LONG-TERM POTENTIATION IN THE BEHAVING RAT

by

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ABSTRACT

Long-term potentiation (LTP) has been postulated to be a mechanism of learning and long-term memory. A popular experimental procedure is to examine LTP in the hippocampus with stimulation of the perforant path (PP) fibers with simultaneous recording in the dentate granule cells of the hippocampus. In vitro studies of LTP using hippocampal slices are numerous. Fewer investigations of LTP have been done in awake rats with chronic electrodes. Noradrenaline is a neuromodulator that has been implicated in numerous cognitive processes including attention, learning, and memory. Although noradrenergic modulation of LTP has been studied in vitro, its effects on LTP induction in the awake rat are unknown. I intended to use implantation of a chronic chemitrode to study the effects of a β-adrenergic antagonist, timolol, on induction of LTP. The chemitrode allows for direct infusion of the drug into the dentate gyrus. However, pilot data suggested the chemitrode itself upset the normal rate of induction of LTP. In Experiment 1 LTP was induced in 3 groups of rats: one group with chronic chemitrodes and an infusion of artificial cerebral spinal fluid, one with chronic chemitrodes, and one with chronic electrodes. The electrode rats exhibited more LTP than the chemitrode groups, suggesting that the presence of the chemitrode might have disrupted LTP. Experiment 2 investigated effects of the β-antagonist propranolol

on induction of LTP in rats with chronic electrodes. A saline control group and 3 drug groups (10, 20, and 40 mg/kg l-propranolol) received intraperitoneal injections and then tetanic stimulation of the PP. No group differences were observed and very little LTP was observed in all groups. I speculated that the ip injection procedure may have disrupted LTP. Experiment 3 tested the hypothesis that behavioural state may effect the variability of evoked potentials. In one group of rats data were collected only when the rat was immobile. These data were compared to the saline group from Experiment 2, which was run in the freely behaving condition. No groups differences were found in either the amount of LTP induced or the amount of variability of evoked potentials.

These results more clearly define the experimental conditions under which LTP may be induced consistently. The results of Experiment 1 suggested that LTP is more reliable when induced in rats with chronic electrodes than in those with chronic chemitrodes. This obviates the use of this preparation in studies of drug effects on LTP in awake rats, with one tetanic stimulation. The results of Experiment 2 suggested that LTP may not be induced reliably when drug injections are given pre-tetanization. The results of Experiment 3 agree with this conclusion. They also suggest that behavioural state is not a factor in either the amount of LTP induced or in the variability of the evoked potentials. These data together suggest that using one tetanic stimulation to induce LTP may not be an appropriate method with which to study drug effects. Future studies will have to be done to test the hypothesis that

LTP can be induced more reliably in freely behaving rats when multiple tetanizations are applied over many sessions.

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Table of Contents

Title Page i

Abstract ii

Table of Contents v

List of Figures vii

List of Tables viii

List of Appendices xi

Acknowledgements x

Introduction 1
- Anatomy of the hippocampus 3
- Characteristics of LTP 4
  - Synaptic LTP vs E-S potentiation 10
  - Cooperativity 13
  - Associativity 14
- LTP in awake rats 15
- Noradrenaline 16
- The use of cannulas to study LTP 20

General Methods 22
- Materials 22
- Surgery 23
- Electrophysiology 23
- Statistical analysis 25
- Histology 26

Experiment 1 27
- Introduction 27
- Methods 27
- Results 28
  - Time effect 28
  - Group differences 29
  - Histology 34
- Discussion 34
  - Part 1. LTP in rats with chemitrodes 37
    - LTP in awake rats (cannula) 37
    - LTP in anaesthetized rats (cannula) 40

Bramham et al 40
Errington et al 41
Conclusions - Part 1 43
Part 2. LTP in rats with chronic electrodes 44
LTP in awake rats (electrodes) 44
Conclusions 47

Experiment 2 48
Introduction 48
Methods 48
Results 49
Time effect 49
Group differences 50
Variability 53
Histology 53
Discussion 55

Experiment 3 57
Introduction 57
Methods 58
Results 58
Time effect 58
Group differences 59
Histology 59
Discussion 59

General Discussion 63
Experiment 1 63
Experiment 2 64
Experiment 3 66
Factors affecting rate of success of LTP 67
Conclusion 68

References 69

List of Figures

Figure 1. Schematic diagram of a transverse section through rat hippocampus and dentate gyrus, a horizontal section. 5

Figure 2. Characteristic perforant path evoked potential as recorded in the granule cell layer of the dentate gyrus of rat hippocampus. 8

Figure 3. Long-term potentiation in the perforant path-dentate gyrus synapse. 11

Figure 4. Population spike (PS) amplitude means (± SEM) plotted at baseline and 3 times post-tetanization for all 3 groups combined in Experiment 1. 30

Figure 5. Population spike (PS) amplitude means (± SEM) plotted at baseline and at 3 times post-tetanization for individual groups in Experiment 1. 32

Figure 6. Coronal section of hippocampus showing tissue damage resulting from chronic implantation of a chemitrode (A) or electrode (B). 35

Figure 7. Population spike (PS) amplitude means (± SEM) plotted at baseline, immediately after drug injection, and at 3 times post-tetanization for saline (n=7) and drug groups in Experiment 2. 51

Figure 8. Population spike (PS) amplitude means (± SEM) plotted at baseline, immediately after saline injection, and 3 times post-tetanization for immobile (n=6) and freely behaving (n=7) groups in Experiment 3. 60

List of Tables

Table 1. A comparison of data, including population spike (PS) amplitude means (± SEM), from two experiments inducing long-term potentiation (LTP) in rats with chronic chemitrodes. 39

Table 2. A comparison of data from a number of studies of induction of long-term potentiation (LTP) in rats with chronic electrodes. 46

Table 3. A comparison of data, including population spike (PS) amplitude means (± SEM), between group 3 of Experiment 1 and the saline group of Experiment 2. 54

List of Appendices

Appendix A. Rationale for various tetanization and sampling I/O protocols. 75

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This thesis is dedicated to my rats, for always behaving themselves.

INTRODUCTION

The field of behavioural neuroscience is involved, in part, in the reduction of "...the vagaries of human thought to a mechanical process of cause and effect" (Hebb, 1949, p*xi*). Behavioural neuroscience has used this reductionist approach to describe the biological basis of numerous psychological processes from visual perception to learning and memory. The specific objective of my research was to examine an aspect of the biological mechanisms that may be involved in the process of learning. Specifically, I examined the conditions under which long-term potentiation can be induced in awake rats.

Learning may be described as the changes in the nervous system that mediate a lasting association between stimulus and response. In reductionist tradition, the response has been quantified at many levels of analysis, from the behavioural reactions of a child in the classroom to the number of quanta released by an interneuron in an invertebrate reflex.

Donald Hebb, in his "cell assembly" theory of 1949, was the first to reduce the psychological process of learning to the level of the neuron. He suggested in his book The Organization of Behavior that the classical stimulus-response (S-R) association necessary for learning could potentially apply to the interactions of neurons: one as the stimulator, the other as the responder. Hebb suggested that learning occurs when the connection between the stimulator and the responder is strengthened. Specifically, Hebb stated:

> "When an axon of cell A is near enough to excite a 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. (Hebb, 1949, p62.)"

Alternatively, this statement can describe a classical learning situation in which an unconditioned stimulus comes to elicit a conditioned response after repeated pairings. Hebb noted that the stimulus and response (S-R) need not refer only to sensory input and motor output, as most commonly implied in psychological theory, but could pertain to any single neuronal connection involved in the manifestation of the learning. This concept therefore describes one biological unit of a learning circuit and is now commonly referred to as a Hebbian synapse. For example, a simple reflex response, e.g. *Aplysia's* gill-siphon withdrawal reflex, can be described as a single Hebbian synapse, whereas a complex cognitive task may involve millions of Hebbian synapses.

The specific phrase from Hebb's statement that forms the theoretical foundation of my thesis research and a plethora of related research is that: **"...some metabolic ...change takes place in one or both cells such that A's efficiency ...is increased."** This phrase illustrates the concept of neuroplasticity: that the nervous system is capable of change.

Long-term potentiation (LTP) is an electrophysiological phenomenon that is a model of increased synaptic efficiency or strength [1]. Since its initial description

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[1] Although LTP is commonly referred to as an increase in synaptic strength, note that changes in neuronal responsiveness may occur at loci other than the synapse on the postsynaptic neuron (see discussion of synaptic LTP vs E-S potentiation, p.8)

by Bliss and Lømo in 1973, LTP has been investigated by researchers world wide. Correlations between LTP and learning have been made on the basis of anatomical, biochemical, and electrophysiological evidence (Green & Greenough, 1986). Behavioural evidence that LTP is correlated with learning, however, has thus far eluded investigators. Therefore, I refrain from suggesting that LTP is **necessary** for learning but, instead, assume that LTP is a **model** of increased synaptic efficacy, which itself is presumed necessary to the process of learning. Although the phenomenon of LTP has been observed in numerous brain sites, the most popular structure investigated in LTP studies, and in my thesis, is the hippocampus.

Anatomy of the hippocampus

The hippocampus is an archicortical structure for which a homologue exists in all vertebrates (O'Keefe & Nadel, 1978). This phylogenetically old cortex is divided into the hippocampus proper and the dentate gyrus, both of which are characterized by three well-defined layers. The layers of the dentate gyrus are the strata moleculare, granulosum and polymorphus. The strata moleculare contains dendrites; cell bodies are located in the granular layer (O'Keefe & Nadel, 1978).

