism and antagonism on LTP induced by high-intensity stimulation are very similar to that following low-intensity trains. All groups that received trains showed substantial late-component potentiation relative to controls, with the PILO/High group showing slightly more LTP.

Figure 2.7 Representative sweeps taken pre- and post-LTP induction for groups that received scopolamine and high-intensity trains (Top) or scopolamine alone (Bottom). Similar to the effect shown in figure 3, both the

SCOP/High and SCOP animals showed similar surface-positive shifts, but the animal that received trains also showed population spike enhancements. This suggests that different processes are responsible for the changes shown by these groups. In fact, the SCOP group change is more indicative of a depression effect than potentiation.

Figure 2.8 Population spike measures. Total population spike height measures are shown following the high-intensity LTP induction regimens for time-points prior to LTP induction (PRE-LTP), 24 h following the induction protocols (POST-LTP) and after a 2-week decay period (DECAY). All groups that received high-intensity trains showed strong population spike enhancements, with the SAL/High group achieving the largest amplitude response by the end of the LTP induction protocol. Interestingly, while the PILO/High group average is larger than that of the SCOP/High group, both groups ultimately achieve equivalent population spike amplitudes despite the fact that the SCOP/High group shows little early-component EPSP amplitude change relative to controls. These population spike measures remain enhanced for at least 2 weeks following the cessation of the LTP induction protocol.

Figure 2.1 ACh Suppresses Various Potassium Currents

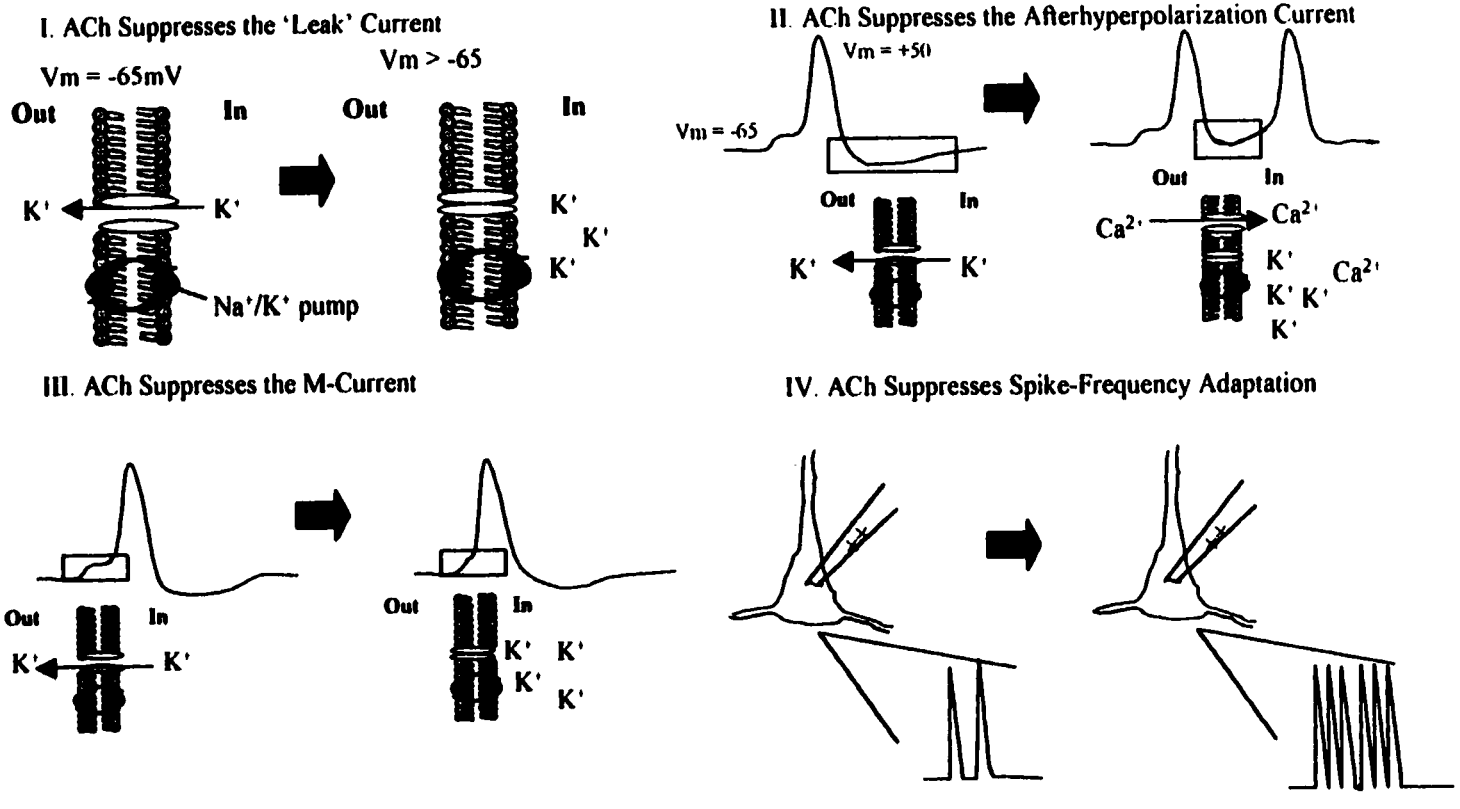
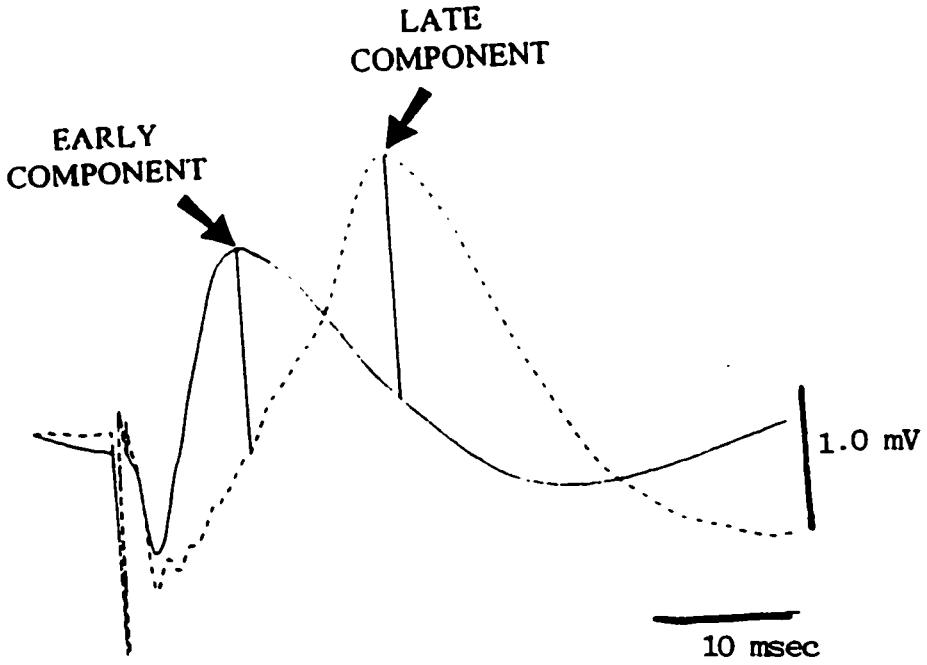


Figure 2.2 Neocortical Field Potentials Evoked by White Matter Stimulation



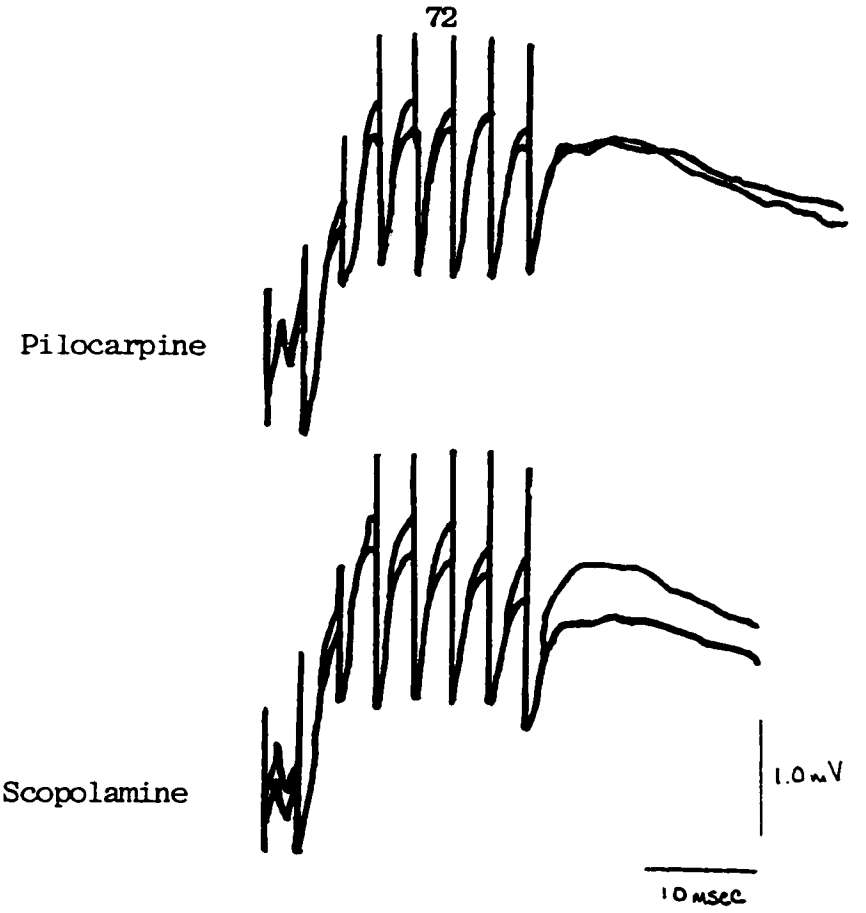


Figure 2.3 The Effect of Pilocarpine and Scopolamine on the Train-Evoked Response.

Figure 2.4 Cholinergic Modulation of Low-Intensity LTP

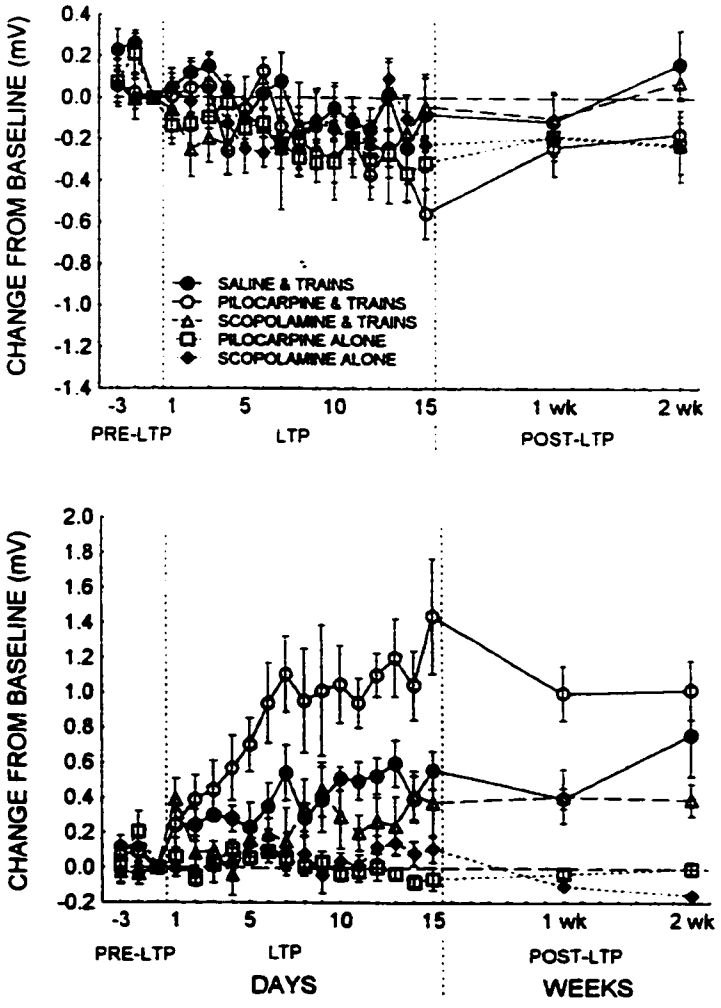


Figure 2.5 Representative Sweeps Taken Pre-and Post-LTP Induction for PILO/low and PILO Groups