The hippocampus is characterized by a trisynaptic circuit, identified initially by Andersen, Blackstad, and Lømo (1966). This circuit starts with the main afferent to the hippocampus; the axons from the entorhinal cortex. These axons, the perforant path (PP), synapse on dendrites of dentate granule cells (see Figure

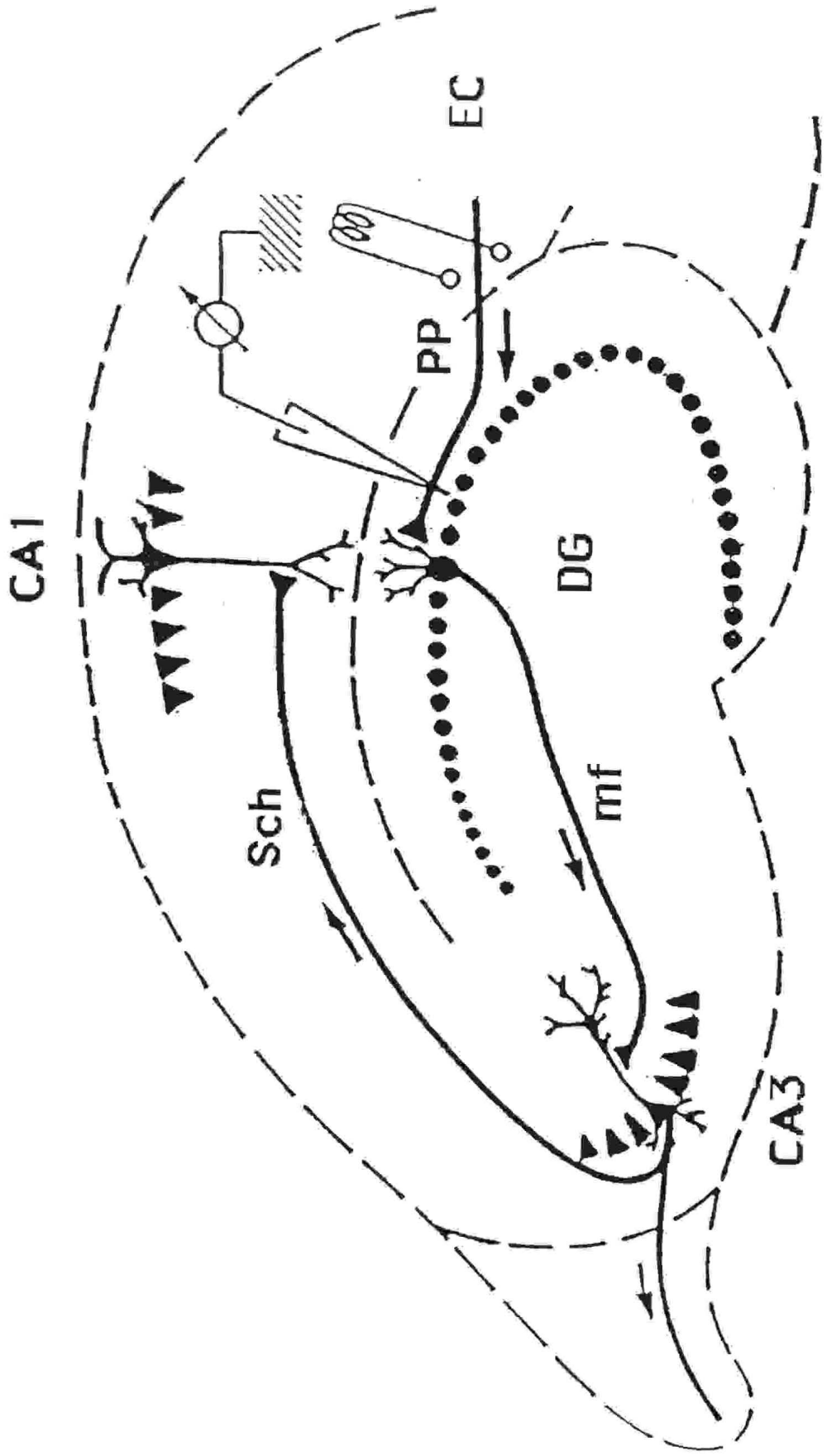
1). Axons of the granule cells, the mossy fibers, project to dendrites of pyramidal cells in area CA3 of the hippocampus proper. CA3 axons project to the septum via the fornix, and their Schaffer collaterals synapse on pyramidal cells in area CA1. Axons of CA1 pyramidal neurons project to the septum via the subiculum and the fornix. This trisynaptic circuit is amenable to in vitro study because horizontal slices taken through the structure can yield all components of the circuit. Of particular interest to this study is the perforant pathway (PP) of the entorhinal cortex and its target the dentate gyrus (DG).

On the basis of electrophysiological data, the PP has been divided into medial and lateral components (Abraham & McNaughton, 1984; McNaughton & Barnes, 1977). These pathways send homotopic projections from the dorsomedial and ventrolateral entorhinal cortex to the middle one-third and outer one-third of the molecular layer of the DG, respectively (Hjorth-Simonsen, 1972; Hjorth-Simonsen & Jeune, 1972). These two pathways are electrophysiologically and pharmacologically distinct (Pelletier, Kirkby & Corcoran, in press). In this thesis, I have concentrated on LTP induced by stimulation of the medial perforant pathway.

Characteristics of LTP

In Bliss and Lømo's seminal publication in 1973, LTP was characterized in anaesthetized rabbit as: "a potentiation in the population response of a target cell group after repetitive stimulation of its afferent fibres." Since 1973, LTP has been

Figure 1. Schematic diagram of a transverse section throught rat hippocampus and dentate gyrus, a horizontal section. Major excitatory pathways of the trisynaptic circuit are indicated: perforant path (PP) fibers from the entorhinal cortex (EC) synapse on dendrites of granule cells of the dentate gyrus (DG); the axons of the granule cells, the mossy fibers (mf) synapse on apical dendrites of the CA3 pyramidal cells; Schaffer collateral (Sch) from CA3 pyramidal cells project to dendrites of pyramidal cells of area CA1. Stimulating and recording electrodes are indicated in PP and dentate granule cell layer, respectively.


CA1
EC
PP
DG
Sch
mf
CA3

described in a variety of species, including crayfish opener muscle (Baxter, Bittner, & Brown, 1985). Most of the research, however, has been performed in rodents, and LTP has been reported in numerous rodent brain sites including neocortex and virtually all limbic sites (Racine, Milgram, & Hafner, 1983). The phenomenon of LTP exhibits numerous characteristics that suggest it is a mechanism of memory formation. These include: longevity (recorded for several weeks by Douglas in 1977) induction of changes in synaptic strength, cooperativity, and associativity.

Bliss and Lømo (1973) applied tetanizing stimulation[2] to the medial PP fibers and recorded responses from the granule cells of the DG of the hippocampus, as I have done. Specifically, Bliss and Lømo noted long-lasting potentiation in 2 parameters of the extracellular evoked potential: the amplitude of the population spike (PS) and the excitatory postsynaptic potential (EPSP); also, the latency of the PS was decreased. These components of the evoked potential are indicated in Figure 2. The EPSP represents a field measure of dendritic depolarization of dentate granule cells. This depolarization is due to activation by glutamate released from the PP terminal boutons. The PS represents the "overall excitability" of the granule cells population (Lømo, 1971); the amplitude of the PS represents the relative number of cells firing, and the width of the PS represents the relative synchrony of cell firing. A larger PS amplitude indicates a larger

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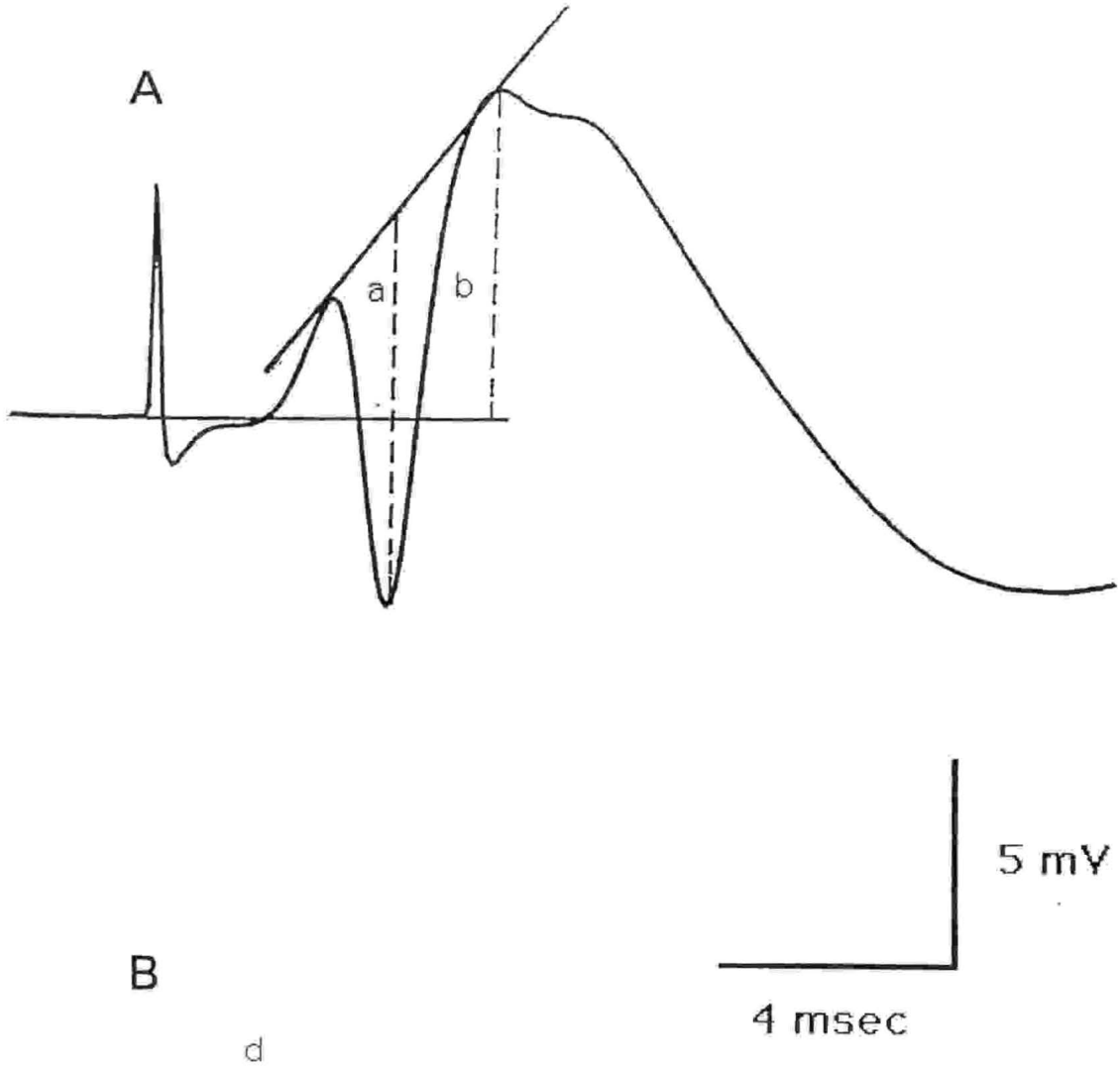
[2] The phrase "tetanic", or tetanizing stimulation was first used by Liley (1956) to refer to potentiation of responses at the rat neuromuscular junction after high-frequency stimulation. This potentiation, referred to as post-tetanic potentiation (PTP), was of very short duration.

Figure 2. Characteristic perforant path evoked potential as recorded in the granule cell layer of the dentate gyrus. Parameters of the evoked potential in A include : a) population spike (PS) amplitude, and b) population excitatory postsynaptic potential (EPSP) amplitude. In B, parameters are c) PS latency, d) EPSP latency, and e) slope of the EPSP.


A
a
b
5 mV
4 msec
B
d
e
c

number of cells firing and a narrower PS width indicates increased synchrony of firing. Figure 3 gives an example of a PP-DG evoked potential measured before and after tetanization.

Potentiation of either the PS or the EPSP amplitudes suggests that the connection between the PP terminals and the DG dendrites is stronger because an unchanged input produces an increased output. In Hebb's terms, the efficiency of the PP (cell A), as one of the cells participating in firing the DG (cell B), has increased. However, if LTP of the PS amplitude could be dissociated from that of the EPSP, this would provide some evidence to suggest that LTP was not solely a synaptic phenomenon. In the years following Bliss and Lømo's observations, several studies have attempted to dissociate potentiation of the EPSP and the PS amplitudes.

Synaptic LTP vs E-S potentiation

LTP has been divided into two main components: synaptic LTP and E-S potentiation. Synaptic LTP refers to LTP of the EPSP amplitude along with that part of the PS amplitude potentiation resulting directly from EPSP activation[3]. The second component of LTP, E-S potentiation, refers to a shift in the relationship between the EPSP and the PS observed after tetanization (Kairiss, Abraham, Bilkey & Goddard, 1987). Briefly, a graph of the PS amplitude plotted

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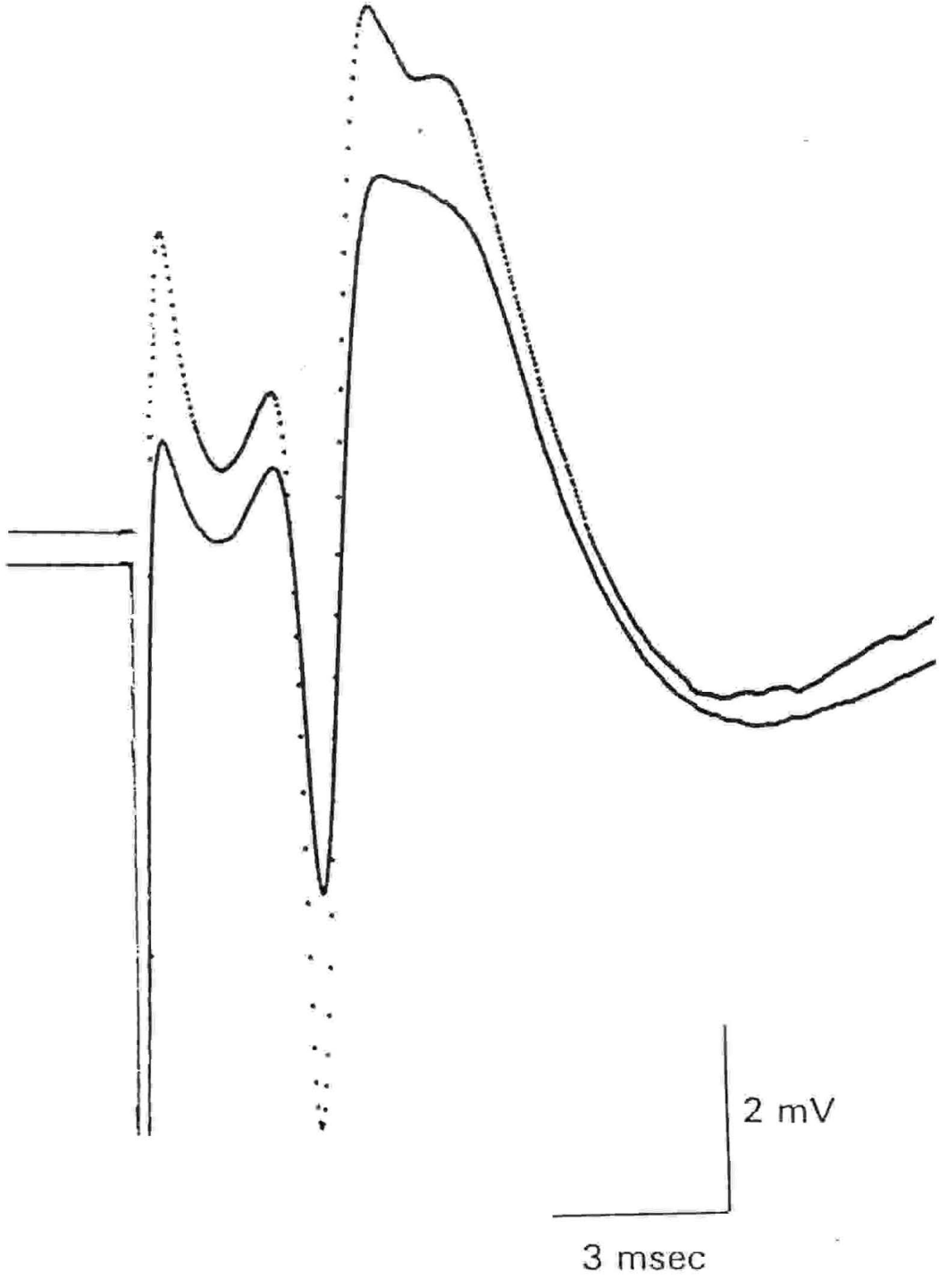
[3]Taube and Schwartzkroin (1988) refer to synaptic LTP as V-E potentiation, or volley to EPSP potentiation, which refers to EPSP potentiation only, with no PS potentiation component.

Figure 3. Long-term potentiation in the perforant path-dentate gyrus synapse. Characteristic perforant path evoked potential recorded in the dentate granule cells at baseline (solid line) and at 60 minutes post-tetanization (dashed line). Tetanization stimulation consisted of 10 trains of stimulation applied at 10 sec intervals. Each train consisted of 8 pulses of 0.1 msec duration at 400 Hz.


2 mV
3 msec

against EPSP slope shifts to the left after tetanization. This shift indicates that a given EPSP produces a larger PS after tetanization. Therefore, E-S potentiation theoretically suggests that some changes must be happening at a locus other than the synapse during LTP induction. However, these 2 components of potentiation usually occur together so that examining one without the other has not yet been accomplished (Bliss & Lynch, 1988). This dissociation of LTP components, however, suggests that discussion of mechanisms of LTP should include postsynaptic loci in addition to the synapse.

Cooperativity

Hebb's postulate suggests that changes in synaptic strength should depend on coactivation of presynaptic and postsynaptic elements. This simultaneous activity should, theoretically, result in a larger global response, a phenomenon referred to as cooperativity. McNaughton, Douglas, and Goddard (1978) tested this hypothesis by determining whether duration or number of pulses in the tetanizing stimulation resulted in more potentiation. If changes in synaptic strength depend on coactivation, then only an increase in the duration of the pulses should result in increased LTP. Indeed, McNaughton et al. (1978) observed that potentiation depended on the duration of the pulses rather than on the number of pulses applied.

Associativity

A type of classical conditioning has been observed in the PP-DG synapse; this has allowed study of the associative properties of LTP. Briefly, most PP axons project to the ipsilateral DG; there is only a sparse projection to the contralateral DG. Therefore, one granule cell receives converging fibers from both the ipsilateral PP and the crossed pathway. Levy and Steward (1979) observed that LTP could be induced in the DG when the ipsilateral PP was tetanized, but not when the crossed pathway was tetanized. The crossed pathway was deemed to be too weak to induce LTP. As well, when the test pulse was applied to the crossed pathway after induction of LTP by ipsilateral tetanization, no LTP was observed. In fact, a depression of the response was observed; this was termed long-term depression (LTD). However, if the ipsilateral and crossed pathways were tetanized concurrently, LTP was recorded in the target cells when the test pulse was later applied to the crossed pathway. This associative pairing was observed to have temporal constraints, as would be expected in a classical conditioning paradigm (Levy & Steward, 1983). Specifically, LTP was greatest when the crossed pathway was stimulated not more than 5 msec before the ipsilateral pathway. LTD resulted when the crossed pathway was stimulated after the ipsilateral pathway.

Steward (1976) elaborated on these studies of associativity by determining its spatial constraints. Specifically, Steward determined that associativity between converging fibers occurs only when the fibers terminate in the same area of the

dendritic tree. Steward observed that associativity did not occur when the crossed lateral PP fibers were activated concurrently with the ipsilateral medial PP fibers. This indicates that associativity occurs only when fibers converge in close anatomical association.

LTP in awake rats

Douglas and Goddard (1975) were the first to observe the effects of daily tetanizing stimulation on the PP-DG evoked potential in awake rats. Potentiation was observed in the majority of animals stimulated, over a wide range of stimulation train frequencies. Many of these animals, however, exhibited afterdischarge (AD). AD represents synchronous, epileptiform firing of cell populations evident in the EEG. Douglas (1977) considered AD to be "a large and uncontrollable response" that may have resulted in the potentiation observed by Douglas and Goddard (1975). Therefore, Douglas (1977) repeated the experiments while controlling for AD induction; this was done by lowering stimulation intensities and omitting rats that exhibited AD. Tetanizing stimulation parameters used by Douglas mimicked normal discharge characteristics of the HPC in freely behaving rats[4]. Douglas found significant potentiation of the PS amplitude 1 day after the fourth session of tetanic stimulation; this is the first report of LTP induced in awake rats, in the absence of AD. Since Douglas's seminal experiment,

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[4] A discussion of research that defined appropriate parameters of tetanizing stimulation is in Appendix A.

most research on LTP using the awake rat preparation has been directed towards determining behavioural correlates of LTP.

A current debate in the LTP literature involves the correlation between learning and LTP. Although this question is not directly relevant to my thesis, the technique is relevant. Studies concerning LTP and learning fall into three categories of experimental approach: new learning should produce an electrophysiological phenomenon similar to LTP; pharmacological manipulations that block LTP should block learning; and new learning should be blocked by saturating LTP. The results from the majority of these studies have been negative; yet research continues with a vengeance (see Korol, Abel, Church, Barnes & McNaughton, 1993; Jeffrey & Morris, 1993; Sutherland, Dringenberg & Hoesing, 1993; Cain, Hargreaves, Boon & Dennison, 1993; all failures to replicate Castro, Silbert, McNaughton & Barnes, 1989). All these studies provide data about LTP in awake rats. My position in this debate is that learning involves increased synaptic strength, and LTP is an appropriate **model** with which to study increased synaptic strength.

Noradrenaline

The research for my thesis was intended to examine the role of noradrenaline (NA) in LTP. In 1970, Kety hypothesized that the functional role of NA in the nervous system was to mediate the state of attention of an animal:

> "1) that the aroused state induced by novel stimuli, or by stimuli genetically recognized as significant, is pervasive and affects synapses throughout the

> central nervous system, suppressing most, but permitting or even accentuating activity in those that are transmitting the novel or significant stimuli; (2) that this state, through one or more of its components, favours the development of persistent facilitatory changes in all synapses that are currently in a state of excitation or have recently been active." (p325)

The first aspect of Kety's hypothesis refers to NA's differential effects on spontaneous firing vs evoked responses of target neurons. Specifically, NA has been reported to depress spontaneous firing while simultaneously enhancing neuronal responses to afferent input (Olpe, Steinmann & Jones, 1985). This comparison of evoked responses to spontaneous firing is referred to as the signal/noise ratio. Kety suggested that, through modulation of signal/noise ratios, NA affects the attentional state of the animal. Although behavioural evidence supporting the role of NA in attentional remain inconclusive, much electrophysiological evidence supports the role of NA as a modulator of signal/noise ratio; this literature will be reviewed shortly.