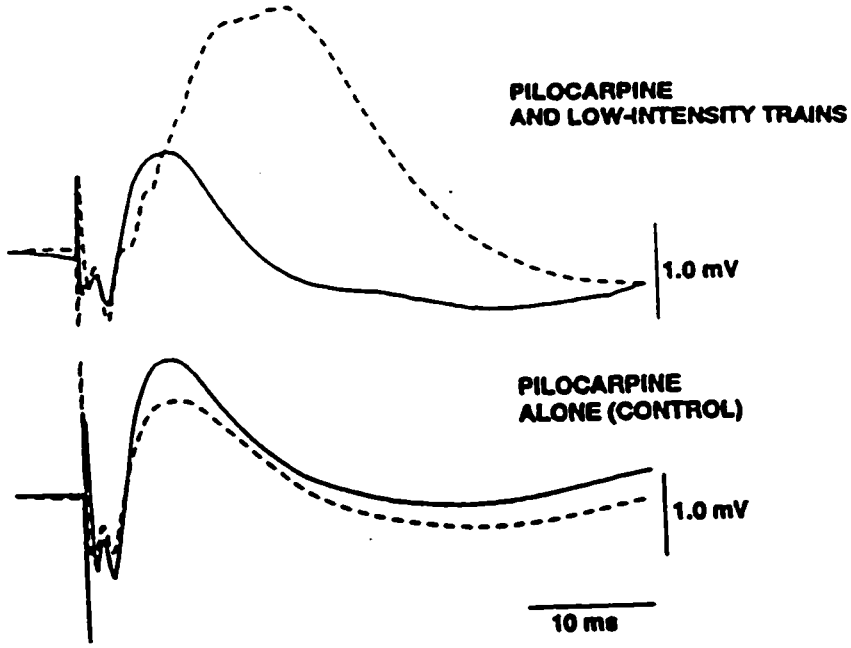


Figure 2.6 Cholinergic Modulation of High-Intensity LTP

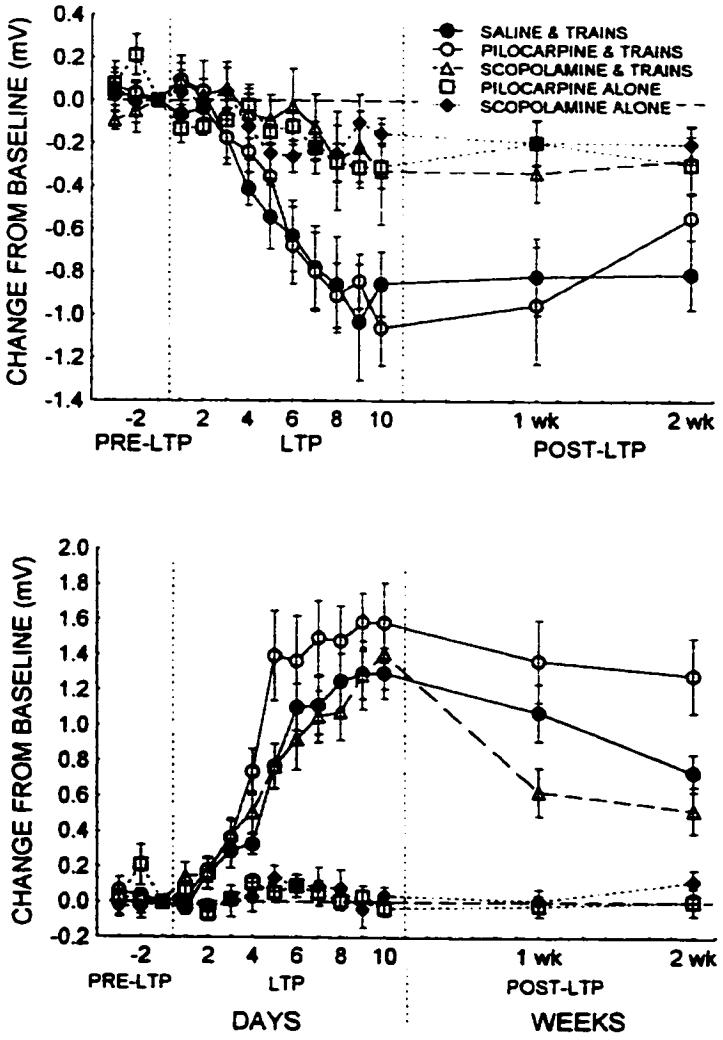
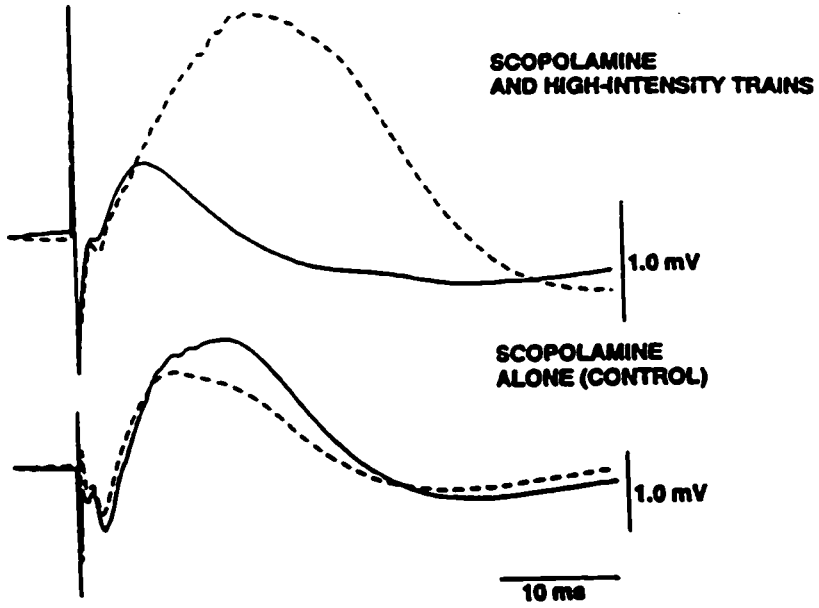


Figure 2.7 Representative Sweeps Taken Pre- and Post-LTP Induction for SCOP/high and SCOP Groups



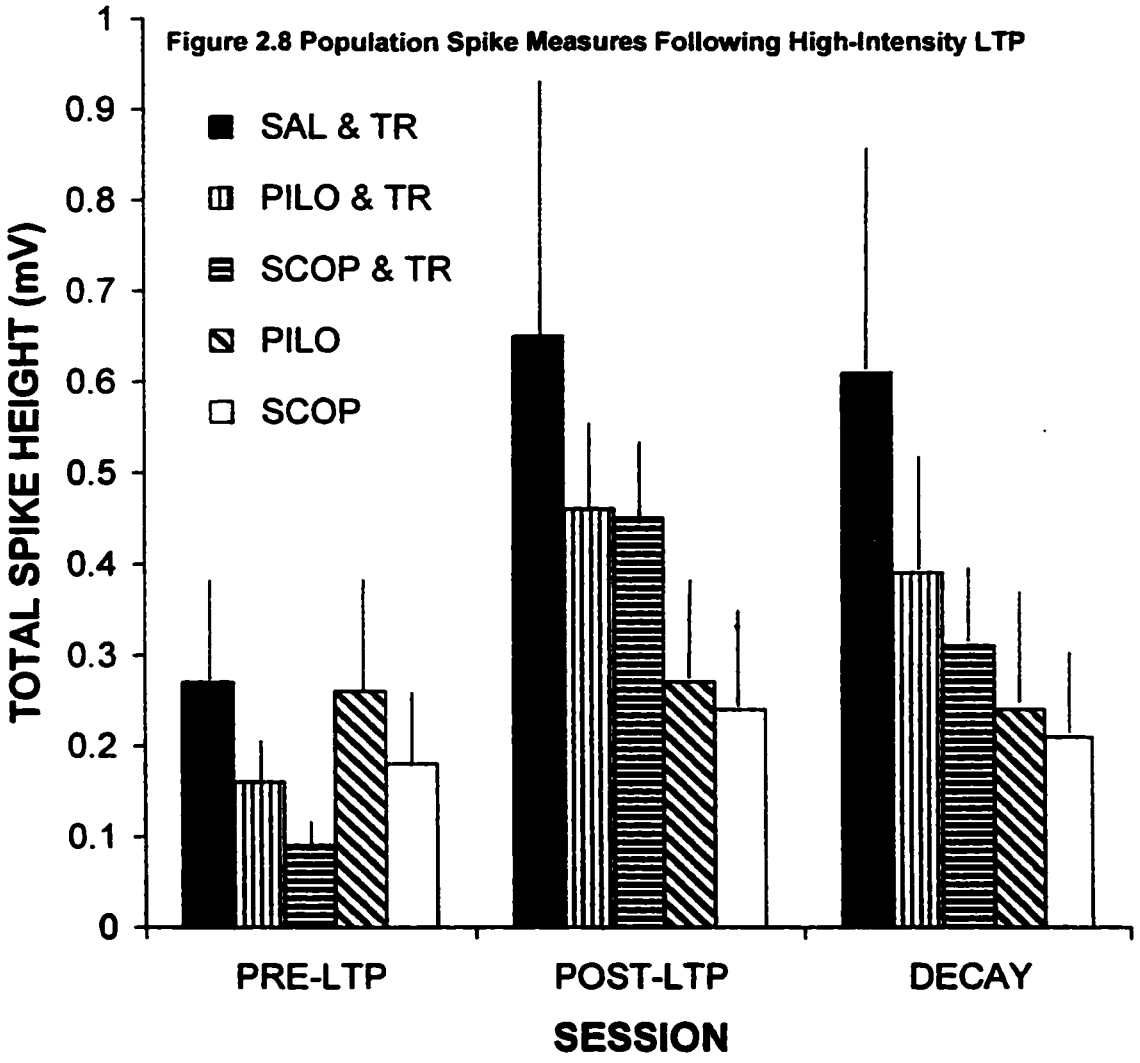


Figure 2.8 Population Spike Measures Following High-Intensity LTP

CHAPTER THREE

NORADRENERGIC MODULATION OF NEOCORTICAL LONG-TERM POTENTIATION IN THE FREELY MOVING RAT

3.1 INTRODUCTION

3.1.1 Noradrenergic Projections to the Neocortex

The catecholamine neurotransmitter noradrenaline (NA) exists both in the peripheral and central nervous systems. In the brain, projections from the locus coeruleus distribute NA to most areas of the cerebral cortex (see figure 2.3; reviewed in Cooper et al., 1996). Bilateral lesions of the locus coeruleus reduce cortical NA by 70 percent (Anlezark et al., 1973).

3.1.2 Noradrenergic Receptors and Signal Transduction

NA binds to two types of receptors: α and β. To date, two subtypes of α adrenoceptors have been identified: α_1 and α_2. β receptors are further segregated into three receptor subtypes: β_1, β_2 and the less-well researched β_3. The cerebral cortex primarily contains β_1 receptors, while the cerebellum contains mostly β_2 receptors (Frazer et al., 1986). When NA binds to a central β_1 or β_2 receptor subtype, G-proteins initiate the adenylate cyclase, c-AMP-dependent protein kinase system (Hieble et al., 1995; see figure 1.5). The α_1 subtype activates the phospholipase C second messenger cascade, while ligand bound to the α_2 receptor inhibits adenylate cyclase (Hieble et al., 1995) opposing the actions of the β_1 or

β_2 receptor. The experiments contained within this chapter focus on β-adrenergic modulation of LTP induction in the freely moving rat.

3.1.3 Noradrenergic Modulatory Actions

Similar to cholinergic muscarinic receptors, β-NA receptors modulate neuronal excitation by preventing spike-frequency adaptation to a depolarizing pulse. This effect has been demonstrated in both hippocampal (Madison and Nicoll, 1986a) and layer V somatosensory cortical slices (Norwicky et al., 1992). Activation of β-noradrenergic receptors prevents spike-frequency adaptation by blocking the Ca^{2+}-activated K^+ I_{AHP} (Madison and Nicoll, 1986a; see figure 2.1). NA binding to β-noradrenergic receptors facilitates glutamate release (Herrero and Sánchez-Prieto, 1996) and initiates the cAMP/PKA cascade (Madison and Nicoll, 1986b). cAMP actions mediate the reduction in calcium-dependent K^+ I_{AHP} and prevent the aforementioned spike-frequency adaptation (Madison and Nicoll, 1986b).

An "inverted-U"-shaped function best describes the dose-dependent way in which NA modulates glutamate-evoked discharges (Devilbiss and Waterhouse, 2000). In fact, high doses of NA depressed glutamate-evoked discharge below control levels in 10% of cells tested (Devilbiss and Waterhouse, 2000).[1] Activation of NA α_1 receptors accounts for the facilitation of glutamate-evoked

[1]The dose-response relationship demonstrated upon glutamate-evoked responses mirrors effects obtained on memory tasks (see section 3.1.4). This converging evidence supports the idea that an optimal amount of NA must exist for proper cellular-and systems- (e.g., learning and memory) level functioning.

responses, while nonspecific activation of α_2 or β receptors mediates the high-dose NA-triggered suppression.

Kety (1972) proposed, "The dependence of learning on attention and on reward or punishment is well-established at the behavioural level, and there is obvious adaptive advantage in a mechanism which consolidates not all experience equally but only those experiences which are significant for survival." The notion that NAergic neuromodulatory activity could filter significant from insignificant experiences and promote memory for the former became known as the Kety hypothesis. Although primarily discussed at the systems level for learning and memory, support for the Kety hypothesis has been demonstrated at the cellular levels and in neural-network models as well. It has been proposed that NA can increase the "signal to noise ratio" by selectively suppressing the background spontaneous neuronal activity in favor of evoked discharge (Haas and Konnerth, 1983; Devilbiss and Waterhouse, 2000). As briefly described above, Devilbiss and Waterhouse (2000) used glutamate-evoked responses to demonstrate that NA facilitates EPSPs in some cells and suppresses EPSPs in others. Irrespective of the direction of NA modulation (i.e., facilitation or suppression), the magnitude of change from control was greatest in cells that received glutamate iontophoretic pulses that depolarized the cell, but did not produce an action potential (subthreshold stimulation) (Devilbiss and Waterhouse, 2000). Evoked responses by suprathreshold iontophoretic application of glutamate were not modified by NA application, whereas NA facilitated or depressed responses to subthreshold

glutamate application[2]. The exact mechanism that governs this neuromodulatory capability is unknown. However, Hasselmo et al., (1995) demonstrated in piriform cortical slices, and simulated in neural network models, a selective NA suppression of spontaneous excitatory synaptic transmission between pyramidal cells (thus, reducing background noise). This, in turn, promoted increased firing in cells receiving afferent input (i.e., enhanced the signal).

These general modulatory principles apply to NA within a specific range of doses, and the effect varies with stimulus parameters, brain structure, and method of investigation. For example, while some researchers report that NA suppresses EPSP amplitude (e.g. Mody et al., 1983 in CA1; Dodt, et al., 1991 in somatosensory cortex; Scanziani et al., 1994 in CA3), others report no change (e.g., Mueller et al., 1981; Madison and Nicoll, 1988 both in hippocampus). By contrast, NA depletion in the freely moving rat has been shown to decrease perforant-path-evoked granule cell population EPSP and increase the population-spike amplitude (Robinson and Racine, 1985).

3.1.4 Noradrenergic Modulation of Memory

The Kety hypothesis linked memory to arousal through noradrenergic modulation of the encoding process (1972). Kety (1972) proposed that drugs that enhance NA release in the brain, or mimic its neuronal actions, would favor consolidation and facilitate memory. Conversely, lesions or drugs that cause NA depletion or blockade at synapses should retard consolidation (Kety, 1972).

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[2] At the systems level, presumably the combined factors that determine whether the response

Since the formulation of the Kety hypothesis, the involvement of NA in learning and memory has been extensively investigated in a variety of tasks. Many studies have confirmed Kety's predictions. For example, blockade of NA synthesis disrupted long-term, retention for a step-down task in rats (Stein et al., 1975) and mice (Randt et al., 1971). Bilateral locus coeruleus ablations have hindered learning of a food-rewarded motor task, but did not impair motor ability or motivation (Anlezark et al., 1973). Intra-peritoneal injection of the non-specific □-receptor antagonist propranolol impaired reactivation of a memory for radial arm maze and footshock-conditioned emotional tasks (Przybyslawski, et al., 1999) as well as memory for an olfactory associative learning task in rat pups (Wilson et al., 1994). Similarly, i.p. injections of propranolol delayed learned maternal behaviour (Moffat et al., 1993). As well, cannula-administered propranolol disrupted formation of post-partum olfactory recognition in ewes (Levy et al., 1990). Finally, when trained on an inhibitory-avoidance task, i.p.-injected propranolol blocked bicuculline-enhanced retention in rats.

Fewer demonstrations of NA-enhanced memory have been shown. Both systemic and intra-amygdalar administration of clenbuterol enhanced retention for an inhibitory-avoidance task in rats (Introini-Collison et al., 1991). Moreover, clenbuterol blocked the impairing effects of the GABA agonist muscimol (Introini-Collison et al., 1994). The non-specific β-receptor agonist isoproterenol expedited learned maternal behaviour in dams (Moffat et al., 1993).

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is facilitated or depressed include behavioural relevance as initially proposed by Kety (1972).

However, not all NAergic modulatory results support the Kety hypothesis. Interestingly, both the agonist isoproterenol and the antagonist propranolol blocked consolidation of olfactory memories when systemically injected after training (Wilson et al., 1994). Additionally, i.p.-injected propranolol had no effect on errors during a reference memory task, but negatively affected learning for a working memory task when given in conjunction with the cholinergic antagonist scopolamine (Kobayashi et al., 1995).

In the same way that NA modulates neuronal excitation in response to glutamate-evoked discharge, Price et al. (1998) suggest that an optimal amount of NA must exist at the time that the unconditioned stimulus (UCS) is presented for learning success. The level of NA present modifies learning and memory in an "inverted-U" relationship. Price et al. (1998) propose that β-noradrenergic agonists supplement NA concentrations and enhance learning if the endogenous levels of NA at stimulus presentation have not exceeded a critical threshold. However, agonists can also block learning if they combine with endogenous levels of NA to exceed the effective level and duration of NA action. This hypothesis may account for some of the inconsistencies seen in NAergic modulatory actions at both the cellular and systems levels.

3.1.5 Noradrenergic Modulation of LTP

NA has also been shown to modulate other forms of brain plasticity. Kasamatsu and Pettigrew first reported that NA depletion blocked ocular dominance shifts following monocular deprivation in kittens (1976). As well, NA

depletion prevented cortical-weight increases in enriched-environment-reared or maze-trained rats. However, this depletion did not impair spatial working memory acquisition (Benloucif et al., 1995).

NA can also produce long-lasting modulations of synaptic efficacy in both the anaesthetized *in vivo* and slice preparations. For example, NA potentiated the dentate gyrus population spike when microiontophoretically applied in the anaesthetized rat (Neuman and Harley, 1983). Additionally, NA dose-dependently increased the population-spike amplitude of the perforant path evoked response in hippocampal brain slices (LaCaille and Harley, 1985). In this experiment, over 50 percent of slices showed an increased EPSP slope and a decreased population spike onset latency in response to NA subfusion (LaCaille and Harley, 1985). These pharmacological effects were mimicked by β-receptor agonism and blocked by β-receptor antagonism (LaCaille and Harley, 1985) suggesting that β receptors mediate these effects within the hippocampus.

Most research investigating NA neuromodulation of LTP has been conducted in the hippocampal slice preparation. In this preparation, NAergic modulatory effects seem to depend upon the frequency of the conditioning stimulation and the hippocampal pathway to which it is applied. In the CA1 region, NA agonists facilitated LTP induced by 10Hz (Katsuki et al., 1997) and 5Hz (Thomas et al., 1996) stimulation. This effect was blocked by the □-antagonist timolol (Katsuki et al., 1997) suggesting that these effects are mediated by □-NA receptors. However, Dunnwiddie (1982) reported that NA had no effect

on 500-Hz-induced LTP in CA1, and isoproterenol, a nonspecific □ agonist, attenuated LTP. Perhaps when combined with high-frequency stimulation, the agonist pushed NA receptor activation beyond the levels where it would be expected to facilitate LTP induction (in keeping with the "inverted-U" function proposed for modulatory action). In conjunction with the lower frequency trains, the agonist can still work within the effective window and produce a synergistic effect on LTP.

In area CA3, NA and isoproterenol were found to increase the magnitude, duration and probability of LTP induction when paired with high-frequency (100Hz) train stimulation (Hopkins and Johnston, 1984). Moreover, NA antagonists propranolol and timolol blocked CA3 LTP induced by 100Hz trains (Hopkins and Johnston, 1984), but timolol did not effect CA3 LTP induced with 50Hz train stimulation (Katsuki et al, 1992). The results of these studies suggest that the optimal combination of neuromodulation and excitation differ between the CA1 and CA3 areas. It also hints that different receptors may mediate NAergic neuromodulation depending upon the frequency used for LTP induction.

Finally, Robinson and Racine (1985) showed in freely moving rats that NA depletion promotes higher levels of perforant path-evoked LTP of the population EPSP, but reduces population-spike potentiation in the dentate gyrus. These results raise the possibility that the complex nature of NAergic modulation may depend on preparation as well as brain area.

Few studies have investigated the effect of NA on LTP in the neocortex. In sensorimotor slices, noradrenergic agonists increased the excitability of layer V sensorimotor cells, but did not increase the probability of LTP (Nowicky et al., 1992). While some researchers have reported a facilitative effect of isoproterenol on LTP induction in visual cortical slices (Kato, 1993), others report no effect (Bröcher et al., 1992). The influence of NA on neocortical LTP remains relatively unexplored particularly in the freely moving rat. The following experiment was designed to determine the modulatory effects of β-adrenergic agonism and antagonism on neocortical LTP in chronic preparations.

3.2 MATERIALS AND METHODS

3.2.1 Animals and Surgery

Thirty-five male, Long-Evans rats, weighing 300-350g, from the Charles River breeding colonies, were used in this experiment. Animals were housed in pairs prior to surgery, individually thereafter, and maintained in a colony as described in the preceding chapter. Electrode construction and implantation was also as described in the previous chapter. Experimentation began no sooner than 10 days after surgery. All experimental procedures were conducted in accordance with the Guidelines of the Canadian Council on Animal Care and approved by the McMaster University Animal Research Ethics Board.

3.2.2 Baseline Measures and Induction of LTP

Electrical stimuli were produced and recorded as described in the preceding chapter. The agonist and antagonist manipulations were conducted on separate occasions. Forty-eight and 24 hours[3] separated each of three baseline field potential I/O measures for the antagonist and agonist data sets respectively. Baseline and experimental I/O measures were collected using the same parameters as described in the previous chapter. The evoked field potentials comprised an early, monosynaptic component and a later, polysynaptic component (Chapman et al., 1998) that was most easily visualized after LTP induction. In some animals, one or more population-spikes were present at latencies corresponding to the early component. Based upon baseline-response morphology, animals were matched in triads and randomly assigned to the three groups (drug plus trains, drug alone, and saline plus trains) in either the agonist or antagonist conditions.

Thirty minutes following each daily injection of agonist, antagonist or saline, LTP groups received 60 high-frequency (300Hz), 24msec, 8-pulse trains delivered to the corpus callosum. Intra-train pulse duration was 0.1msec. Animals in the propranolol (antagonist) manipulation received high-intensity trains of 1260μA. Based upon a previously conducted pilot experiment, however, 1260μA proved too high to allow any dissociation between the agonist (clenbuterol) and the control groups. Consequently, the stimulation intensity for this group was reduced to 795μA.

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[3] Due to scheduling constraints.

Due to the time required to run the large number of animals in the propranolol experiment on a daily basis, it was determined that an I/O measure would be taken every third day of experimentation and a final measure after 10 LTP sessions. A total of 10 I/O measures were collected: 3 to establish baselines (I/Os 1-3), 5 during LTP induction and one per week for two weeks to measure decay of the response. I/O measures were taken prior to every third conditioning session during LTP induction. After the 10^{th} LTP session, data were analyzed and it was suggested that the experiment should continue to determine if the PROPTR and SALTR groups would further diverge. Standard injection and train-stimulation protocol resumed the following day. A total of 12 daily conditioning sessions were administered. LTP decay was monitored for 2 weeks. For the clenbuterol manipulation, daily LTP conditioning stimulations were administered for 11 days. Scheduling conflicts forced minor alterations in the experimental protocol (see table 1). The final I/O measurement was collected 1 week after the final LTP induction I/O to monitor decay of the response. EEG was monitored during the experiment as described in the preceding chapter.

3.2.3 Noradrenergic Agonism and Antagonism

Twenty-four hours after the third baseline test, animals were injected with the antagonist propranolol hydrochloride (10mg/kg, i.p.) or the agonist clenbuterol (5mg/kg, i.p.) (both obtained from Sigma-Aldrich Canada, and dissolved in saline), or an equal volume of saline. Propranolol has broad-spectrum effects targeting both β_1 and β_2 adrenoceptors nonselectively. While

clenbuterol has equal affinity for β_1 and β_2 receptors (Frazer et al., 1986), it only activates β_2 receptors (O'Donnell, 1997). Though the majority of cortical β receptors are type β_1, clenbuterol has been shown to significantly increase cyclic AMP production in cortex (Frazer et al., 1986) indicating that it acts potently at nascent cortical β_2 receptors.

The animals were divided into six groups: saline + trains, (SALTR: n=8 for antagonist and n=3 for agonist), propranolol + trains (PROPTR: n=8), propranolol only (PROPO: n=9), clenbuterol + trains (CLENTR: n= 4) and clenbuterol only (CLENO: n=3). Thirty minutes after the injection, SALTR, PROPTR and CLENTR groups received LTP-inducing stimulation.

3.2.4 Analyses

Field potentials and population spikes were analyzed as described in the preceding chapter. One animal from the PROPTR group was discarded from analysis due to artifact contamination. One SALTR animal, run in conjunction with the clenbuterol experiment, was an outlier and thus discarded from analysis. After discarding this animal, only two SALTR animals remained for population-spike comparison analysis in the clenbuterol manipulation. So, SALTR animals from the propranolol and clenbuterol experiments were combined and compared to all other clenbuterol experimental groups through the one-week decay point.

Animals groomed themselves and gained weight normally throughout the experiment. Experiments were conducted in a plexiglass running box as described previously. Following completion of the experiment, animals were

intracardially perfused with saline followed by 10% formalin. The brains were sliced and stained with cresyl violet to verify electrode placements. In all animals, stimulating and recording electrodes were positioned within their target structures (Paxinos and Watson, 1997).

3.3 RESULTS

3.3.1 Response Morphology

The responses were similar to those previously reported (Trepel and Racine, 1998; Chapman et al., 1998) and those described in chapter two. In some animals there was a very short-latency, deep-negative, population-spike response, which previous experiments have shown to be a mix of antidromic and orthodromic effects (Chapman et al., 1998). The population spike was followed by an early response component with a mean latency-to-peak of 7.60 msec and 8.49 msec for the propranolol and clenbuterol groups respectively. The late peak, representing polysynaptic activity, emerged at a mean latency of 23.58 and 19.40 msec for the propranolol and clenbuterol groups respectively.

Figure 3.1 depicts the effect of both clenbuterol and propranolol on the train-evoked response in a representative animal. Both clenbuterol and propranolol depressed the train-evoked response.

3.3.2 β-Noradrenergic Antagonism Fails to Attenuate LTP Induction

The population-spike amplitudes were summed across groups in the antagonist manipulation. A repeated-measures ANOVA yielded a significant

main effect for session $F_{(2,42)} = 9.68$, $p < 0.001$. However, no interaction effect was obtained $F_{(4,42)} = 0.98$, $p = 0.42$ (data not shown). All groups showed a modest increase in summed population spike amplitude, but there were no significant group effects.

Figure 3.2 shows the effect of propranolol on early-component LTP induction. A repeated-measures ANOVA of the early-component measures revealed a significant group-by-session interaction effect $F_{(14, 154)} = 6.53$, $p < 0.001$. Both PROPTR and SALTR differed significantly from PROPO ($p < 0.001$ in both cases), but not from each other ($p = 0.81$). The PROPO group hovered around baseline for the duration of the experiment, while both groups that received conditioning trains diverged from baseline indicating that the LTP trains had an effect on both the SALTR and PROPTR groups.

The late-component data analysis also revealed a significant group-by-session interaction effect ($F_{(14,154)} = 12.34$, $p < 0.001$). Figure 3.3 depicts these data. The SALTR group shows more potentiation than PROPTR during LTP induction, with the greatest divergence occurring after 5 days of trains. The difference between the SALTR and PROPTR groups approached significance ($F_{(7, 98)} = 1.96$, $p = 0.06$), suggesting that the antagonist may have had some suppression effect on the late-component LTP induction. The PROPO group shows no change throughout the experiment. Both the PROPTR and the SALTR groups differed from the PROPO group ($p < 0.001$ in both cases).

3.3.3 β-Noradrenergic Agonism Both Facilitates and Attenuates LTP Induction

Neither significant main effects nor significant group-by-session interaction ($F_{(4,28)} = 1.30$, $p = .29$) effects were obtained for the population-spike amplitude measurements owing to the small sample size of the CLENTR and CLENO groups (data not shown). The CLENTR group showed a non-significant increase in population-spike activity over LTP induction days. This group also showed relatively little decay in the week following the final train session. The population-spike activity in the CLENO group changed very little over the course of the experiment.

Clenbuterol facilitated LTP induction in the monosynaptic, early component (see figure 3.4). A repeated-measures ANOVA of the early-component measures yielded a significant overall group-by-session interaction $F_{(22,77)} = 3.76$, $p < 0.001$. CLENTR showed significantly more potentiation than SALTR ($F_{(11,55)} = 2.07$, $p < 0.05$). However, SALTR and CLENO did not differ significantly ($p = 0.10$) indicating that the trains alone were not sufficient to induce LTP. It appears that the SALTR group was beginning to diverge from the CLENO group. Thus, it is reasonable to speculate that more sessions of stimulation would eventually drive the two groups apart. Indeed, train intensity as low as 160uA has induced

LTP in the chronic preparation (Trepel and Racine, 1998). However, in that study, the induction protocol required 14 sessions for complete divergence.

Figure 3.5 shows that clenbuterol attenuated LTP induction of the polysynaptic, late component. A repeated-measures ANOVA resulted in a significant overall group-by session-interaction ($F_{(22,77)} = 3.17$, $p < 0.001$). SALTR showed normal potentiation for the late component, and this potentiation was significantly greater than the CLENTR ($F_{(11,55)} = 2.35$, $p = 0.01$) and CLENO ($F_{(11, 44)} = 6.22$, $p < 0.001$) groups.

3.3.2 Decay

The PROPTR group showed little decay during the initial week following LTP train cessation, but showed early- and late-component decay during the second week that was comparable to the SALTR group. Clenbuterol seemed to preserve early-component potentiation during the first week after trains ceased, as well. The SALTR group showed marked early- and late-component decay while the CLENTR group remained at a stable level.

3.4 DISCUSSION

Kety predicted that NA blockade would disrupt learning and memory (1972). If Kety was correct, and if LTP reflects a memory mechanism, propranolol might be expected to attenuate LTP. In our protocol, however, it did not. There are several possible reasons for this lack of effect including, but not limited to: 1) The drug did not interact with central receptors; 2) the intensity of

LTP trains was not optimal for visualization of the modulatory actions of NA; 3) NA antagonism promotes compensatory actions from other modulatory systems; 4) NA does not play a role in LTP induction in this preparation; 5) LTP and memory do not share mechanisms.

It is unlikely that the drug failed to interact with central receptors. The antagonist propranolol both crosses the blood-brain barrier and binds with high potency to central receptors within the cortex and cerebellum (Tondo et al., 1985) and hippocampus (Garvey and Ram, 1975) when injected systemically.

It is possible that expression of the modulatory effects of propranolol depends upon the train intensity. Repeated 1260μA stimulation typically produces an asymptotic level of LTP in chronically prepared rat sensorimotor cortex (Trepel and Racine, 1998), leaving room for expression of attenuating effects. However, this high-intensity stimulation may overpower any subtle modulatory effects contributed by NA antagonism. Such effects may be more clearly seen at lower stimulation intensities.

Some neuromodulatory actions of NA and ACh are similar: both transmitter systems reduce spike-frequency adaptation and suppress K^+ currents. Reduction in one transmitter system's modulatory effects may initiate compensatory actions in partially redundant systems to ensure an organism's continued ability to adapt to the world. Additionally, noradrenergic and cholinergic systems have been shown to modulate memory and synaptic plasticity synergistically (Bröcher et al., 1992; Dalmaz et al., 1993; McGaugh et al., 1993;), with subeffective doses of

each neurotransmitter combining to produce a large result. Perhaps the effect of propranolol would have been enhanced if paired with a cholinergic antagonist such as scopolamine (e.g., Kobayashi et al., 1995). Systemic injection of a "drug cocktail" containing cholinergic and noradrenergic antagonists could shed some light on the ability to induce LTP in the absence of these important neurotransmitters and prevent compensatory modulatory actions.