The second part of Kety's hypothesis can be interpreted as suggesting that NA actively participates in changing synaptic strength. This second statement is so reminiscent of Hebb's terminology that I could not miss the chance to bring the two theories together. Specifically, Hebb suggested that learning may occur when the synapse is strengthened. Kety suggested that NA "favours the development of" changes in the synapse, when it is active. LTP provides a model with which to study synapses in the process of facilitatory change. Therefore LTP is an appropriate model to study the effects of NA on active synapses; Hebb's theory meets Kety's theory at the level of the synapse. The anatomy of noradrenergic

projections provides other circumstantial evidence for a role of NA in cognitive functions.

Noradrenergic projections originate in the locus coeruleus (LC) of the midbrain and project diffusely to all parts of the brain. Noradrenergic axons end in varicosities, with no distinct postsynaptic elements (Descarries, Watkins, & Lapierre, 1977). The anatomical distribution of noradrenergic axons suggests that NA may have some widespread effects on the general cognitive state of the animal.

Noradrenergic enhancement of signal/noise ratio has been documented in rat visual cortex, lateral geniculate nucleus, lateral hypothalamus, superior colliculus, and hippocampus (review by Waterhouse, Sessler, Cheng, Woodward, Szizi, & Moises, 1988). Sara and Segal (1991) recorded firing of LC neurons in response to a tone. They reported increases in neuronal firing when the significance of the tone changed from non-rewarding to rewarding and visa versa during learning of an appetitive task. Firing rate remained constant once the animal had learned the task. Electrophysiological recordings of spontaneous LC cell firing also provides circumstantial evidence for the hypothesis that NA has a role in attention. Aston-Jones (1985) reported that firing rate varied depending on the state of vigilance of the animal. For example, firing rate was high when novel stimuli were presented, and lower when the animal was engaged in non-vigilant behaviours such as grooming. Paradoxically, the LC was virtually silent during REM sleep, when the rest of the brain exhibits high levels of neuronal firing.

From these data one can speculate, as Kety did, that NA modulates

neuronal responses depending on the biological significance of the input. This speculation directly implicates NA in mechanisms of learning. Since LTP has been implicated as a model of learning, it follows that NA may modulate LTP induction.

Plethora of data exists on the effects of NA on the hippocampus. Lacaille and Harley (1985) observed effects of NA superfusion on PP evoked potentials in dentate gyrus in vitro. NA application resulted in an increase in the PS amplitude, and a β-antagonist blocked the increase. Dahl and Sarvey (1989) also found that NA induced long-lasting potentiation of the PS amplitude in medial PP evoked responses. The potentiation was blocked by the β-antagonist, propranolol. Stanton and Sarvey (1985) demonstrated effects of noradrenergic modulation on the induction of LTP. They found that the nonspecific β-antagonist propranolol and the β_1-antagonist metoprolol both reduced the amount of LTP in the DG; neither drug affected baseline evoked potentials.

LTP is a postulated mechanism of learning and long-term memory. Since NA has also been implicated, theoretically at least, in learning, it is appropriate to examine possible effects of NA or its antagonists on induction of LTP. The purpose of my thesis was to determine the effects of noradrenergic antagonists on induction of LTP in vivo. To avoid involvement of extrahippocampal noradrenergic fibers, an intradentate infusion of drug was used. This necessitated implantation of a chemitrode into the dentate gyrus.

The use of cannulas to study LTP

Errington, Dolphin, and Bliss (1983) first described the push-pull cannula technique used to study LTP in urethane anaesthetized rats. The push-pull cannula allows for in vivo perfusion of a specific brain region and collection of that perfusate; field potentials can be recorded from the same site simultaneously. Subsequent studies from their laboratory were generally aimed at determining whether the presynaptic or postsynaptic locus is more important in the induction of LTP. Although this question is not directly relevant to my thesis, the technique of cannula implants into the DG is relevant.

In the initial description of the push-pull cannula technique, Errington, Dolphin, and Bliss (1983), reported stable recordings of potentials from the granule cell layer for 5-6 hours during constant local perfusion. They concluded that cannulas provide "a powerful method for the analysis of synaptic activity in the hippocampus...". Bliss, Dolphin, Errington, and Lynch (1986) used this method to collect perfusate after LTP induction. They reported potentiation of the EPSP slope 2 hours after tetanization. Lynch and Bliss (1986) reported 199 percent PS amplitude potentiation. Errington, Lynch, and Bliss (1987) succeeded in depressing the amount of potentiation seen in control rats by adding a glutamate receptor antagonist, 5-aminophosphonovalerate (APV). This literature suggests that the technique of acute cannulation in anaesthetized rats is an appropriate method for the study of LTP.

More evidence in support of this conclusion comes from Bramham,

Milgram, and Srebro (1991a,b). In both these studies evoked potentials were recorded from an electrode attached to a push-pull cannula. They obtained characteristic recordings from the medial and lateral perforant pathways; this replicates a previous study done with electrodes alone (McNaughton & Barnes, 1977). The authors reduced the amount of control LTP by infusing either APV (1991a) or naloxone (1991b). Therefore, both Errington's and Bramham's laboratories appear to have successfully induced LTP in anaesthetized rats with cannulas. However, the technique I used in Experiment 1 involves chronic chemitrode, so that LTP could be observed in awake rats. I know of only two studies using this technique.

Laroche, Doyere, and Bloch (1992) and Cain, Boon, and Hargreaves (1992) both studied LTP in awake rats with chronic cannulas; Laroche et al used a push-pull cannula and Cain et al used a chemitrode identical to that in my thesis. Laroche et al reported successful LTP in 2 of 4 rats infused with saline. Cain et al reported successful LTP in 100 percent of tetanized rats.

According to the literature presented above, use of chronic chemitrodes appears to be an appropriate method to study LTP. Experiment 1 involved use of chronic chemitrodes in rats, in order to examine the effects of infusion of a β-adrenergic antagonist on induction of LTP. In experiment 2, chronic electrodes are used to study effects of an intraperitoneal injection of the β-adrenergic antagonist propranolol on induction of LTP. Experiment 3 examine whether behavioural state my effect the amount of potentiation observed in awake rats.

GENERAL METHODS

Materials

Subjects were male Long-Evans hooded rats weighing 250-450 grams, housed individually with food and water *ad libitum* and a 12/12 hour light/dark cycle.

Electrodes were made from enamel insulated nichrome wire 127 μm in diameter. The insulation was burned off the ends of each wire and the ends were soldered into gold-plated amphenol pins. Both electrodes were made from 2 strands of wire. Each pole of the PP electrode was inserted into a separate pin, but both poles of the DG electrode were inserted into a single pin. Thus the PP electrode was bipolar, whereas the DG electrode was monopolar. PP electrodes were implanted into all rats. Chemitrodes were implanted in the DG of groups 1 and 2 of Experiment 1 and electrodes were implanted in all other rats. The chemitrode consisted of a DG bipolar electrode with a guide cannula attached. The guide cannula was constructed from a 23-gauge needle, cut to a length of 16 mm +/- 0.2 mm; it was 850 μm wide. The DG electrode was glued to the guide cannula using epoxy and the electrode extended 1.0 mm beyond the end of the guide cannula. Ground electrodes were made from insulated stainless steel wire. The insulation was burned off both ends of the wire, one end was soldered into an amphenol pin and one end was soldered onto a jeweller's screw.

Surgery

Bipolar and monopolar electrodes were surgically implanted into the PP and DG of the hippocampus, respectively, using standard stereotaxic techniques. Rats were anaesthetized with sodium pentabarbital at 65 mg/kg, and were given 1.0 mg/kg scopolamine methylbromide. Electrodes were implanted using co-ordinates determined from Paxinos and Watson (1986) (PP: AP:-7.9, L:4.2, V:-3.1; DG: AP:-3.5, L:2.0, V:-3.2). Measurements were taken from bregma with the incisor bar set at +3.9 mm. The electrodes were inserted to a depth of 2.0 mm from the surface of the cortex, and from there lowered at a rate of 0.1 mm/20s, while monitoring evoked potentials. The frequency of tests pulses was 0.05 Hz with intensity set at 800 μA. Each test pulse consisted of one 0.1 ms monophasic cathodal square wave pulse. The signal was filtered to a 0.1 Hz - 1 kHz frequency range. The final depth of the electrodes was determined by the location of the maximal PS. Electrodes were cemented in place with dental acrylic, then inserted, along with the ground screws, into plastic pedestals. The pedestal was cemented to the skull and the surgical wound was sutured. An obturator of length 16.5 mm was inserted into the guide cannula of the chemitrodes. Animals were given at least one week recovery.

Electrophysiology

After the recovery period, the PS thresholds were determined. Animals with PS thresholds higher than 550 μA were omitted from the experiments.

Evoked potentials were monitored at a variety of intensities to determine the input/output curve relations (I/O) for each animal; input refers to the intensity of the test pulse and output refers to the amplitude of the PS. Specifically, 5 intensities were chosen for which the PS amplitude was: maximum amplitude and approximately 80 percent, 60 percent, 40 percent and 20 percent of maximum.

During each LTP session the PP was stimulated with a test pulse at a frequency of 0.03 Hz. Each I/O within a session comprised of 5 evoked potentials recorded at each of the predetermined 5 intensities. Data were collected at a sampling rate of 32 Khz and stored and analyzed by Brainwave Systems software. Analysis of the evoked potential was completed offline on the average of 5 evoked potentials collected at each intensity.

Parameters collected included the amplitude and latency of the PS and EPSP, and the slope of the EPSP. The PS amplitude was calculated as the distance from the minimum of the spike to a tangent drawn from the two maxima of the EPSP. EPSP slope was measured as the slope of the initial portion of the EPSP. The parameter of particular interest to this study was the PS amplitude. At each intensity, the averaged PS amplitudes were expressed as a percentage of the baseline PS amplitude. This was necessary to allow comparison between individuals because of large intersubject variability.

The tetanization stimulation consisted of 10 trains with an intertrain interval of 10 seconds. Each train consisting of 8 pulses, each 0.1 ms in duration, at a frequency of 400 Hz (Douglas, 1977). The intensity of stimulation was either

1500 μA or 800 μA. In Experiment 1 all tetanizations were applied first at 1500 μA. If any behavioural signs of AD were evoked with the first train of the tetanization, the stimulus intensity was reduced to 800 μA for the remaining nine trains. In Experiment 2, stimulation intensity was 1500 μA for those animals with a PS threshold higher than 300 μA, and 800μA for those with PS threshold lower than 300 μA. In Experiment 3, EEG was monitored and the intensity of the stimulus reduced to 800 μA if AD was observed.