The possibility that NA does not affect neocortical LTP induction in the freely moving rat cannot be overlooked, but the clenbuterol results, together with the results of a manipulation employing the α_2 agonist clonidine, suggest that this is not the case. α_2 receptor agonists prevent the release of NA from the synaptic terminal. Systemic administration of the α_2 agonist clonidine attenuates late-component, but not early-component LTP induction in the freely moving rat (Boyd et al., 1999) suggesting that NA plays a role in late-component LTP (see figure 3.6)

As reviewed in the General Introduction and the Introduction of this chapter, LTP and learning appear to share similar mechanisms. Although this does not mean that LTP is *the* learning mechanism, it suggests that they are related. Nevertheless, it remains to be determined whether neocortical LTP and memory that depends on the tested neocortical system responds similarly to the manipulation of NA pathways.

Clenbuterol promoted LTP in the early component. The group receiving saline did not differ from the control group that only received the drug. This

result suggests that clenbuterol increased the probability of LTP induction and is consistent with the Kety hypothesis. This result agrees with some agonist studies in the hippocampus (Katsuki et al., 1997; Thomas et al., 1996; Hopkins and Johnston, 1984) and neocortex (Kato, 1993). However, it stands in contrast to others in sensorimotor cortex slice (e.g., Nowicky et al., 1992) and visual cortex slice (Bröcher et al., 1992).

Unexpectedly, clenbuterol attenuated late-component LTP. A couple of factors may account for this result: 1) the "inverted-U" dose-response NAergic neuromodulatory relationship seen at both the systems and cellular levels or 2) receptor changes resulting from repeated drug administration.

As explained previously, the neuromodulatory effects of NA depend upon the brain area investigated, frequency of electrical stimulation, concentration of NA, and preparation in which the manipulation is conducted. Robinson and Racine (1985) have demonstrated that dentate gyrus LTP lasting for days or weeks is unaffected by NA depletion. And, as just discussed, propranolol came close to attenuating late-component LTP, while clonidine did attenuate late-component LTP in awake rat neocortex. The attentuation of late-component LTP produced by clenbuterol may have resulted from the same mechanisms that promote the inverted-U relationship governing cellular responses (Devilbiss and Waterhouse, 1998;) and learning and memory success (Price et al., 1998). In this case, the combination of NA and excitation exceeded the optimal level of modulatory action resulting in an attenuation of LTP. The early component, on

the other hand, showed the predicted facilitation of LTP suggesting that the concentration of NA and electrical stimulation was within the optimal range for that component. The dose-response relationships may differ for the two components.

Repeated administration of clenbuterol has been shown to down-regulate the number of β_2 receptors in both the cerebellum and cortex (Frazer et al., 1986). It has also been suggested that clenbuterol may act as a β_1 antagonist in the cortex (Frazer et al., 1986). The combined effect of β_2 receptor down regulation, and β_1 receptor antagonism could account for the attentuated LTP in the late component.

The opposing effects seen in the early and late component suggest that the synapses mediating those components have different modulatory requirements. It would be instructive to test these modulatory effects separately on major neocortical pathways (e.g., thalamocortical, callosal and horizontal).

LTP induced in conjunction with both clenbuterol (early and late) and propranolol (late) showed consistently less decay within the first week than LTP induced with saline. It is tempting to speculate that NA agonism may lead to a more prolonged LTP effect. However, the PROPTR animals also decayed minimally during the first week after train cessation. Thus, it seems reasonable to suggest that the "preservation" effect is actually a result of β_1 antagonism in the cortex. Future experiments should include specific β_1 antagonism (e.g., atenolol) and agonism (e.g.,xamoterol) in conjunction with LTP to test this hypothesis.

Table 1. Timetable for the β-Noradrenergic Experiments

Clenbuterol				**Propranolol**			
Date	I/O	Inject	Trains	Date	I/O	Inject	Trains
8/21	IO01	No	No	2/19	IO01	No	No
8/22	IO02	No	No	2/21	IO02	No	No
8/23	IO03	No	No	2/23	IO03	No	No
8/24	No	Yes	Ses. 1	2/24	No	Yes	Ses. 1
8/25	IO04	Yes	Ses. 2	2/25	No	Yes	Ses. 2
8/26	IO05	Yes	Ses. 3	2/26	IO04	Yes	Ses. 3
8/27	IO06	Yes	Ses. 4	2/27	No	Yes	Ses. 4
8/28	IO07	Yes	Ses. 5	2/28	No	Yes	Ses. 5
8/29	IO08	Yes	Ses. 6	2/29	IO05	Yes	Ses. 6
8/30	IO09	Yes	Ses. 7	3/1	No	Yes	Ses. 7
8/31	IO10	Yes	Ses. 8	3/2	No	Yes	Ses. 8
9/1*	No	No	No	3/3	IO06	Yes	Ses. 9
9/2	IO11	Yes	Ses. 9	3/4	No	Yes	Ses. 10
9/3*	No	Yes	Ses. 10	3/5**	IO07	No	No
9/4	IO12	Yes	Ses. 11	3/6	No	Yes	Ses. 11
9/5	IO13	No	No	3/7	No	Yes	Ses. 12
9/12	Decay	No	No	3/8	IO08	No	No
				3/15	Decay	No	No
				3/22	Decay	No	No

*Scheduling conflicts forced experimentation protocol modifications for the clenbuterol group. Due to the time required to run the large number of animals in the propranolol experiment on a daily basis, I/O measures were taken every third day. **After the 10th LTP session data were analyzed and it was determined that the experiment should continue in the hopes that the PROPTR and SALTR groups would further diverge. Standard injection and train protocol resumed the following day.

FIGURE CAPTIONS

Figure 3.1 The effect of clenbuterol and propranolol on the train-evoked response. Propranolol (top) and clenbuterol (bottom) depressed the train-evoked response.

Figure 3.2 The effect of propranolol on early-component LTP. The X-axis depicts the response amplitude change from baseline in millivolts. The Y-axis depicts baselines, LTP induction sessions, and decay measurements corresponding to table 1. SALTR and PROPTR showed marked and equivalent potentiation of the early component. Both groups differed from the PROPO group ($p < 0.001$ in both cases), but not from each other ($p = 0.81$). Propranolol failed to attenuate LTP of the early component as predicted.

Figure 3.3 The effect of propranolol on late-component LTP. X- and Y-axes are as in figure 3.2. Both the SALTR and PROPTR groups showed potentiation and differed from the PROPO group ($p < 0.001$ in both cases). The SALTR group showed slightly more potentiation than the PROPTR group. The difference between these two groups approached significance ($F_{(7, 98)} = 1.96$, $p = 0.06$).

Figure 3.4 The effect of clenbuterol on early-component LTP. X- and Y-axes are as in figure 3.2. Clenbuterol facilitated LTP induction in the monosynaptic, early component. CLENTR showed significantly more potentiation than SALTR

($F_{(11,55)} = 2.07$, $p < 0.05$). However, SALTR and CLENO did not differ significantly ($p = 0.10$) indicating that the trains alone were not sufficient to induce LTP. 48 hours elapsed between LTP sessions 8 and 9. Session 9 was followed 48 hours later by an I/O measure. See table 1.

Figure 3.5 The effect of clenbuterol on late-component LTP. X- and Y-axes are as in figure 3.2. Clenbuterol attenuated LTP induction of the polysynaptic, late component. SALTR showed normal potentiation for the late component, and this potentiation was significantly greater than the CLENTR ($F_{(11,55)} = 2.35$, $p = 0.01$) and CLENO ($F_{(11,44)} = 6.22$, $p < 0.001$) groups. Two days elapsed between I/Os 10 and 11 during only one of which the animals received LTP trains (see table 1). This seemed to cause a dramatic shift in both the SALTR and CLENTR groups. Two days elapsed between I/Os 11 and 12. During both days, the animals received LTP-inducing trains. The SALTR group recovered to subsequent levels, but the CLENTR group plummeted toward baseline levels. Interestingly, the CLENTR group showed complete recovery 24 hours later and maintained a consistent level of potentiation during the post-inductive experimental phase.

Figure 3.6 The effect of clonidine on late-component LTP. The $\square_2$ adrenergic agonist clonidine (0.3 mg/kg i.p.) was given in conjunction with high-intensity, high-frequency trains. The Y-axis depicts the change in response amplitude from

baseline in millivolts. The X-axis demarcates the sessions beginning with three baselines and continuing through LTP induction. No decay measures were collected. LTP induction was carried out following the spaced and repeated trains protocol. Clonidine blocked late-component LTP.

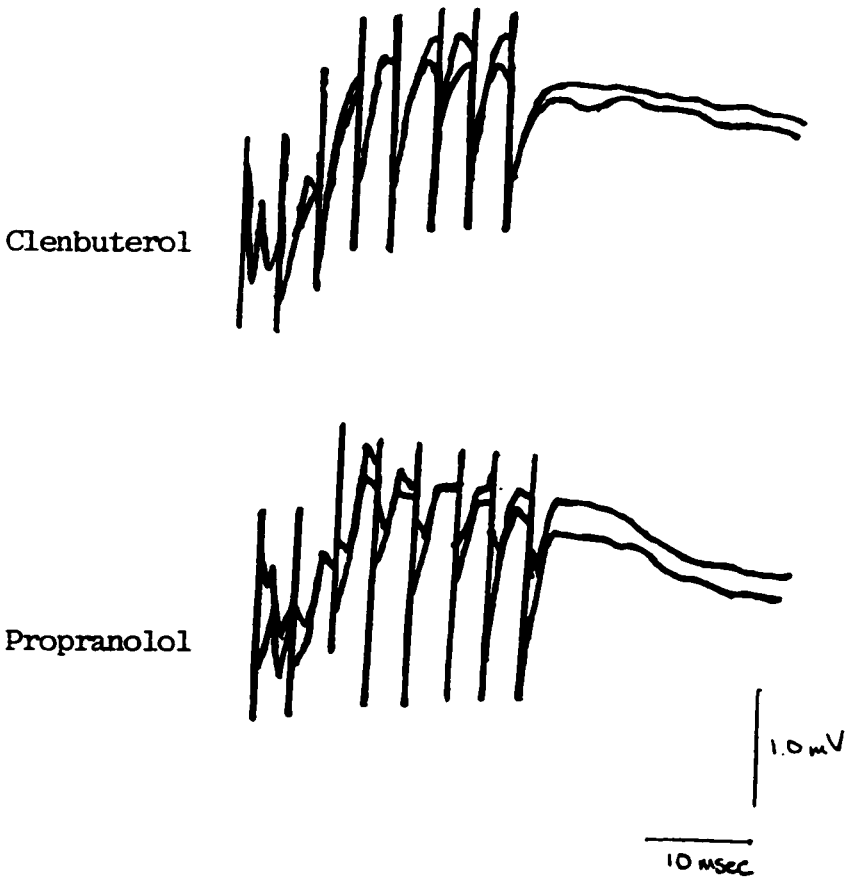


Figure 3.1 The Effect of Clenbuterol and Propranolol on the Train-Evoked Response.

Figure 3.2 The Effect of Propranolol on Early-Component LTP

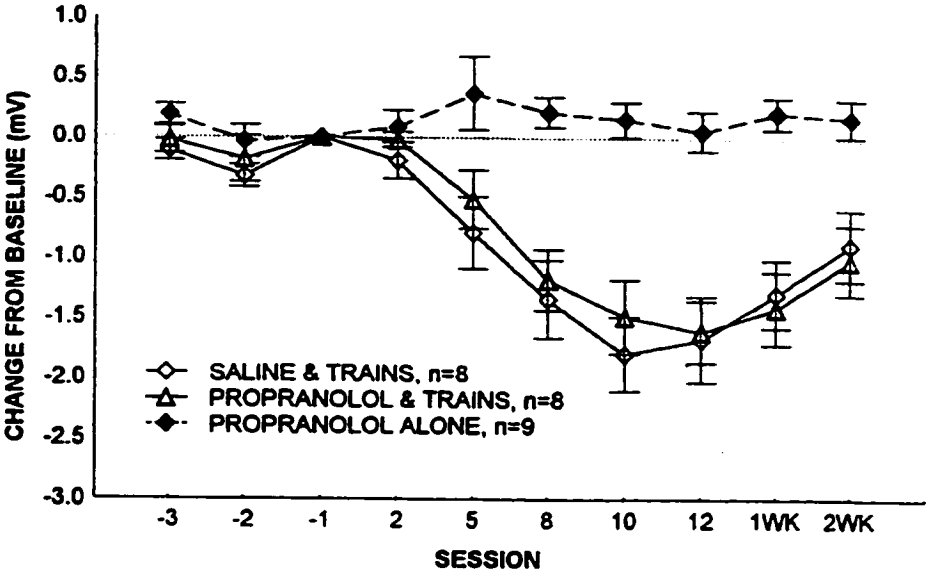


Figure 3.3 The Effect of Propranolol on Late-Component LTP

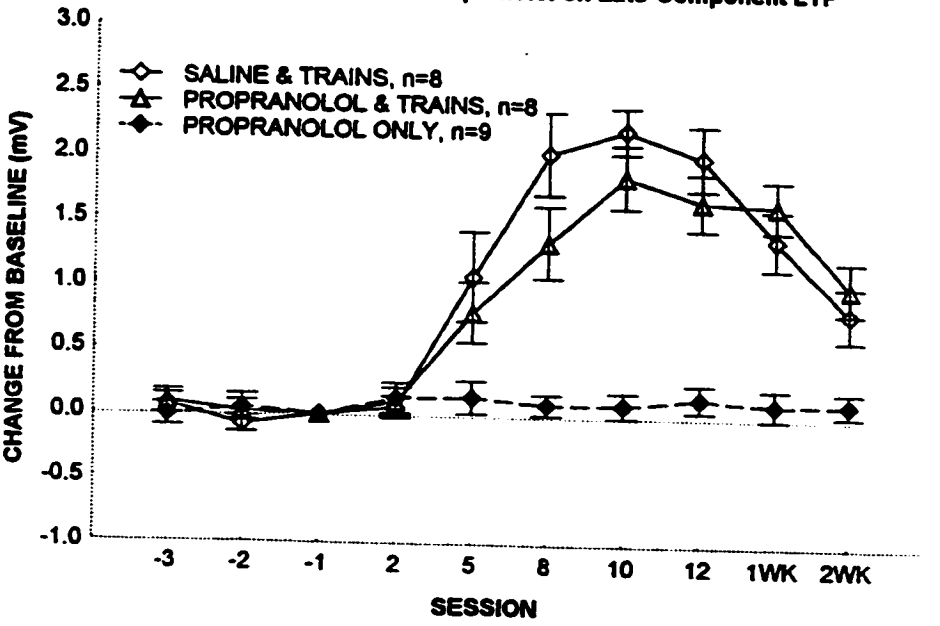


Figure 3.4 The Effect of Clenbuterol on Early-Component LTP

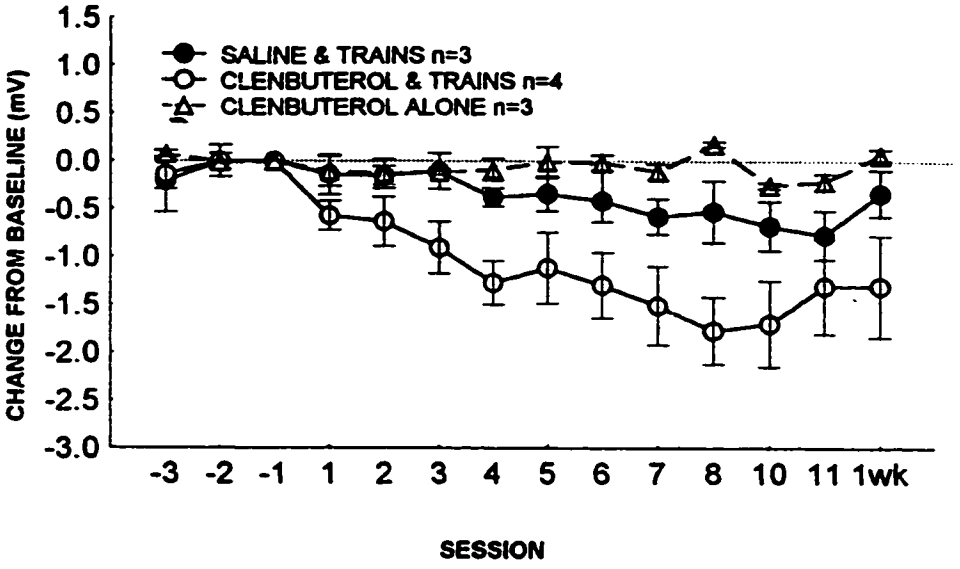


Figure 3.5 The Effect of Clenbuterol on Late-Component LTP

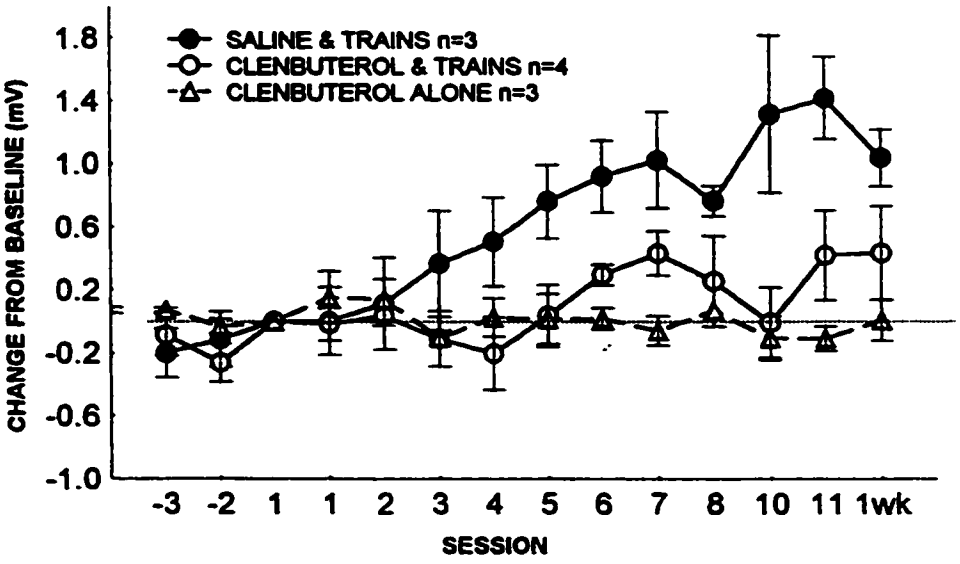
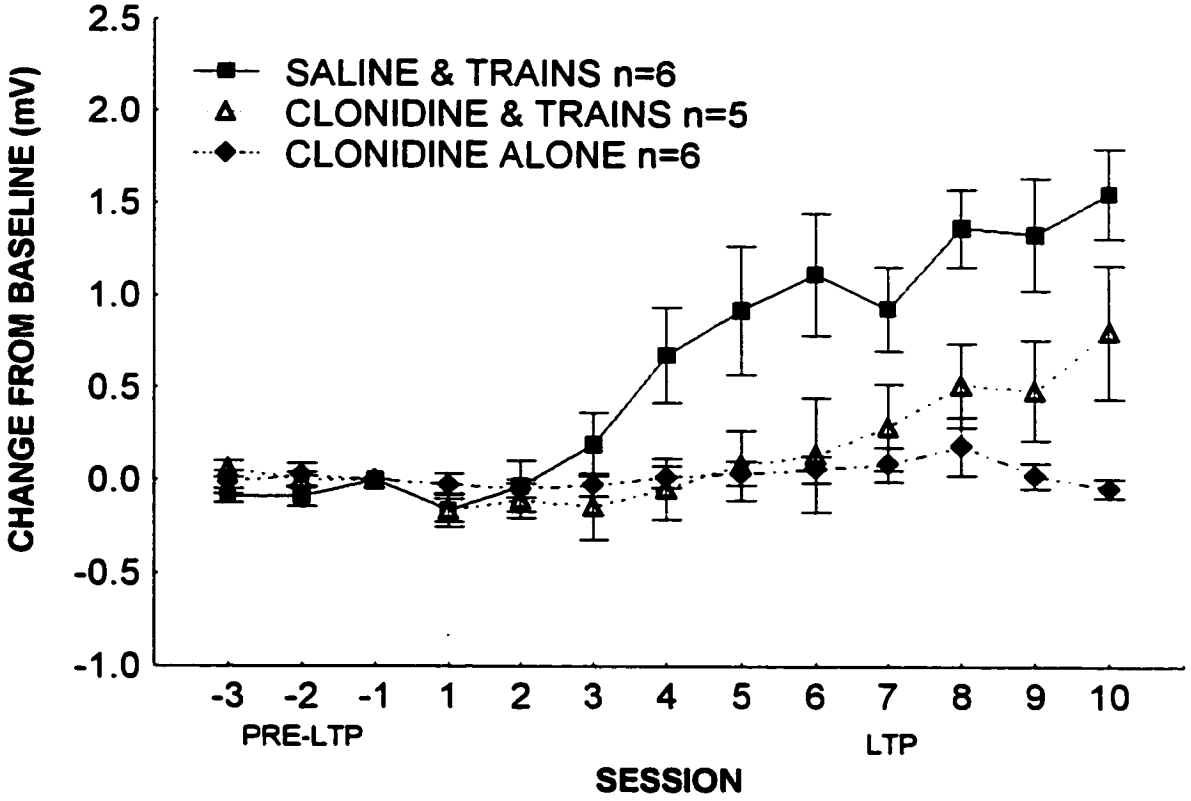


Figure 3.6 The Effect of Clonidine on Late-Component LTP



CHAPTER FOUR

DOPAMINERGIC MODULATION OF NEOCORTICAL LONG-TERM POTENTIATION IN THE FREELY MOVING RAT

4.1 INTRODUCTION

4.1.1 Dopaminergic Projections

Cooper et al. (1996) adopted a nomenclature to distinguish central dopaminergic (DAergic) efferent projections based upon fiber length. Long-length ventral tegmental area and substantia nigra systems project to the neostriatum (caudate and putamen), mesocortical targets (medial prefrontal, cingulate, and entorhinal) and mesolimbic targets (olfactory tubercle, nucleus accumbens septi, amygdaloid complex, and piriform cortex) (see figure 1.4).

The neostriatum receives glutamatergic inputs from many different areas of cortex (Berke and Hyman, 2000). Additionally, dopaminergic inputs from the substantia nigra terminate in the neostriatum near the cortical inputs (see figure 4.1). Ninety to 95 percent of these striatal neurons are GABAergic cells (Berke and Hyman, 2000).

The prefrontal cortex receives excitatory input from the thalamus (Conde et al., 1990), and the hippocampus (Carr and Sesack, 1996) and is richly innervated by dopaminergic projections from the ventral tegmental area (Emson and Koob, 1978). Within the prefrontal cortex, DAergic axons target pyramidal cells (van Eden et al., 1987) and GABAergic interneurons (Sesack et al., 1995).

Most of the regions receiving DAergic input have been implicated in memory formation, making them suitable for an analysis of DA modulation of LTP. *In situ* hybridization has shown that the prefrontal and entorhinal cortices possess the highest cortical levels of DA mRNA (Weiner et al., 1991). The experiment contained within this chapter focuses on LTP in prefrontal cortex.

4.1.2 Dopaminergic Receptors and Signal Transduction

Five DA receptors have been identified and grouped into two families. The D_1-like receptors include subtypes $D_1$and D_5 while the D_2-like receptors include subtypes D_2, D_3 and D_4. The D_1 and D_2 receptor subtypes have been more extensively studied than the D_3-D_5 subtypes. D_1 receptors positively couple to adenylyl cyclase while D_2 receptors negatively couple to adenylyl cyclase (Kebabian and Calne 1979).

In the primate cortex, D_1 receptors are concentrated in pyramidal cell (layers II-VI) (Smiley et al., 1994). In primate prefrontal cortex, the D_1 family of receptors is an order of magnitude more abundant than the D_2 family of receptors (Muly et al., 1998). In rat prefrontal cortex, D_1 receptors are mainly concentrated in deep cortical layers (Smiley et al., 1994). The density of D_1-receptor positive cells is approximately 0.1 per 1000μm^2 in layers II/III of prefrontal cortex versus 0.22 per 1000μm^2 in layers V/VI (Vincent et al., 1993).

The D_2 receptor exists in two isoforms: D_2 short and D_2 long. The D_2 short isoform is thought to be an autoreceptor (Khan et al., 1998; Usiello et al., 2000), while the D_2 long is localized postsynaptically (Usiello, et al., 2000). The density

of D_2–receptor positive neurons is approximately 0.13 per 1000μm^2 in layers II/III of prefrontal cortex versus 0.24 per 1000μm^2 in layers V/VI (Vincent et al., 1993). D_2 receptors have also been localized to GABAergic interneurons and astroglia (Khan et al., 2001).

Dopaminergic receptors interact to modulate brain function. The D_2 short isoform has been implicated in negatively regulating D_1 receptor-dependent responses (Usiello et al., 2000). The D_2 long isoform mediates synergistic or cooperative activity with D_1 receptors (Usiello et al., 2000).

4.1.3 Dopaminergic Modulatory Actions

The effect of dopamine on prefrontal cortical cells has been the subject of debate. Both excitatory and inhibitory actions have been demonstrated. Dopamine has been shown to increase prefrontal cortical cell excitability. In cortical brain tissue surgically excised from epileptic children, D_1 agonists and dopamine increased the amplitude of NMDA-mediated potentials thereby enhancing cortical activity and in some cases inducing epileptogenic activity (Cepeda et al., 1999). In primate prefrontal cortical slices, DA increased the excitability of layer III pyramidal cells in response to depolarizing current steps by reducing action potential threshold and decreasing the first interspike interval (Henze et al., 2000). This effect was mediated by D_1 receptors: the D_1 antagonist SCH 23390 blocked this response to DA but the D_2 antagonist sulpiride had no effect (Henze et al., 2000). Yang and Seamans, (1996) obtained an identical result on interspike interval and excitability using dopamine or the D_1 agonist

SKF 38393. They determined that this modulatory activity is accomplished through the enhancement of a slowly inactivating sodium current and attenuation of a slowly inactivating outwardly rectifying potassium current. Taken together, these three experiments suggest that dopamine enhances excitatory activity in the brain and that this effect is mediated by D_1 dopaminergic receptors.

By contrast, iontophoretic application of DA depressed spontaneous firing of medial prefrontal cortical neurons (Bernardi, et al., 1982). As well, DA application to layer V pyramidal cells reversibly decreased the number of spikes evoked with depolarizing current injection (Gulledge and Jaffe, 1998). These authors showed that the reduction in evoked spikes resulted from a DA-induced decrease in membrane resistance and is mediated through D_2 receptors (Gulledge and Jaffe, 1998). The initial decrease was followed by a transient increase in excitability after the DA was washed out (Gulledge and Jaffe, 1998).

Dopamine receptors have been shown to interact in prefrontal cortex, producing a net increase in GABAergic cell excitability. Seamans et al. (2001b) demonstrated though whole-cell patch clamping that dopamine has a biphasic effect on evoked IPSCs. DA initially produced an abrupt decrease in IPSC amplitude mediated by D_2 receptors (mimicked by D_2 agonism) followed by a delayed increase in IPSC amplitude mediated by D_1 receptors (mimicked by D_1 agonism). In this experiment, the D_1 agonist SKF-81297 enhanced spontaneous IPSCs, (sIPSCs) but did not affect miniature IPSCs (mIPSCs) suggesting that

these drugs increased the intrinsic excitability of GABAergic interneurons (Seamans et al., 2001b).

Zhou and Hablitz (1999) reported that dopamine application increased spontaneous firing in inhibitory layer I neurons and enhanced spontaneous inhibitory synaptic transmission. D_2 regulation of GABAergic release has been replicated *in vivo*. *In vivo* microdialysis showed that systemic administration of apomorphine, a non-specific DA agonist, increased extracellular GABA levels (Grobin and Deutch, 1998). The D_2 agonist quinpirole also increased GABA concentration, but SKF 38393, a D_1 agonist, did not. Moreover, sulpiride, a D_2 antagonist, blocked the GABA increase, but SCH 23390, a D_1 antagonist, did not (Grobin and Deutch, 1998).

Some investigators report that dopamine modulates cellular excitability in an inverted-U dose-response function. In prefrontal cortical neurons, low concentrations of DA increased the NMDA response through D_1 receptors. In contrast, high concentrations depress the NMDA response via D_2 receptors (Law-Tho et al., 1994). Similarly, NMDA, and low doses of the D_1 agonist SKF38393 combined, increased layer V pyramidal cell excitability recorded via whole-cell techniques (Wang and O'Donnell 2001). This synergistic effect was blocked by the D_1 antagonist SCH 23390, protein kinase A inhibitors and the calcium chelator BAPTA (Wang and O'Donnell, 2001) supporting the idea that dopaminergic increases in pyramidal cell excitability are mediated through D_1 receptors, but also implicating a calcium-dependent pathway involving protein

kinase A. Alternatively, Seamans et al., (2001a) found no inverted-U dose response effect. DA agonists applied in both low and high concentrations increased the NMDA-component of the EPSCs in pyramidal cells (Seamans et al., 2001a).

4.1.4 Dopaminergic Modulation of Memory

The prefrontal cortex is an area of association cortex that has been implicated in working memory (Goldman-Rakic, 1990). Working memory, a term coined by Goldman-Rakic, specifies a type of memory for information that is kept active for only a short period of time and is only transiently relevant (Goldman-Rakic, 1990). Delayed-response learning tasks are widely thought to engage working memory processes. Prefrontal cortical neurons fire during the delay periods of the tasks (Fuster and Alexander, 1971). This activity has been repeatedly hypothesized to represent the cellular counterparts of the mnemonic event (Fuster and Alexander, 1971; Kubota and Niki, 1971, Goldman-Rakic, 1990, Seamans et al., 2001a). Recently, Seamans et al. (2001a) reported that deep layer prefrontal cortical neurons maintain their activity during delay periods of working memory tasks and suggest that the activity may represent the mnemonic event as well as planned subsequent action by the animal. The prefrontal cortex receives DA projections from the ventral tegmental area and sends glutamatergic projections to the striatum. It has been shown that this system, the mesocortical DA system, is important for the learning of delay-dependent memory tasks (Bubser and Schmidt 1990; Sawaguchi and Goldman-Rakic 1994).