Statistical analysis

The data were analyzed by repeated measures analysis of variance (ANOVA) (SYSTAT, Wilkinson 1990) with group as a categorical independent variable, and time as a dependent variable. Specifically, the analysis was done with repeated measures taken at the second baseline, as well as at 60 minutes, 1 day, and 1 week post-tetanization. The alpha level for acceptance of significance was 0.05. If time effect was significant, specific contrasts were analyzed. Contrasts of interest were baseline vs the three post-tetanizations measures; "specify" (Wilkinson, 1990) was used to determine specific contrasts. A Bonferroni adjustment was used for determining the alpha level for contrasts, that is α=0.05 $\div$ number of contrasts.

Success rates of potentiation were defined as the percentage of rats within a group that exhibited at least 20 percent potentiation for all three times analyzed, that is 60 minutes, 1 day, and 1 week post-tetanization.

Histology

All animals were euthanized with sodium pentobarbital and brains were removed and kept in 10 percent formalin. Frozen sections 80 μm thick were taken and stained with cresyl violet.

EXPERIMENT 1

INTRODUCTION

Experiment 1 was initially intended to examine the effects of a β-adrenergic antagonist on induction of LTP. All animals were to receive intradentate infusions of either artificial cerebrospinal fluid (ACSF) or timolol followed by tetanization. My hypothesis was that the ACSF group would show significant potentiation of the PS, and the drug groups would show less or no potentiation. However, pilot data suggested that the chemitrode or the infusion disrupted LTP. I therefore altered the design of Experiment 1 to test this hypothesis. Group 1 had a chemitrode in the DG, and an infusion of ACSF. Group 2 animals had a chemitrode, but received no infusion. Group 3 received an electrode in the DG, instead of a chemitrode. Tetanization was applied to all groups.

METHODS

Each animal was habituated to the experimental cage for approximately one hour to eliminate effects of excessive motor activity and stress. One LTP session for groups 2 and 3 consisted of two baseline I/Os, followed by tetanizing stimulation, and 7 post-tetanization I/Os taken immediately, 15, 30, 45, 60 minutes, 1 day, and 1 week after the tetanization. No stimulation was applied between I/Os.

One LTP session for Group 1 consisted of two baseline I/Os followed by the infusion. The infusion consisted of ACSF with concentrations in mMol: NaCl

125, KCl 3.0, $CaCl_2.2H_2O$ 2.4, $NaHCO_3$ 26, glucose 10, $MgSO_4$ 1.3, H_2PO_4 1.25. An infusion of 0.5 μl of ACSF was delivered over 4 min 30 sec. The injection cannula was left in place for five minutes after the end of the infusion to facilitate passive diffusion. The injection cannula was then withdrawn and the obturator put back in place to prevent reflux up the cannula. A post-infusion I/O was collected 5 minutes after the beginning of infusion. Tetanization stimulation was given 20 minutes after the beginning of infusion. Seven post-tetanization I/Os were then collected, as for groups 2 and 3.

RESULTS

The rate of success of finding a characteristic DG-PP evoked potential during surgery was much lower in rats with chemitrodes than in rats with electrodes alone. Success rates were approximately 50 percent for chemitrodes vs approximately 80 percent for electrodes. All 23 rats included in the experiment had characteristic PP-DG evoked potentials for which the maximum PS threshold was 550 μA; the mean PS threshold was 250 μA. Each rat was included in only one group. Final sample sizes within each group were: group 1, n=8; group 2, n=8; and group 3, n=7.

Time effect

The criterion for occurrence of LTP was that the PS amplitude at baseline must be significantly lower than the PS amplitude at one or more of the post-

tetanization intervals. The PS amplitude at baseline was found to be significantly different from that measured 1 day and 1 week post-tetanization when data from all 3 groups were pooled. Statistical analysis of all the data from groups combined indicated that there was a significant time effect: [$F(3,18)=6.547$, $p=0.003$]. The three specific contrasts tested were: baseline vs 60 minutes, baseline vs 1 day, and baseline vs 1 week. The two latter contrasts were significant: [$F(1,22)=10.491$, $p=0.004$] and [$F(1,22)=11.241$, $p=0.003$], respectively. The contrast between baseline and 60 minutes was nonsignificant; however, the p value is very close to the alpha level [$F(1,22)=6.457$, $p=0.019$, $\alpha=0.017$] (see Figure 4).

When the data of the 3 groups were analyzed separately, the changes in PS amplitude across time were nonsignificant. That is, none of the 3 groups by itself showed significant LTP: group 1 [$F(3,5)=0.622$, $p=0.631$], group 2 [$F(3,5)=3.584$, $p=0.102$] and group 3 [$F(3,4)=2.354$, $p=0.213$]. However, a graph of results from each group (see Figure 5) clearly indicates that PS amplitudes after tetanization were much larger in all groups than at baseline.

Group differences

The amplitude of the PS did not differ significantly among the 3 groups. The interaction between groups and time was also nonsignificant. Neither the group effect, nor the time by group interaction were significant: [$F(2,20)=1.423$, $p=0.264$] and [$F(6,36)=0.819$, $p=0.704$], respectively. Although statistically significant group differences did not occur, graphical presentation of the data

Figure 4. Population spike (PS) amplitude means ($\pm$ SEM) plotted at baseline and at 3 times post-tetanization for all 3 groups combined in Experiment 1. All PS amplitudes are plotted as a percentage of the first baseline measurement. B2, T60min, T1D, and T1W represent the second baseline measure, 60 minutes post-tetanization, 1 day post-tetanization, and 1 week post-tetanization. The PS amplitude at baseline is compared to the three post-tetanization measures. Significant contrasts are indicated by * ($p < 0.17$).

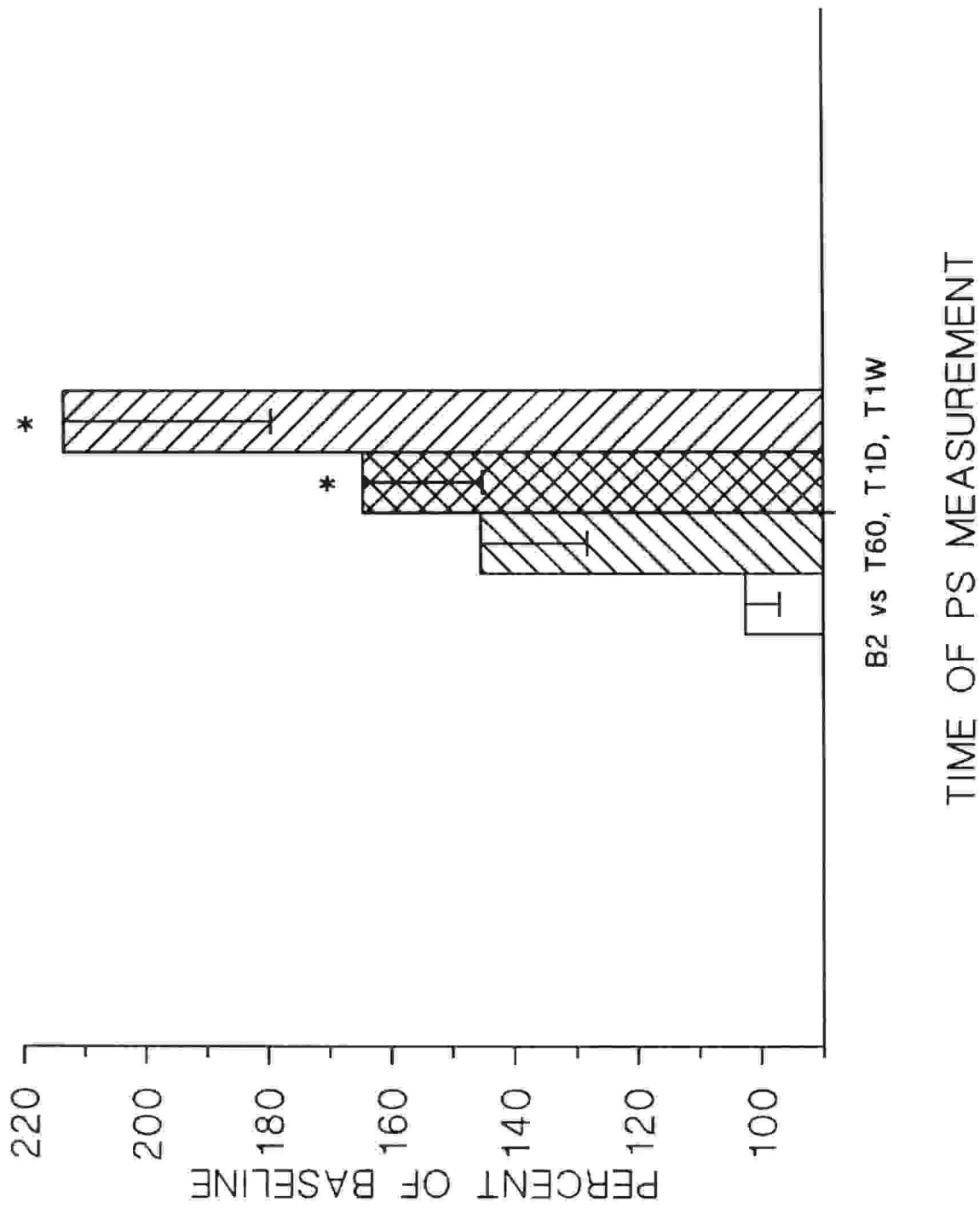
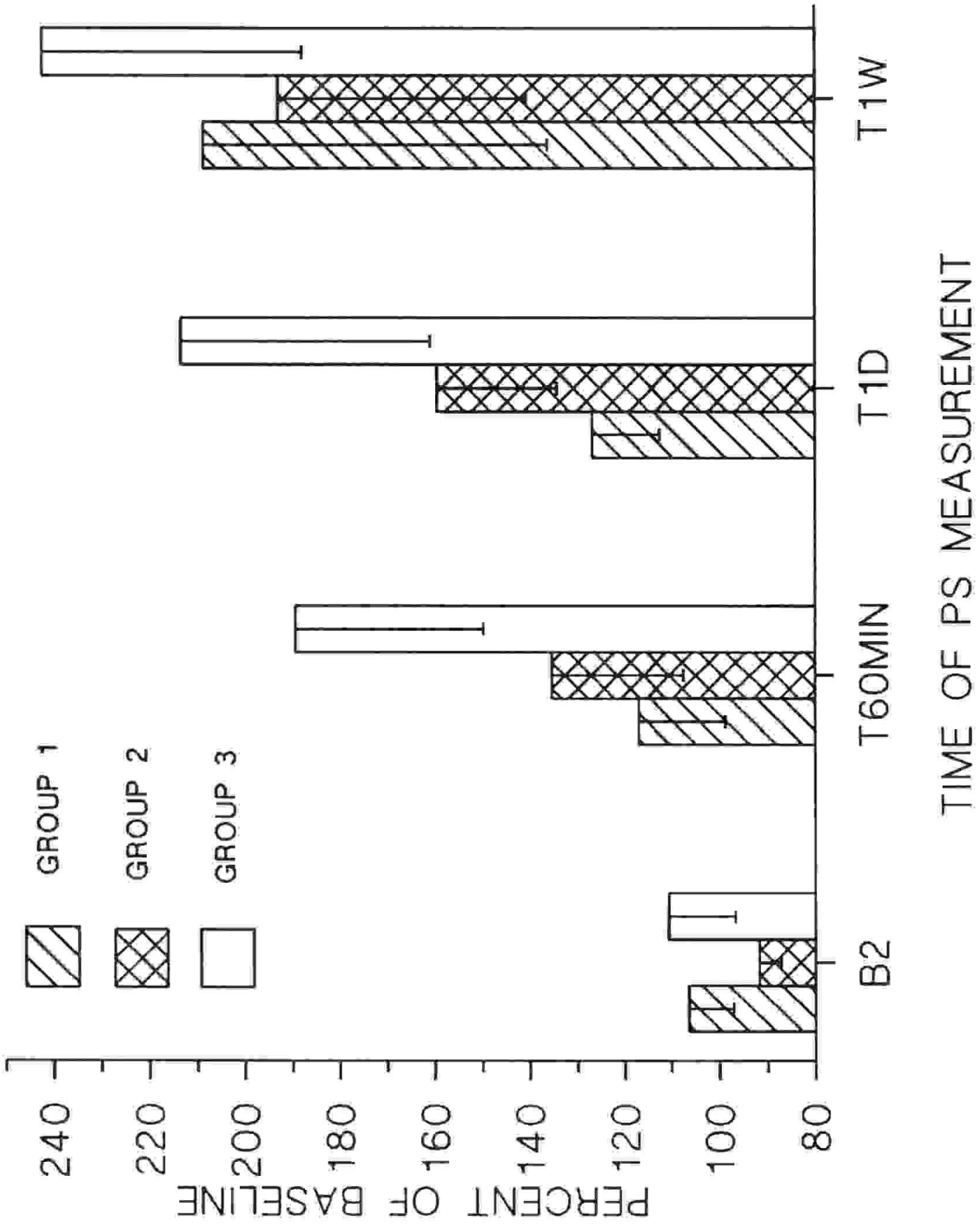

220
200
180
160
140
120
100
PERCENT OF BASELINE
*
*
B2 vs T60, T1D, T1W
TIME OF PS MEASUREMENT

Figure 5. Population spike (PS) amplitude means (± SEM) plotted at baseline and at 3 times post-tetanization for individual groups in Experiment 1. PS amplitudes are plotted as percentages of the first baseline measure for all rats in Experiment 1. B2, T60MIN, T1D, and T1W represent the second baseline, 60 minutes post-tetanization, 1 day post-tetanization, and 1 week post-tetanization. Group 1 had chemitrodes and infusions (n=8), group 2 had chemitrodes (n=8) and group 3 had electrodes (n=7). The PS amplitude at baseline was not statistically different from that at any of the post-tetanization times within any group.


GROUP 1
GROUP 2
GROUP 3
240
220
200
180
160
140
120
100
80
PERCENT OF BASELINE
B2
T60MIN
T1D
T1W
TIME OF PS MEASUREMENT

demonstrates obvious group differences (see Figure 5). Group 3 exhibited the largest amount of LTP, followed by group 2; and group 1 exhibited perhaps the least consistent LTP. Group differences in success rates of potentiation were also apparent. Group 3 had a 58 percent success rate, whereas groups 1 and 2 had relatively low success rates of 25 percent and 12.5 percent, respectively.

Histology

Histological analysis indicated that all PP placements were within the area of projections from the entorhinal cortex to the DG molecular layer. All DG placements were within the granule cell layer of the DG. Tissue damage was apparent in sections from all rats with chronic chemitrodes. Figure 6 shows representative sections from chemitrode and electrode implantations.

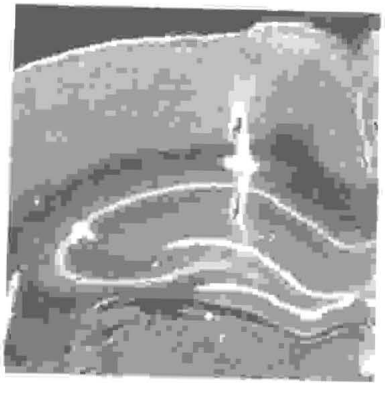
DISCUSSION

The data collected in Experiment 1 support the hypothesis that the chemitrode or the infusion disrupts LTP. Although group differences were not statistically significant, graphical representation and success rates indicate that the chemitrode groups exhibited less LTP than the electrode group. A second important finding of Experiment 1 is that even the electrode group did not show statistically significant LTP. First I will examine how the data from the chemitrode group compare to previous literature and reasons why the rats with chemitrodes showed less LTP than the electrode group. Then I will compare the

Figure 6. Coronal section of hippocampus showing tissue damage resulting from chronic implantation of a chemitrode (A) or electrode (B). The photomicrographs are negatives of sections 80 μm thick, stained with cresyl violet.

A

B



data from the electrode group to previous published data.

PART 1. LTP IN RATS WITH CANNULAS

LTP in awake rats (cannula)

I have found only two studies in which LTP was studied in awake rats with chronic cannulas. Laroche, Doyere, and Bloch (1989) implanted push-pull cannulas in 8 Sprague-Dawley rats, infused either 5-APV or saline into the DG, and applied tetanizing stimulation. Only 2 of 4 saline-infused rats were reported to show LTP, although no specific data were given. Many methodological differences between the Laroche study and my own exist. These include differences in intertrain intervals within the tetanizing stimulation and the use of saline as an infusion medium instead of ACSF. As well, Laroche's group repeatedly tetanized the PP until LTP saturation was reached[5]. Therefore this study suggests that LTP can be induced in rats with chronic cannulas. However, the authors do not provide statistical data to allow comparison with my results.

Cain, Boon, and Hargreaves (1992) delivered infusions into the DG through a chemitrode (identical to the one I used) during a number of stimulation procedures. Seventeen animals were divided into 3 groups: 5 were given tetanizing stimulation, followed by partial kindling; 5 received two tetanizing

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[5] LTP saturation refers to the protocol of giving multiple (usually daily) tetanizing stimulations to one animal. The amount of LTP increases with each tetanizing stimulation until a "ceiling" is reached. Laroche et al (1989) used a criterion of three stimulations, after which time they considered the animal's LTP to be saturated. Note that the protocol of my experiment included only one tetanization.

stimulations, followed by partial kindling; and 7 received partial kindling. Tetanizing stimulation was then applied to all rats in the absence of APV. Cain et al reported that 100 percent of the rats exhibited LTP. A comparison of PS amplitude means (± SEM) from Cain's experiment and my own is shown in Table 1. Comparisons cannot be made, however, without considering certain differences in experimental protocols between these two experiments.

First, the rats in Cain's experiment received at least 3 sessions of AD-evoking stimulation and some received two additional tetanizing stimulations. These previous stimulations may have accounted for elevated baseline PS amplitudes and must be considered when comparing PS amplitudes at 60 minutes and 24 hours post-tetanization. Another difference worth noting is that Cain et al. reported 100 percent success rate of PS potentiation at 60 minutes post-tetanization (17/17); I reported only a 50 percent success rate (8/16). My criterion for potentiation was a 20 percent increase over baseline; Cain's criterion was not stated. Notwithstanding my lower success rate, the means of both experiments are comparable and, the SEMs in my experiment are actually smaller than in Cain's.

In light of my own data and those of the two other publications using chronic chemitrodes in rats, some observations can be made. First, only a very small proportion of the plethora of studies on LTP involves chronic chemitrodes. These data demonstrate that LTP can occur using this preparation; however, the amount of LTP is either unknown (Laroche et al, 1989) or small (Cain et al., 1992). My own results compare well to those of Cain et al. and are just barely

Table 1. A comparison of data, including population spike (PS) amplitude means (± SEM), from two experiments inducing LTP in rats with chronic chemitrodes.

	Jones	Cain, Boon & Hargreaves
n	16	17
Baseline	99.1 ± 5.3%	118 ± 40%
60 minutes	126 ± 16%	155 ± 40%
1 day	143 ± 14%	135 ± 40%
p value (time effect)	0.05	0.00001
# reported to have potentiated at 60 min post-tet	8	17

significant ($p=0.05$). I cannot rule out the fact that some methodological problem with the chemitrode may have been responsible for my low success rate of LTP induction. To identify possible reasons for this I examined research using acute cannulas.

LTP in anaesthetized rats (cannula)

In my general introduction I referred to the technique of push-pull cannulas in anaesthetized rats (Errington, Dolphin, & Bliss, 1983; Bramham, Milgram, & Srebro, 1991a,b). In light of the results of Experiment 1, I reexamined the data from these two laboratories, Bramham's first followed by Errington's.

Bramham et al.

Bramham's laboratory examined the effect of APV on induction of LTP. Their results initially appear to support the hypothesis that LTP can be induced robustly in cannula preparations: the PS amplitude was reported to potentiate to 400 $\pm$ 118 percent (1991a) and 307 percent (1991b) of baseline. However, upon reexamination, I found that : "The criterion for LTP in individual rats was a statistically significant increase in field potential values obtained during the 20- to 30-min post-train period following the tetanus, relative to baseline values" (p.1302 & p.44, respectively). Bramham et al (1991a) went on to suggest that the APV group exhibited less LTP (77 $\pm$ 76 percent) than the control group. However, my conclusion is that no conclusions can be drawn, since the control group was

apparently selected on the basis of showing statistically significant LTP whereas the experimental group was unselected.

Errington et al.

Three issues arise when reading reports from Errington's laboratory: the correlative nature of their research, the lack of data concerning the success rate of LTP induction, and the possibility that cannulas may cause tissue damage. First, most of the research from Errington's laboratory is aimed at correlating the presence of LTP with changes in the concentration of various endogenous chemicals. In the first report using push-pull cannulas, Dolphin, Errington, and Bliss (1982) infused labelled glutamine and collected newly synthesized glutamate from the perfusate post-tetanization; an increase in glutamate release was reported. Bliss, Douglas, Errington, and Lynch (1986) reported that potentiation is correlated with increased release of endogenous glutamate. Understandably, success rates of potentiation were not reported since it was not germane to the question at hand; unfortunately, this means the data are incomparable.

One exception to this correlative type of study is that of Errington, Lynch, and Bliss (1987). They examined the effect of APV on induction of LTP. APV was reported to block LTP after the first tetanization. However, when a second tetanizing stimulation was given in the absence of APV, LTP did occur. The only data reported were that the PS amplitude was potentiated to 150 percent of baseline at 60 minutes post-tetanization. Unfortunately, SEMs and statistical analysis were

not reported, and so it is impossible to determine how their success rate of potentiation compares to mine.