Similar to the modulatory actions of noradrenaline, too little or too much DA receptor stimulation impairs cognition (reviewed in Arnsten, 1997). In aged monkeys, systemic injections of the D_1 receptor agonists A77636 and SKF81297 produced an inverted "U" shaped improvement in a delayed-response task (Cai and Arnsten, 1997) with low doses promoting significant improvements over baseline and high doses impairing performance below baseline levels. These improvements were blocked if the monkeys were pre-treated with the D_1 antagonist SCH 23390 (Cai and Arnsten, 1997) suggesting that the D_1 receptor modulates working memory processes.

The D_1 antagonist SCH 23390 was infused via cannula prior to, immediately post training, or after an elapsed period of time to test the involvement of this dopaminergic receptor in working, short-term and long-term memory respectively for a step-down inhibitory avoidance task (Izquierdo, et al., 1998). When infused into the prefrontal cortex, SCH 23390 blocked working memory and long-term memory for the task (Izquierdo et al., 1998), suggesting that D_1 transmission is required for proper prefrontal working memory and LTM functioning. Additionally, SCH 23390 enhanced short-term memory for the task when infused into CA1 (Izquierdo et al., 1998).

Seamans et al., 1998 demonstrated in rat hippocampal-prefrontal circuitry that the D_1 antagonist SCH 23390, but not the D_2 antagonist sulpiride disrupted performance on a delayed-foraging task when microinjected into the prelimbic region of the prefrontal cortex (Seamans et al., 1998). In the same experiment,

unilateral hippocampal injections of SCH 23390 on one side and lidocaine injected contralaterally disrupted the ability of rats to utilize previously learned spatial information and apply that knowledge to a radial maze task (Seamans et al., 1998). Taken together, these results confirm the importance of D_1 receptors in working memory function as well as identify a role for hippocampal inputs to the prefrontal cortex in executing actions based upon the information held in working memory.

Typical neuroleptic drugs block D_2 receptors. Chronic D_2 receptor blockade simultaneously up regulates these receptors and down regulates D_1 receptors in the simian prefrontal cortex (Lidow and Goldman-Rakic, 1994). Monkeys receiving chronic haloperidol treatment showed memory impairments on both a spatial and object working memory task (Castner et al., 2000). Administration of a D_1-selective agonist in conjunction with continued haloperidol treatment reversed the memory impairment (Castner, et al., 2000). These results support the idea that D_1 and D_2 receptor function is linked (Usiello et al., 2000), and that the D_1 receptor is important for working memory functioning. These results also provide an example at the systems level of dopaminergic excitability enhanced through D_1 receptors and inhibition enhanced through D_2 receptors (i.e., Yang and Seamans, 1996; Zhou and Hablitz 1999; Henze et al., 2000; Seamans et al., 2001b;)

However, not all investigations into prefrontal DAergic modulation of working memory yield uniform results. The DA agonist, apomorphine, and the

neuroleptic, cis-flupenthixol, infused directly into the prefrontal cortex of rats, did not affect short-term memory for an operant delayed match-to-position test. However, cholinergic blockade did disrupt memory for the task (Broersen et al., 1994).

4.1.5 Dopaminergic Modulation of LTP

Despite the implication of the prefrontal cortex in working memory, most of the investigations of DAergic modulation of LTP have been conducted in the hippocampus, likely because the hippocampus receives a large DAergic innervation from the ventral tegmental area and the ease with which this area expresses LTP. Both D_1 and D_2 receptors have been implicated in hippocampal LTP.

D_1 agonists have been shown to induce LTP lasting more than 2-3 hours in the hippocampus while D_1 antagonism blocked hippocampal LTP (Huang and Kandel, 1995). Additionally, D_1 agonists protect against depotentiation protocols applied to potentiated cells (Otmakhova and Lisman, 1998). As well, D_1 knockout mice do not show hippocampal LTP (Mathies et al., 1997). Frey et al. showed in CA1, that the D_1 antagonist, SCH 23390, prevented late-stage LTP (>1-2 hours) and decreased population spike amplitude (1991). These investigators have also shown that the activation of D_2 receptors is necessary for the maintenance of LTP (>4 hours) in CA1 (Frey et al., 1990). Additionally, they found that SCH 23390, the D_1 antagonist, had no effect when administered after LTP induction (Frey et al., 1991) suggesting a critical period during which DA

can modulate synaptic plasticity effectively. The neuroleptic, trifluoperazine, blocked LTP in hippocampal slices stimulated in the CA1/CA2 border and recorded in CA1 (Dunwiddie et al., 1982). However, trifluoperazine also blocks CaMKII activity. The D_2 antagonist sulpiride, which does not have an effect on CaMKII, had no effect on LTP (Dunwiddie et al., 1982) suggesting that the inhibition of CaMKII actions may be responsible for diminished LTP rather than DAergic modulation per se.

Gurden and colleagues showed that LTP is critically dependent upon D_1, but not D_2 receptors in the hippocampal-prefrontal cortex pathway of the anaesthetized rat (2000). D_1, and not D_2, antagonists impaired induction, and the adenylate cyclase activator forskolin mimicked a D_1 agonism facilitation effect. Additionally, the protein kinase inhibitor Rp-cAMPs attenuated LTP (Gurden et al., 2000).

In the entorhinal cortex to dentate gyrus pathway of freely moving rats, Krug et al. (1983) demonstrated that the neuroleptic haloperidol augmented population spikes in response to LTP tetanus. Haloperidol had no effect on evoked EPSPs in the absence of trains or in conjunction with tetanus (Krug et al., 1983) contrasting the work of others (Dunwiddie et al., 1982; Frey et al., 1990). Like Krug et al., (1983), Jibiki et al. found that haloperidol had no effect on evoked potentials in chronically prepared rabbit perforant path dentate gyrus circuitry (1993). However, they also showed that haloperidol blocked LTP induction (Jibiki et al., 1993) confirming other reports (Dunwiddie et al., 1982;

Frey et al., 1990). Similar to Frey et al. (1991), these authors found that haloperidol had no effect on LTP if administered after tetanus implying a critical time window during which neuroleptics can block LTP induction (Jibiki et al 1993).

LTP, within the glutamatergic pathway from the prefrontal cortex to the neostriatum has also been investigated. Corticostriatal LTP in Mg^{2+}-free bathed slices is blocked by the D_1 antagonist SCH 22390 but not by the D_2 antagonist remoxipride (Kerr and Wickens, 2001). Additionally, DA depletion prevented LTP induction in this pathway, but the D_1 agonist SKF 38393 restored it (Kerr and Wickens, 2001) suggesting that corticostriatal LTP requires DAergic activity at the D_1 receptor.

Working in the goldfish Mauthner cell, Kumar and Faber (1999) showed that broad-spectrum DA antagonists failed to block LTP suggesting that DA is not required for tetanus-induced LTP. However, when DA application preceded tetanus, the probability of LTP increased 25% (Kumar and Faber, 1999) implying a facilitatory role for DA in Mauthner cell LTP. Moreover, DA combined with tetanic stimulation, potentiated synaptic responses in the presence of the calcium chelator BAPTA suggesting that this effect is not critically dependent upon elevated calcium (Kumar and Faber, 1999).

DA has been implicated in anti-Hebbian plasticity modulation in addition to LTP. DA applied to layer I/II prefrontal cortex slices in conjunction with high-frequency tetanus produced a long-term depression (LTD) of the monosynaptic

EPSP in 12 of 19 layer V cells, and no change in the remaining 7 cells (Law-Tho et al., 1995). In this same experiment, LTP was not induced suggesting that DA application favors LTD rather than LTP induction in prefrontal cortical slices. Law-Tho tested the dependency of this effect on calcium and also found that the LTD effect was not dependent upon cytosolic calcium (1995). By contrast, Thomas et al (2000) also induced NMDA-receptor-dependent LTD in the corticostriatal pathway but found that neither DA nor the DA antagonists SCH 23390 (D_1) or sulpiride and haloperidol (D_2) affected the induction.

The following experiment was designed to explore the involvement of dopamine in prefrontal cortical LTP in the freely moving rat.

4.2 MATERIALS AND METHODS

4.2.1 Animals and Surgery

Twenty-three male, Long-Evans rats, weighing 300-350g, from the McMaster University and Charles River breeding colonies were used in this experiment. Animals were housed in pairs prior to surgery, individually afterwards, and were maintained in a colony as described previously. Electrode construction was also as previously described, but the implantation sites were altered to target DA-rich termination fields. The recording electrode was implanted into cortical area corresponding to the prefrontal cortex (Paxinos and Watson, 1997): 3.0mm anterior to Bregma, 0.6mm lateral to the midline and 3.5mm ventral to skull surface. The stimulating electrode was implanted

ipsilaterally into the corpus callosum, 2.0mm anterior to Bregma, 2.0mm lateral to the midline and 3.5mm ventral to skull surface. Experimentation began no sooner than 14 days after surgery

4.2.2 Baseline Measures and Induction of LTP

Forty-eight hours separated each of three baseline field potential I/O measures. Biphasic pulses were delivered to the corpus callosum at 10 ascending intensities as administered in previous experiments. The responses evoked in the cortex were then amplified, digitized and averaged over each intensity. Animals were matched according to response morphology and randomly assigned to one of five experimental and control groups.

LTP induction began four days after the final baseline collection. Animals in the LTP groups first received an i.p. injection of drug or vehicle followed fifteen minutes later by conditioning trains. Animals in the drug control groups received injections only. Each LTP session comprised 30, 8-pulse, 1260□A trains at 300 Hz and was administered daily for 10 days. I/O field potential measures were collected every second day of train and drug treatment. After LTP induction, decay of the responses was monitored once at 48 hours post final train stimulation and then weekly for three weeks. EEG activity was monitored during train delivery to ensure that epileptiform discharges were not triggered.

4.2.3 Dopaminergic Agonism and Antagonism

This experiment comprised five groups: three received LTP-inducing stimulation and two did not. The non-specific DA agonist, apomorphine was

dissolved in distilled water, heated to boiling, allowed to cool to room temperature and then administered (1.0 mg/kg i.p.) in conjunction with LTP conditioning trains (APOTR n=6) and alone to animals serving as a drug control group (APO n=3). The neuroleptic haloperidol was dissolved in DMSO and administered (1.0 mg/kg i.p.) in conjunction with LTP stimuli (HALTR n=6) or alone (HAL n=3). Another control group received LTP-inducing trains and equal volume of distilled water injections (H2OTR n=5).

4.2.4 Analyses

Field potentials were analyzed as described previously. Experiments were conducted in a plexiglass running box. After decay measures were collected, animals were intracardially perfused with saline followed by 10% formalin. The brains were sliced and stained with cresyl violet to verify electrode placements. In all animals, recording electrodes were positioned within prefrontal cortex or Frontal Area 2 (Paxinos and Watson, 1997). Stimulating electrodes were positioned within the corpus callosum.

4.2.5 Behaviour

Rats receiving the agonist apomophine frequently displayed tremors and increased activity following injection. Following injection of the antagonist haloperidol, rats consistently displayed a distinctive "wobble" in their hind legs. Twenty-four hours after injection, all animals appeared normal. Animals in the apomorphine and distilled water groups gained weight normally throughout the

experiment. However, animals in the haloperidol group lost an average of 10-15 grams (less than 5% of body weight) during the experiment.

4.3 RESULTS

4.3.1 Response Morphology

The responses resembled those previously reported (figure 2.1) (Trepel and Racine, 1998; Chapman et al., 1998). However, in some cases, the late component peak in these prefrontal responses was clearly visible prior to high-frequency stimulation (see figure 4.2). The early-component peaked at a latency of 8.53 msec. The late component peaked at 21.00 msec.

The effect of apomorphine and haloperidol on the high-frequency train-evoked response in a representative animal can be seen in figure 4.3. Both apomorphine and haloperidol produced a small enhancement of the train-evoked response.

4.3.2 Dopaminergic Effect on Early-Component LTP

A repeated-measures ANOVA of the population spike measures revealed a significant main effect of session ($F_{(2,36)} = 9.47$, $p < 0.001$). Although all groups receiving LTP trains showed increases in population spike measures over the course of induction, there was no significant interaction effect ($p = 0.29$, data not shown).

Neither dopaminergic blockade at D_2 receptors nor non-specific DA agonism significantly influenced early-component LTP development (figure 4.4). A repeated-measures ANOVA yielded a main effect of session ($F_{(10, 180)} = 8.94$, p

< 0.001), but there was no group-by-session interaction effect ($F_{(40, 180)} = 1.06$, $p = .37$). Although the H2OTR, APOTR and HALTR groups all diverged from the two groups that did not receive LTP trains, the result was not significant. HALTR showed the largest trend toward LTP induction.

4.3.3 Dopaminergic Modulation Produces Late-Component LTP and LTD

The late-component results yielded both a facilitation of LTP by apomorphine and a neuroleptic-induced LTD. Figure 4.5 depicts these data. A repeated-measures ANOVA revealed a significant group-by-session interaction effect ($F_{(40, 180)} = 2.97$, $p < 0.001$) indicating that the LTP induction protocol as well as the drug protocol affected the experimental outcome. Further repeated-measures ANOVAs comparing pairs of groups showed that H2OTR differed significantly from both APOTR ($F_{(10, 90)} = 2.41$, $p < 0.05$) and HALTR ($F_{(10, 90)} = 2.35$, $p < 0.05$). Both HALTR and APOTR completely diverged from the H2OTR group after 4 days of high-frequency trains. However, the APOTR group began to show a facilitation effect while the HALTR responses began to depress at that point. The APOTR facilitation effect increased steadily as the LTP induction protocol progressed. The HALTR depression effect reached asymptote at approximately 20 percent of baseline measures.

4.3.4 Decay

During the period following tetanization, responses decayed most rapidly during the initial week. Subsequently, the APOTR group returned to near early–component baseline levels by week three. The H2OTR group decayed slightly

more slowly, but approached baseline levels. In contrast, HALTR animals' decay levelled off after the first week and remained at approximately 140% of baseline. The two drug control groups showed a small downward drift during the experiment, but remained near baseline. These differences are consistent with a background of potentiation in the groups receiving trains, but, as previously described, there were no significant interaction effects.

Similar to the early component, most of the overall late-component decay occurred in the first week following tetanization. Responses in the APOTR group decayed from approximately 280% of baseline at peak induction to approximately 160 % of baseline following three weeks of decay. H2OTR decayed from approximately 200% of baseline (measured 48 hours after the final LTP session) to 150% of baseline. The depressed HALTR response peaked at 20% of baseline on the final day of LTP induction and decayed approximately back to baseline levels after three weeks.

4.4 DISCUSSION

The early-component results showed a non-significant surface positive shift across all three groups of animals that received LTP-inducing trains. Because the summed population spike measures showed a non-significant increase from baseline to LTP cessation, the change in mV values represents a trend toward potentiation rather than a depression in the apomorphine groups. After 10 days of LTP induction, it appeared that the HALTR and APOTR groups had not reached

asymptotic levels. The lack of significance in the LTP effect is likely due to the relatively small number of animals.

Even with the small group sizes, however, apomorphine, in combination with high-frequency stimulation, resulted in enhanced LTP in the late component. This result is not surprising given the demonstrated facilitatory effect of D_1 receptor activation on LTP induction in other preparations (Huang and Kandel, 1995; Mathies et al., 1997; Frey et al., 1991; Gurden et al., 2000). Although apomorphine is non-selective, its actions at the D_1 receptor combined with excitatory high-frequency stimulation overcame any potential inhibitory actions initiated by apomorphine binding to the D_2 receptor.

It was expected that haloperidol would block LTP induction as other investigators have found (Dunwiddie et al., 1982; Jibiki et al., 1993). Surprisingly, haloperidol induced *LTD*. Lisman (1989) first proposed the idea that Ca^{2+} influx can determine whether LTP or LTD induction occurs. He theorized that the induction of LTP depends upon large calcium influx in postsynaptic neurons through NMDA receptors while LTD induction follows from a lesser influx of calcium possibly through voltage-dependent calcium channels. Hirsch and Crepel showed that prefrontal cortical neurons require differential levels of Ca^{2+} influx *in vitro* to express LTP and LTD (Hirsch and Crepel, 1992). Their result supports the idea that Ca^{2+} influx can act as a molecular switch, determining whether LTP or LTD induction will occur in DA-rich areas of the brain.

LTD is typically induced using low-frequency stimulation. Chen et al. (1996) found that low frequency stimulation produced an NMDA-dependent LTD in CA1 slices that was blocked by D_1 antagonism (SCH 23390) or D_2 agonism (LY 171555). Further, this LTD was enhanced by D_1 activation by SKF 38393 or D_2 blockade using sulpiride (Chen et al., 1996) suggesting that D_1 and D_2 receptors cooperate through opposing actions, to induce LTD when paired with low-frequency stimulation.

In the present experiment, it appears that the late component is subject to expressing LTP or LTD in the presence of high-frequency stimulation depending upon the modulatory context. Neuroleptics have been shown to decrease calcium influx (Deffois et al., 1996). As well, haloperidol blocks quinpirol-induced intracellular calcium concentration elevation (Yang et al., 1995). It is possible that the high-frequency stimulation induced the same amount of calcium influx in APOTR and HALTR animals. However, because haloperidol blocks activity of CaMKII (Robinson et al., 1984), a depression instead of an enhancement was seen in the HALTR animals. Future experiments could determine whether the LTD induction results from the blockade of calcium entry (via calcium channel blockade) or phosphorylation-related actions (via kinase-activity blockers).

FIGURE CAPTIONS

Figure 4.1 Basal ganglia-cortical circuits. The striatum receives glutamatergic (excitatory) projections (depicted as solid black arrows) from many areas of the neocortex, hippocampus and amygdala. These projections make synaptic contact close to dopaminergic projections from the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) depicted as a large blue arrow. GABAergic projections from the striatum to the globus pallidus internal (Gpi) and substantia nigra pars reticulata (SNr) primarily contain D_1 receptors. While GABAergic striatal projections to the globus pallidus external (GPe) principally contain D_2 receptors. Figure and text adapted from Berke and Hyman, 2000.

Figure 4.2 A representative animal with a visible late-component peak prior to potentiation. In some animals, the late component was apparent prior to LTP induction (broken line). Despite its presence before LTP, the late component showed further increase after induction (solid line) in this H2OTR animal.

Figure 4.3 Dopaminergic effects on the train-evoked response. Both apomorphine (top) and haloperidol (bottom) depressed the train-evoked response.

Figure 4.4 The effect of apomorphine and haloperidol on early-component LTP. Neither apomorphine nor haloperidol significantly influenced early-component LTP development. The Y-axis represents the change in response

amplitude from baseline measured in millivolts. The X-axis shows sessions beginning with baselines, continuing through LTP induction and decay. During LTP induction, two train stimulation sessions occurred between each field potential measure for the HALTR, H2OTR and APOTR. Although there was a main effect of session ($F_{(10, 180)} = 8.94$, $p < 0.001$), there was no group-by-session interaction effect ($F_{(40, 180)} = 1.06$, $p = 0.37$). The three groups that received trains diverged from the two groups receiving drug-only injections with haloperidol showing the greatest trend toward potentiation.

Figure 4.5 The effect of apomorphine and haloperidol on late-component LTP. Apormorphine facilitated late-component LTP induction while haloperidol induced an LTD effect. X- and Y-axes are the same as in figure 4.4. Both groups diverged from baseline after only two train sessions. The H2OTR group also showed a slowly developing potentiation that had not reached asymptote at the end of the induction period.

Figure 4.1 Basal ganglia – cortical circuits

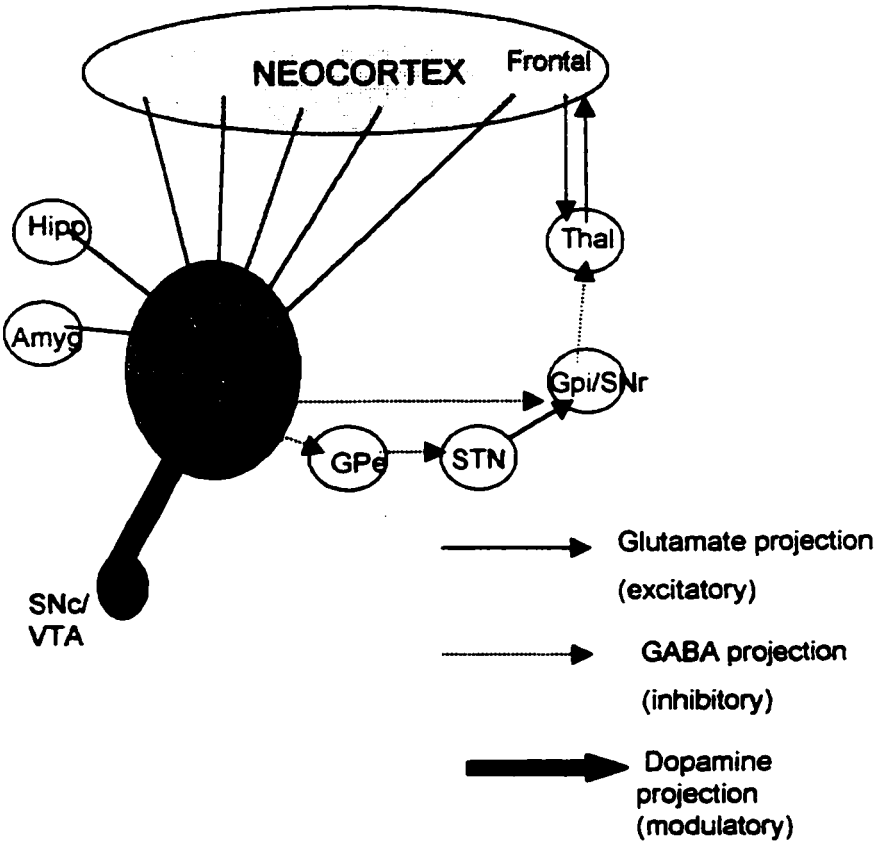
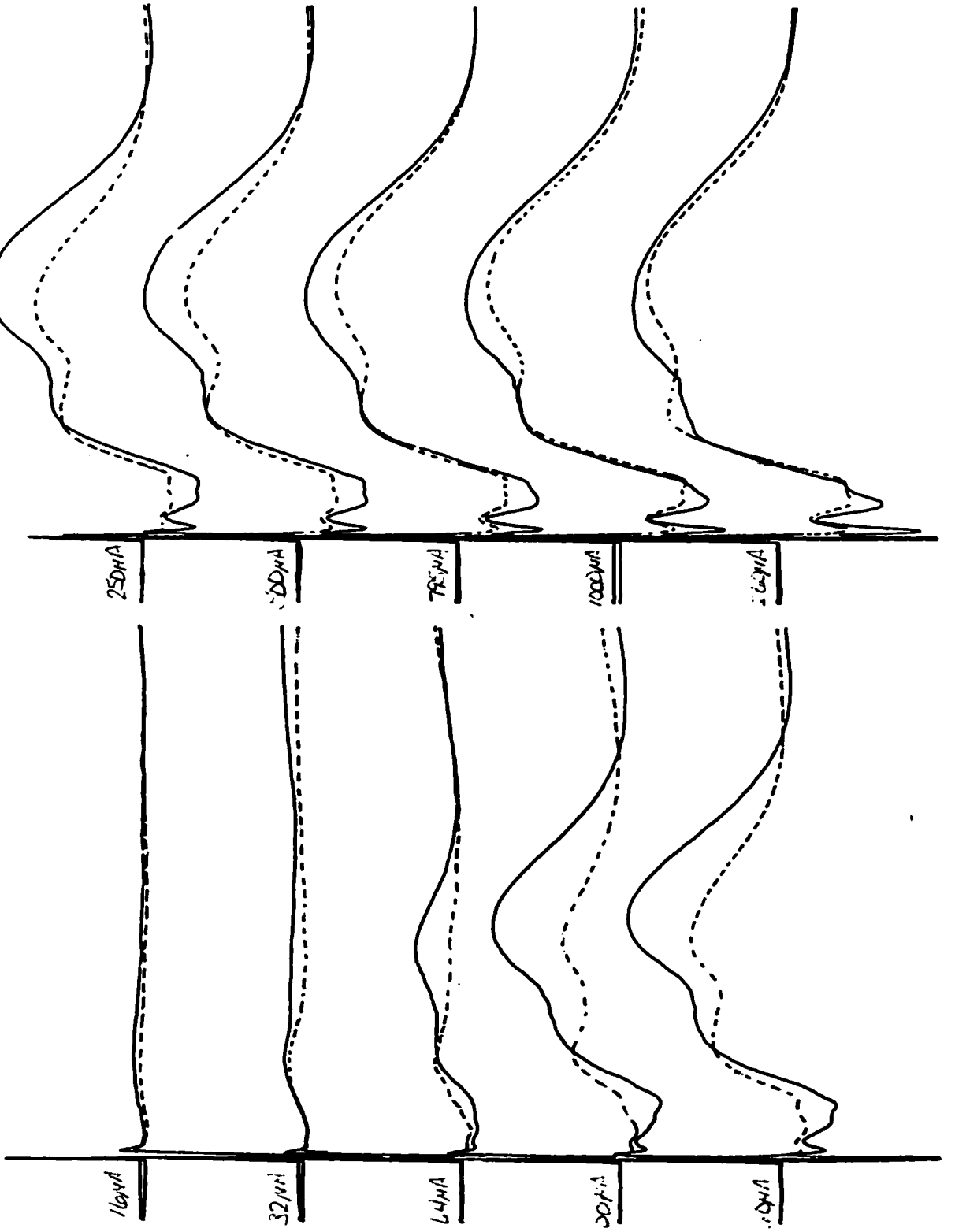


Figure 4.2 Representative Sweeps Showing a Visible Late-Component Prior to Potentiation



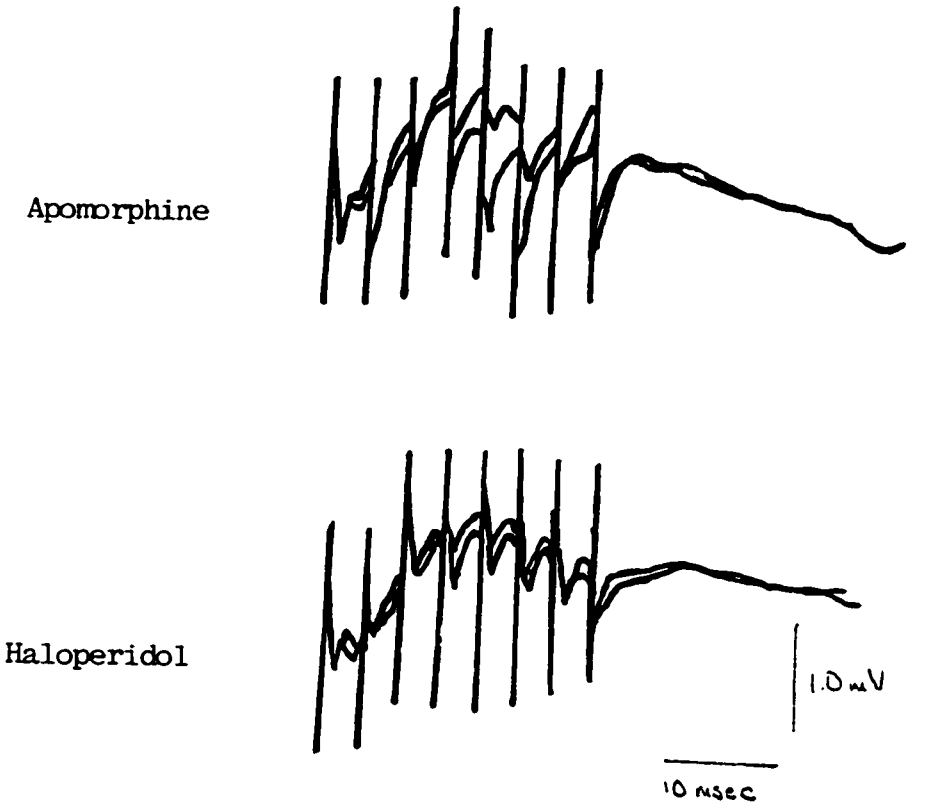


Figure 4.3 The Effect of Apomorphine and Haloperidol on the Train-Evoked Response