The third issue suggested by data from Errington's laboratory is that cannulas cause tissue damage. In the initial description of the technique, Errington et al (1983) reported stable recordings of evoked potentials during 5-6 hours of continual perfusion. From this they concluded that the push-pull cannula is an appropriate method to study hippocampal plasticity; however, no SEMs were given to substantiate this claim. Using the same preparation, Fazeli, Errington, Dolphin, and Bliss (1988) found that tetanization correlates with an increase in protein efflux. The authors concluded that this increased protein efflux originates from proteolytic breakdown of blood clots formed in response to cannula implantation. Fazeli, Errington, Dolphin, and Bliss (1990) later confirmed this hypothesis by identifying the protein as haemoglobin. They observe that the tetanizing stimulation increases protease activity, which in turn increases breakdown of blood clots. Of course the presence of blood clots does not necessarily indicate tissue damage. As well, there was no control group for this experiment, as the cannula was necessary to collect the perfusate.

Interestingly, Aniksztgejn, Roisin, Amsellem, and Ben-Ari (1989) failed to find the increase in glutamate levels reported by Bliss et al (1986). They indicated that Bliss et al (1986) based their results on a comparison between glutamate levels in the controls and tetanized groups; no difference was found between glutamate levels measured in the tetanization group before and after the tetanization.

Aniksztgejn et al (1989) also suggested that their "open" push-pull cannula may have caused less tissue damage than the "closed" cannula used by Bliss et al (1986). The open cannula avoids large changes in local pressure that may result in tissue damage; as well, Aniksztejn et al (1989) did not observe blood in the perfusate, as did Bliss et al (1986). The chemitrode I used may be described as an open cannula; the width of the chemitrode is identical to that of both the open and closed cannulas.

The conclusion I draw from these findings is that some physiological change, neural or otherwise, occurs with acute cannula implantation. The fact that my data came from rats that had cannulas in place for at least two weeks leads me to suspect, if not expect, that tissue damage occurs. Errington et al (1983) noted that damage to the tissue in acute preparations was not great, as determined histologically. However, my chronic cannulas resulted in a great deal of damage as evidenced by Figure 6. These micrographs were taken from brains of animals that showed stable recordings. I speculate that the sequelae of events initiated by cannula implantation upsets normal synaptic plasticity.

Conclusions - Part 1

Acute cannulation preparations may be appropriate for the study of certain aspects of LTP, for example the correlates of LTP. However, the purpose of my thesis was to examine noradrenergic modulation of LTP induction. In my hands, the success rate and amount of LTP elicited in rats with chronic chemitrodes was

insufficient to allow study of induction. Previous literature using this preparation is meagre; the few relevant studies either give insufficient data to allow comparison, or in fact report similar amounts of LTP. Explanation for the lack of reliable LTP with this method are also meagre. I speculate that tissue damage resulting from implantation upsets normal synaptic transmission.

Therefore, I chose to proceed with my original intent of studying noradrenergic modulation of LTP in awake rats. However, I chose to use electrodes and interperitoneal injections instead of chronic chemitrodes and intradentate infusions. Before proceeding to Experiment 2, however, I shall review previous literature examining LTP in awake rats with chronic electrodes to determine how my data for that group compare well with previous literature.

PART 2. LTP IN RATS WITH CHRONIC ELECTRODES

As stated earlier, graphical representation of data from my electrode group indicated that LTP occurred. However, time effect was not statistically significant. As LTP has long been considered a robust, reliable phenomenon (Steward, White, Korol & Levy, 1988) I examined previous literature to determine possible reasons for the apparent lack of statistically significant LTP.

LTP in awake rats (electrodes)

While perusing previous studies using awake rats with chronic electrodes, I quickly became aware of one main problem: most experiments using chronic

electrodes involve saturation of LTP. This means that repeated tetanizing stimulations are applied, usually daily. The endpoint of these experiments often comes only after numerous tetanizing stimulations. In my protocol, on the other hand, only one stimulation was applied. Therefore, the only data reported in these experiments with which I can compare my data are those measured at 60 minutes and 1 day post-tetanization. These comparisons are presented in Table 2. Perusal of this table uncovers one clear finding: the PS amplitude means and SEMs of my data do not differ greatly from those in other published studies.

This finding is somewhat disturbing because it suggests that application of one tetanizing stimulation in awake rats does not result in statistically significant LTP. However, the possibility still remains that some procedural variable accounted for my lack of statistically significant LTP within group 3. Some studies in Table 2 reported smaller SEMs than mine. A reduction in the SEMs might result in statistically significant LTP in my procedure. Therefore, I inspected the previous literature for any information concerning the experimental protocols that might suggest a way to improve my success rate.

The rationale for various procedures used in LTP studies, from tetanization parameters to sampling protocols, is detailed in Appendix A. I found that the main difference was the number of sampling points measured post-tetanization. Most researchers sampled only at 60 minutes and 1 day post-tetanization. Therefore in Experiment 2, I chose to eliminate sampling points immediately, 15, 30, and 45 minutes post-tetanization from my sampling protocol.

Table 2: A comparison of data from a number of studies of induction of long-term potentiation (LTP) in rats with chronic electrodes.

AUTHORS	n	BASELINE	60 MIN	ENDPOINT
Jones	7	110 ± 13%	189 ± 39%	213 ± 52% (1 day)
Douglas, 1977	24	100%[1]		148% (after 4 tets)
Racine, Milgram & Hafner, 1983	12	100% (76-133)		132 (100-305) (3 tets)
Skelton, Miller & Phillips, 1983	6	100%		158 ± 14% (6 days)
Castro, Silbert, McNaughton & Barnes, 1989	8	100%[1]	170 ± 60%	
Jeffery & Morris, 1993	8	100%[1]	220 ± 60% (T30)	316 ± 43% (3 tets)
Korol, Abel, Church, Barnes & McNaughton, 1993	12	100%[1]		140% after (14 tets)
Cain, Hargreaves, Boon & Dennison, 1993	12	100 ± 19%[1]	135 ± 19%	120 ±19% (1 day)

[1] Values from these papers had to be determined from graphs or text within each paper.

CONCLUSIONS

Experiment 1 was designed to test the hypothesis that chronic chemitrodes or infusions may disrupt LTP. The results from this experiment did support this hypothesis: rats with chemitrodes exhibited less LTP and lower success rates of LTP than rats with electrodes. Although these results are not supported statistically, graphical representation of the data clearly indicates differences.

The amount of LTP induced in rats with chronic chemitrodes in this experiment and in other published reports was quite small. This technique was initially intended to study noradrenergic modulation of LTP by β-antagonists. Since the β-antagonists would be expected to decrease the amount of LTP induced, I conclude that the use of chemitrodes is an inappropriate way to answer this question.

More LTP was induced in rats with electrodes than in rats with chemitrodes, yet LTP was still nonsignificant in this group. The means and SEMs, however, compared closely with previous literature. One change in sampling protocol was made in Experiment 2 in an attempt to reduce the variability. It was hoped that this procedural change would result in statistically significant LTP for the control group of Experiment 2. Specifically, fewer I/)'s ere collected post-tetanization.

EXPERIMENT TWO

INTRODUCTION

Experiment 2 examined noradrenergic modulation of LTP, by use of intraperitoneal injections of the nonspecific β-antagonist l-propranolol. Doses were chosen based on results from the previous literature. Harley, Milway, and Lacaille (1989) found that 20 and 30 mg/kg doses of propranolol blocked potentiating effects of LC activation, when the LC was activated by glutamate but not when it was activated by electrical stimulation. Oishi, Watanabe, Ohmori, Shibata, and Ueki (1979) found that 5 mg/kg propranolol reduced the inhibitory effect of LC stimulation on olfactory bulb evoked potentials. Sullivan and Wilson (1991) found that 10, 20, and 40 mg/kg propranolol had no effect on learned olfactory behaviour. Based on this information, four groups were included in Experiment 2: a control, receiving saline injection, and 3 drug groups receiving doses of 10, 20 and 40 mg/kg of propranolol.

A second objective of Experiment 2 was to reduce the variability of the evoked potentials, in hopes that the saline group would show statistically significant LTP. One change was made in the experimental procedure to decrease the variability, the change was to reduce the number of I/Os measured post-tetanization from 7 to 3.

METHODS

One LTP session comprised two baseline I/Os, followed by a 1 ml/kg

injection of either 0.9 percent saline, or 10, 20, or 40 mg/kg l-propranolol. A third I/O was measured 15 minutes after the injection, and tetanization followed 30 minutes after injection. The animals were left in the testing apparatus until the last I/O was taken 60 minutes post-tetanization. Two more post-tetanization I/Os were measured 1 day and 1 week later.

Data were analyzed using repeated measures of responses taken at baseline, 60 minutes, and 1 day post-tetanization. Data collected 1 week post-tetanization were not included in statistical analysis because of a high percentage of attrition in the control group. Four groups were analyzed; the drug groups were not pooled.

RESULTS

All 22 animals had characteristic PP-DG evoked potentials; maximum PS threshold was 500 μA and the mean PS threshold was 156 μA. Final group sizes were: group 1 (control), n=7; group 2 (10 mg/kg), n=4; group 3 (20 mg/kg), n=5; and group 4 (40 m/kg), n=5.