Figure 4.4 DA Modulation of Early-Component LTP

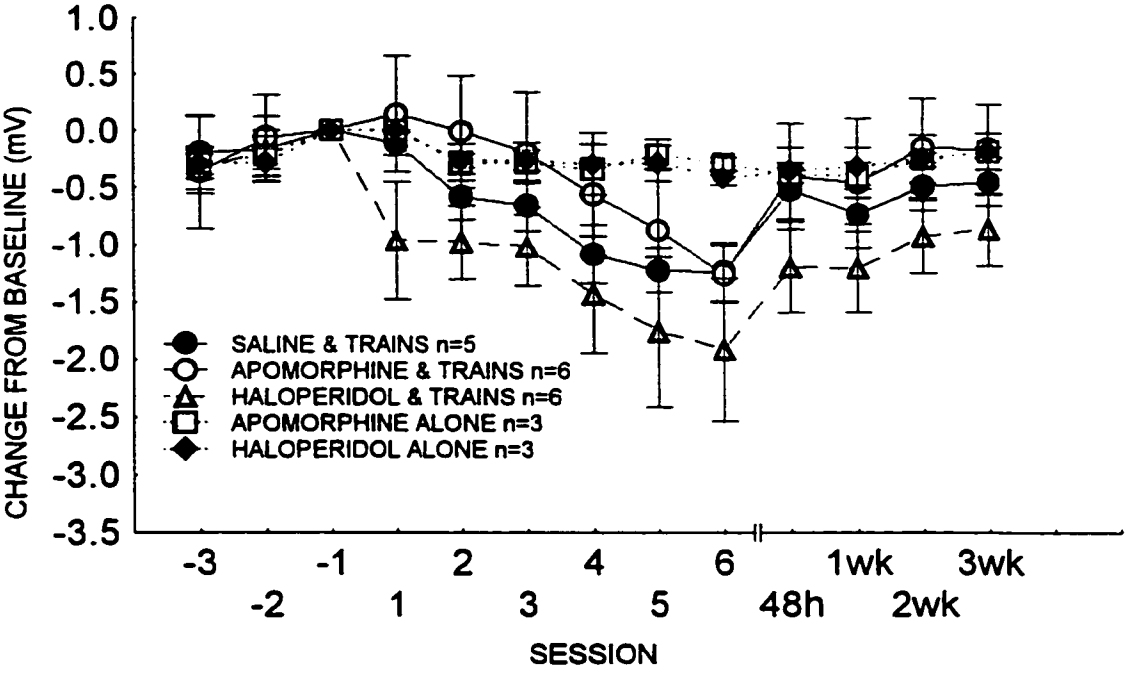
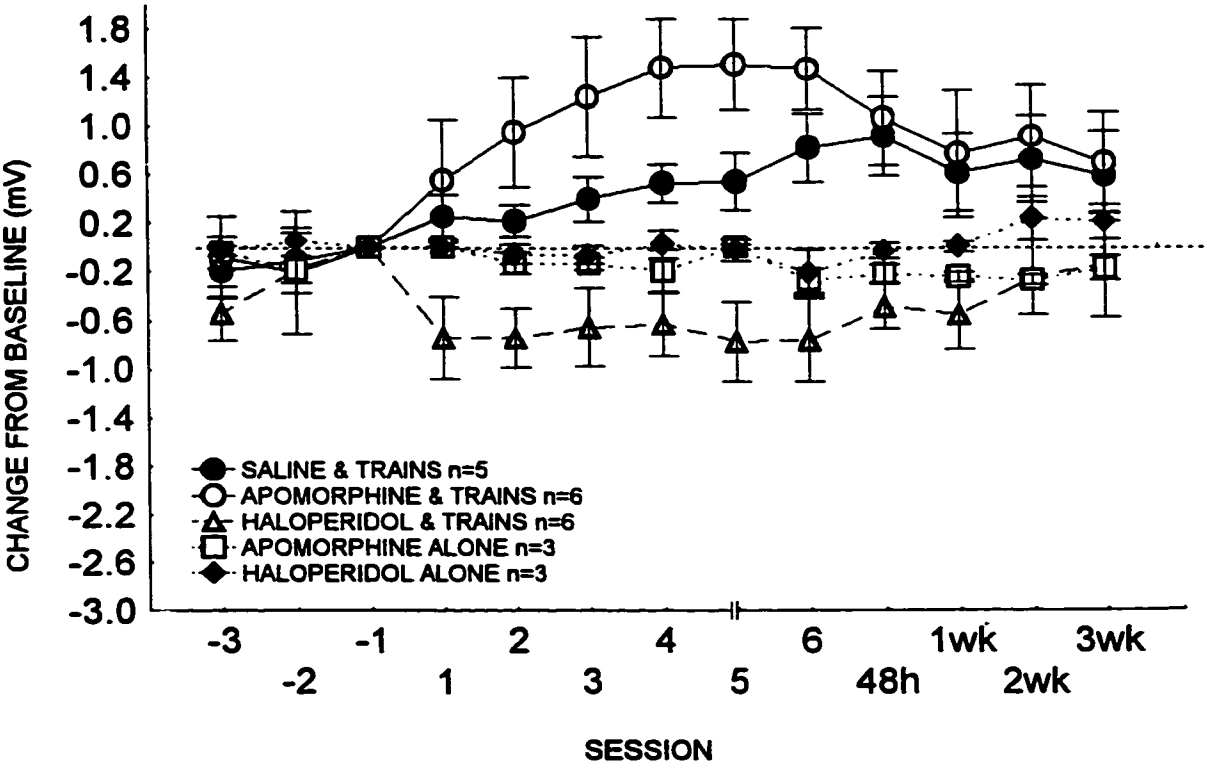


Figure 4.5 DA Modulation of Late-Component LTP



CHAPTER FIVE

GENERAL DISCUSSION

5.1 NEUROMODULATION OF LTP

The discovery of the spaced-and-repeated-trains protocol, (Racine et al., 1995; Trepel and Racine, 1998) showed that the neocortex is readily amenable to LTP induction *in vivo*, providing that the appropriate stimulation regimen is applied. Because the neocortex is widely thought to be the major repository of long-term memory, it is reassuring that it can support LTP effects that last for weeks or months. Such demonstrations lend additional credibility to the use of LTP as a learning and memory model. The direct relationship, if any, between LTP and memory is a topic fervently debated among researchers. Although definitive proof that LTP underlies information encoding and storage remains elusive, much evidence suggests that LTP is a viable neuronal candidate for a learning and memory mechanism. Elucidating the nature of the relationship between LTP, learning, and memory requires further investigation into the characteristics of LTP in the freely moving animal. Recent work from Racine and colleagues (Trepel and Racine, 1998; Chapman et al., 1998; Trepel and Racine, 2000; Ivanco et al., 2000) has served to flesh out some of these characteristics. It is in this spirit that the present work was undertaken — an exploration of the

effects of agonism and antagonism of endogenously occurring neuromodulators on LTP in the behaving rat.

Neuromodulators are widely known to play a role in normal cognitive function. Their effect on learning and memory has also been extensively investigated. If LTP underlies learning and memory, it is reasonable to suggest that neuromodulators may have similar effects on both LTP and learning and memory. This assumption guides the present work as well as similar *in vitro* and *in vivo* work conducted in this field.

5.2 SUMMARY OF RESULTS

This thesis examined the effects of three modulatory systems on the induction of neocortical LTP in the adult, freely moving rat: cholinergic, noradrenergic and dopaminergic. Each experiment tested neuromodulator agonism and antagonism on monosynaptic and polysynaptic LTP. The agonist and antagonist groups were compared to a vehicle-control group that also received LTP-inducing tetani. As well, drug-only groups were implemented to ascertain whether the drugs alone influenced baseline evoked responses. Predicted effects of the modulatory actions on LTP were largely based upon previous studies of these same modulatory systems' influence on learning and memory. Table 2 delineates the results of the experiments contained within this thesis relative to confirmation of the hypotheses guiding them. In all cases,

agonists were predicted to increase LTP, while antagonists were predicted to decrease LTP.

In the cholinergic and noradrenergic experiments, significant group-by-session interactions were obtained in the early-component (monosynaptic) measures of LTP induction. Scopolamine, the cholinergic antagonist, blocked LTP while clenbuterol, a β-noradrenergic agonist, enhanced LTP. The measure of LTP taken from the polysynaptic component resulted in significant group-by-session interactions for four modulatory manipulations. Pilocarpine, the cholinergic agonist, and apomorphine, the dopamine agonist, facilitated LTP. Clenbuterol attenuated LTP. Additionally, the neuroleptic, haloperidol induced LTD.

Interestingly, no injected neuromodulator significantly affected the early and late components identically. In fact, significant opposing results occurred in the noradrenergic experiment where the agonist facilitated monosynaptic LTP and attenuated polysynaptic LTP.

In contrast to the behavioural literature where blockade of learning by antagonists is more readily seen than enhancement, all three agonists facilitated the induction of LTP in either the monosynaptic or polysynaptic component. By contrast, clenbuterol attenuated LTP in the late component. This result raises the possibility that the early and late components possess different "inverted U" dose-response functions.

The neuroleptic-induced LTD in conjunction with high-frequency tetanus came as a surprise. As discussed previously, LTD typically results from low-frequency stimulation. However, the combination of D_2 blockade and high-frequency stimulation not only blocked LTP, but also produced a depression effect. A similar result was obtained when rats were given the NMDA antagonist CPP, where 10mg/kg blocked LTP and promoted a depression effect (Trepel and Racine, 1998). The blockade of NMDA receptors prevents the requisite levels of Ca^{2+} from entering the cell and inducing LTP, but may allow sufficient Ca^{2+} influx to trigger LTD. Haloperidol attenuates CaMKII activity, thus also reducing the rise in intracellular calcium required for LTP induction. These two experimental results provide support for the calcium-switch hypothesis (Lisman, 1994): large amounts of intracellular calcium promote LTP, while smaller amounts promote LTD.

5.3 CLINICAL IMPLICATIONS

Research that sheds light on the neurochemicals involved in information encoding and storage can benefit clinical populations including those with Alzheimer's disease, addictions, or schizophrenia. For example, age- and Alzheimer's-disease related memory loss has been linked to cholinergic dysfunction (Drachman and Leavitt, 1974; Giacobini, 1990; Greenamyre and Maragos, 1993), and, dopaminergic modulation of synaptic plasticity has been

proposed to underlie addiction. It is hypothesized that drug-enhanced release of dopamine in the striatum initiates action at D_1 receptors. Subsequent second-messenger action causes gene expression and LTP-like changes, resulting in addiction (reviewed in Berke and Hyman, 2000). One study suggests that the site of modulation may lie within the VTA. Ungless et al. (2001) found that a single exposure to cocaine enhanced AMPA-mediated responses *in vitro*, in the VTA. This LTP-like effect was still present five days after cocaine exposure (Ungless et al., 2001). Finally, schizophrenics display a range of positive symptoms (e.g., auditory hallucinations) and negative symptoms (e.g., blunted affect). Because neuroleptics ameliorate positive schizophrenic symptoms, dysfunctional dopamine transmission has been implicated in this disorder. Schizophrenics have also shown deficits on both short-term and long-term memory (Lussier and Stip, 2001). It has been reported that the brains of schizophrenics express elevated levels of the D_4 receptor (Seeman et al., 1993). The neuroleptic clozapine binds with high-affinity to this receptor (Van Tol et al., 1991).

5.4 CHALLENGES AND FUTURE DIRECTIONS

5.4.1 Single Dose vs. Dose-Response Curves

In the slice preparation, modulatory effects on LTP are typically determined with logarithmic dose-response curves. Because more than one slice can be tested in the span of a single day, this preparation is amenable to thorough exploration of

different dose ranges and their effects in short periods of time. As well, after the slice undergoes "wash-out", and evoked responses have returned to baseline, slices can sometimes be recycled for additional manipulations. Chronic experiments on rats, can require up to several months. Because the rat's neurochemistry and synaptic connectivity are slowly altered following LTP and drug regimens, rapid data collection is not an option. Nevertheless, it would be useful to determine dose-response curves for both systemic and local administration of these neuromodulatory drugs in the chronic preparation. This knowledge, for example, could be used to build on the work contained within this thesis to confirm whether the "inverted-U" shaped function that best describes NAergic and DAergic modulation of memory, also describes their modulation of LTP in the freely moving rat. Establishing the dose-response functions would enhance the parallel investigation of learning and memory.

5.4.2 Systemic Injection vs. Local Application

The systemic injection of drugs precludes localization of the modulatory effect. That is, it is impossible to determine whether the agonists and antagonists affected the neocortical cells directly, or affected subcortical structures that in turn modulated the neocortex. Local application of the drugs to the neocortex would answer this question.

Systemic administration was chosen for this thesis to allow for stronger comparison of the results to extant learning and memory studies where the

modulatory action is also diffuse. As well, this route of administration most closely resembles the way drugs are administered to humans for the treatment of cognitive dysfunction.

Ideally, once dose-response curves have been established for drug administration and LTP induction, these results can be compared to results obtained by testing the same doses on a variety of learning tasks that are reportedly mediated by the neocortex. Determining if the dose-response effects on LTP are consistent with those affecting learning would provide further information about the relationship between LTP and memory mechanisms.

Systemic investigation is necessary, but is not sufficient for thorough understanding of the complex modulatory actions on LTP. Subsequent research should compare the results of systemic application with results from local application of neuromodulators as a step towards determining the site of action and underlying mechanism.

5.4.3 Compatibility of Chosen Drug Dose with Behavioural Tests

All agonist and antagonist doses were chosen based upon investigations into learning and memory tasks in an effort to further elucidate the connection between LTP, learning and memory. However, some of the doses chosen may have affected an animal's ability to execute a learned task. For example, pilocarpine caused diarrhea in most animals. Haloperidol induced a "wobble" in most animals and apomorphine promoted hyperactivity. One way to eliminate

this problem is to administer the drugs near the recording electrode via a cannula. However, this modification assumes that the modulatory effects are contained within the cortex. If the effects are not contained within the cortex, administration of the drug through cannulae may not affect LTP, or may affect it differently than systemic administration. In any case, knowledge of threshold dosages for induction of reliable LTP effects might lead to lower effective drug dosages for testing modulatory effects.

5.4.4 Interaction of Modulatory Systems

While it is important to consider the individual contributions of neuromodulatory systems to LTP and cognitive functioning, modulatory systems are complex and do not operate in isolation. Because of the initial prominent role given to ACh in age-related and Alzheimer's disease dysfunction, most investigations into the interaction of neuromodulatory systems focus upon ACh interactions with other neuromodulators.

Interactions between ACh and NA have been studied at the cellular and systems levels. For example, NA modulates cortical responses to iontophoretically applied ACh (Waterhouse et al., 1981; Jones and Olpe, 1984). The β-NA agonist, phenylephrine, facilitated excitatory synaptic transmission in single-unit recordings from 12 of 14 forelimb cells in response to contralateral paw stimulation. As well, it enhanced neuronal responsiveness to ACh application in 15 of 24 cells (Waterhouse, et al., 1981). However, NA has also

been shown to decrease release of ACh from cholinergic neurons *in vivo* and *in vitro* (Vizi, 1980; Moroni et al., 1983). In cortical slices, NA reduced a ouabain-stimulated release of ACh (Vizi, 1980). As well, the spontaneous and evoked release of ACh was higher in cortical slices from locus coeruleus-lesioned brains (Vizi, 1980). Cholinergic depletion for 28 days decreases NA synthesis and release in the hippocampus by 50 percent (Hortnagl et al., 1987). A sub-effective dose of scopolamine, when combined with locus coeruleus-lesioned animals produced profound impairments on an active-avoidance learning task (Kruglikov, 1982). Propranolol has been shown to potentiate the inhibitory effects of scopolamine on both Morris Water Maze tasks and inhibitory-avoidance tasks (Decker et al., 1990).

ACh and DA have also been shown to interact. ACh agonists increased striatal DA turnover in the nucleus accumbens, olfactory tubercule and striatum by 68, 45 and 110 percents respectively (Weinstock et al., 1979). L-propranolol reduced the agonist-induced increase in the DA metabolite homovanillic acic (HVA) by 57 percent in the nucleus accumbens (Weinstock et al., 1979). Interestingly, a low dose of d-propranolol decreased the ACh agonist-induced increase in HVA, but had no effect at a high dose (Weinstock et al., 1979). The memory-enhancing effects of the cholinergic agonist oxotremorine are blocked by haloperidol (Baratti et al., 1983). As well, systemic scopolamine administration reduced the dopamine metabolites HVA and 3,4-dihydroxyphenylacetic acid

(DOPAC) in the frontal cortex and hippocampus, but not in the striatum or nucleus accumbens (Memo et al., 1988). In this same experiment, the levels of DA were not changed. This effect was dose-dependent and the time course for reduction paralleled memory deficits in a passive avoidance task (Memo et al., 1988). I.c.v. infusion of ethylcholine aziridinium reduced levels of acetylcholine and promoted a dose-dependent reduction in DA and its metabolites in the striatum (Hortnagl et al., 1987).

While this thesis made no attempt to test the effects of neuromodulatory interactions on LTP, doing so would promote a more thorough understanding of modulatory processes and may also inspire new treatments for cognitive disorders.

Table 2: Results Summary Relative to Hypothesis Confirmation

	Early Component	Confirmation?	Late Component	Confirmation?
ACh Agonist	No effect	No	N/A	N/A
ACh Antagonist	Attenuated LTP	Yes	No effect	No
NA Agonist	Increased LTP	Yes	Decreased LTP	No
NA Antagonist	No effect	No	No effect	No
DA Agonist	No effect	No	Increased LTP	Yes
DA Antagonist	No effect	No	Induced LTD	Somewhat

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