Time effect

As in Experiment 1, in order to show that LTP had occurred, the PS amplitudes at baseline were compared to those measured at 60 minutes and 1 day. The time effect was significant for all groups combined [$F(2,36)=3.378$, $p=0.032$]. Specific contrasts within the time effect, however, showed that the differences between baseline vs 60 minutes [$F(1,21)=0.054$, $p=0.819$] or baseline

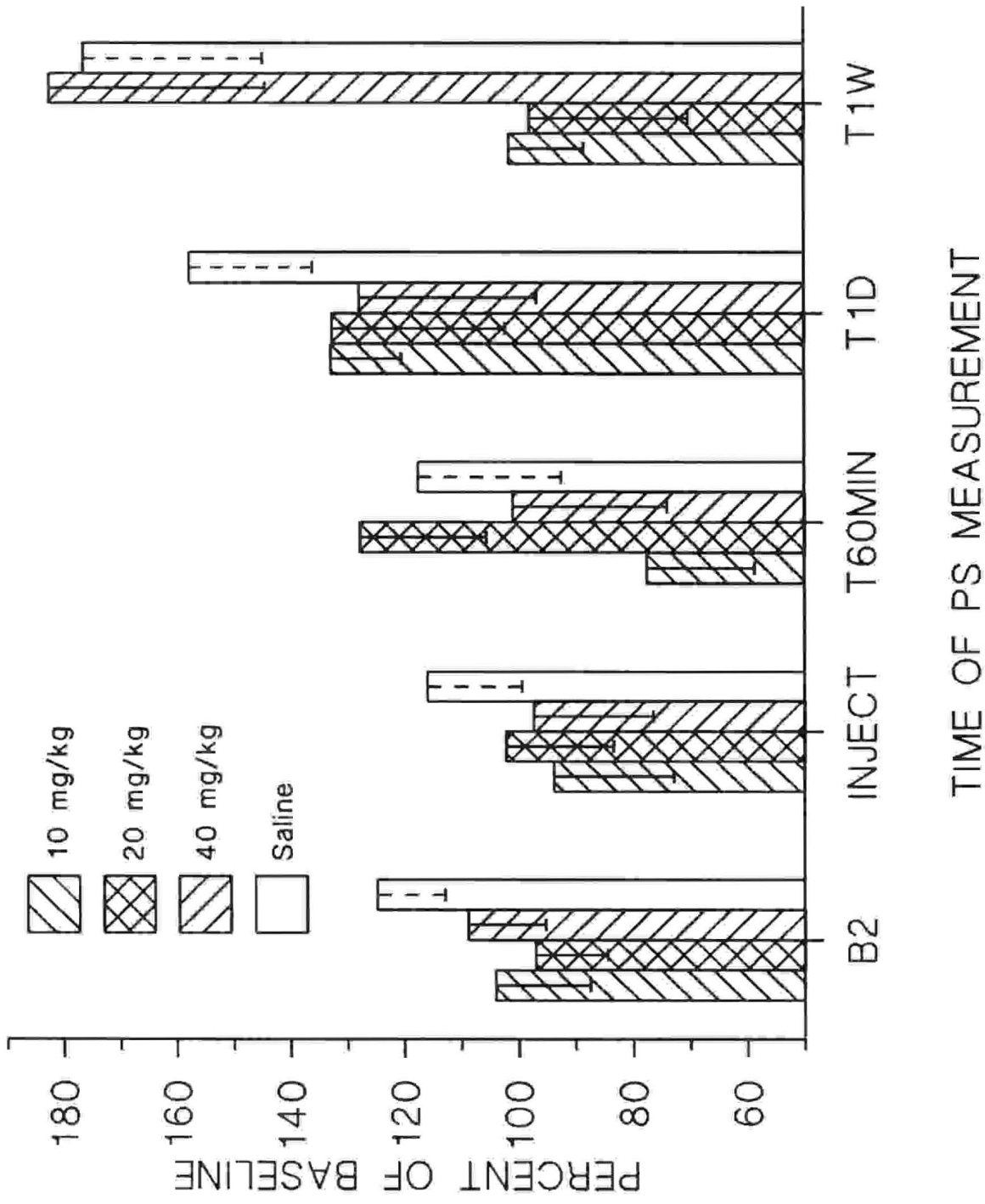
vs 1 day [$F(1,21)=4.893$, $p=0.038$] were not statistically significant; the α level is 0.025. Statistical analysis indicated that no LTP occurred when the data of all groups were combined. Figure 7 agrees with the statistics: no potentiation is apparent in any group 60 minutes post-tetanization. At 1 day, only the saline group showed LTP. At 1 week, the saline and 40 mg/kg groups showed LTP. In order to determine the reason why time effect was significant, one additional contrast between potentiation at 60 minutes and 1 day post-tetanization was analyzed. This contrast was significant [$F(1,21)=7.391$, $p=0.013$].

The saline group appeared to exhibit the largest amount of LTP overall; therefore, this was the only group in which time effects were analyzed. The time effect was significant, but specific comparisons between measures at baseline and post-tetanization indicated that LTP was not successfully induced. Analysis within this group indicated that there was a statistically significant time effect [$F(2,5)=7.904$, $p=0.028$], yet no significance was found for the contrasts of baseline vs 60 minutes [$F(1,6)=2.55$, $p=0.161$] or baseline vs 1 day [$F(1,6)=0.057$, $p=0.819$]. This means that LTP did not occur in any of the 4 groups.

Group differences

As in Experiment 1, the groups were not found to be different and no interaction between the groups and the time of measurements was found. The group effect was nonsignificant [$F(3,18)=0.733$, $p=0.546$], as was the group by

Figure 7. Population spike (PS) amplitude means (± SEM) plotted at baseline, immediately after drug injection, and at 3 times post-tetanization for saline (n=7) and drug groups in Experiment 2. L-propranolol was injected at doses of 10 (n=4), 20 (n=5), and 40 (n=5) mg/kg. PS ampitudes are plotted as percentages of the first baseline measures. B2, DRUG, T60MIN, T1D and T1W represent second baseline, post-injection, 60 minutes post-tetanization, 1 day post-tetanization, and 1 week post-tetanization.


10 mg/kg
20 mg/kg
40 mg/kg
Saline
180
160
140
120
100
80
60
PERCENT OF BASELINE
B2
INJECT
T60MIN
T1D
T1W
TIME OF PS MEASUREMENT

time interaction [$F(6,36)=0.459$, $p=0.833$]. Graphically, group differences arenot obvious (see Figure 7). I might speculate that propranolol reduced the amount of LTP 1 day and 1 week post-tetanization when compared to control values. However, the propranolol groups also appear to have smaller baseline PS amplitudes, which minimizes these differences.

Variability

The second objective of Experiment 2 was to reduce the SEM within the control group. Table 3 compares the results of the electrode group from Experiment 1 with the saline group from Experiment 2. This table indicates that the SEM was reduced. Unfortunately, this decrease in SEM did not result in a statistically significant LTP effect within the saline group. The reason for this lack of statistical significance is apparent when the means of these two groups are compared (see Table 3). The saline group in this experiment exhibited much less potentiation than the electrode group in Experiment 1. The success rates of these two groups follow the same trend: 58 percent for the electrode group in Experiment 1 and 12.5 percent for the saline group in this experiment.

Histology

All DG electrodes were located in the granule cell layer of the DG. All PP electrodes were located within the area of projections from the EC to the DG molecular layer.

TABLE 3. A comparison of data, including population spike (PS) amplitude means (± SEM), between group 3 of Experiment 1 and the saline group of Experiment 2.

	EXP 1 - GROUP 3	EXP 2 - SALINE
n	7	7
Baseline	110 ± 13.8%	124 ± 11.9%
60 min	189 ± 39.6%	117 ± 25.1%
1 day	213 ± 52.4%	157 ± 21.6%
1 week	242 ± 54.4%	176 ± 31.6%

DISCUSSION

The two objectives of Experiment 2 were to examine drug effects on LTP induction and to minimize variability. With respect to the first objective, comparison of data from the drug groups vs the saline group was inconclusive. I might speculate that propranolol reduced LTP 60 minutes and 1 day post-tetanization. However, no statistical support exists for this speculation and graphical support is also meager.

The variability of the saline group, when compared to that for the electrode group in Experiment 1, is in fact smaller (see Table 3). Unfortunately, this decrease in variability did not result in a statistically significant LTP within the saline group. This is because the means of this group were much smaller than the electrode group in Experiment 1. Treatment of the saline group differed from the electrode group from Experiment 1 in only two ways: saline was injected and fewer I/Os were collected post-tetanization. The possibility exists that the saline injection stressed the animal. This may have initiated some chain of physiological events resulting in decreased PS amplitude one hour later. This possibility cannot be ruled out; some animals did show some signs of stress during the injection, although these behaviours ended abruptly with the end of the injection. Alternatively, the continual I/O sampling in Experiment 1 may have induced LTP. This seems unlikely since Skelton et al. (1983) found that test pulses could be applied at a frequency of 0.04 Hz without causing potentiation; the frequency used in Experiment 1 was 0.03 Hz.

To investigate this lack of reliability of LTP, I performed one additional experiment. The only other factor alluded to in the literature that could potentially affect LTP or the variability of evoked potentials is the behavioural state of the animal. Experiment 3 examines possible effects of behavioural state on the variability of evoked potentials.

EXPERIMENT THREE

INTRODUCTION

Some evidence suggests that behavioural state may affect the amplitude of DG-PP evoked potentials. In particular, differences in evoked potentials have been noted when the animal is immobile compared to when it is walking. Immobilility is defined as the state of being awake, sitting quietly with the head held up against gravity. Walking usually refers to forced walking, as on a treadmill. Racine and Milgram (1983) reported that evoked potentials in the PP were smaller when the animal is immobile than when walking. Green, Barnes, and McNaughton (1993) found no difference between evoked potentials measured when the rats were either on or off a treadmill. Hargreaves, Cain, and Vanderwolf (1993) compared evoked potentials measured during 3 behavioural states: immobile, wheel running, and freely behaving. They found that evoked potentials measured while the animal was immobile were significantly larger than when the animal was wheel running. They reported no statistical comparison of immobile vs freely behaving states; the graphs of the results suggested there were no differences.

Results from Experiment 2 indicated that LTP may not be a particularly reliable phenomenon in awake rats with application of one tetanic stimulation. The purpose of Experiment 3 was to examine whether behavioural state may be a factor affecting either the reliability or the variability of LTP. Two groups were compared: freely behaving vs immobile rats. Data for the freely behaving group were those from the saline group in Experiment 2.

METHODS

Data for the freely behaving group were taken from the control group of Experiment 2. The immobile group was treated the same as the freely behaving group except it was given at least three hours of habituation time in the testing chamber over at least two pretreatment sessions. Saline was injected in all rats, at a dose of 1 ml/kg. The immobile group was treated similarly to the freely behaving group except that evoked potentials were collected only when the rat was immobile. In general, rats sat quietly; if they did move, no test pulses were applied. In general all rats sat quietly while the tetanic stimulation was applied.

RESULTS

All 13 rats had characteristic DG-PP evoked potentials. The largest PS threshold and the mean threshold were 500 μA and 171 μA, respectively. Final group sizes were: group 1 (freely behaving), n=6; group 2 (immobile), n=7. In general, the freely behaving group spent equal amounts of time either sitting quietly or walking slowly around the testing apparatus. The immobile group spent approximately 75 percent of the session sitting quietly and 25 percent of the time walking around.

Time effect

PS amplitude varied significantly with time, yet no LTP occured. The time effect was significant when both groups were combined [$F(2,11)=12.748$,

p=0.001]. However, neither the specific contrast at baseline vs 60 minutes or baseline vs 1 day were significant: [F(1,12)=0.23, p=0.640] and [F(1,12)=2.409, p=0.147], respectively. The specific contrast between 60 minutes and 1 day post-tetanization was significant [F(1,12)=19.299, p=0.001]; this may account for the significant time effect. Statistically significant LTP did not occur when the data from either group were analyzed independently. The time effect was significant for group 1 but not for group 2 and none of the specific contrasts were significant.

Group differences

The behavioural state of the animal was unrelated to either the amount of LTP or the variability. That is, the group effect was nonsignificant [F(1,11)=0.054, p=0.821]. No group differences were evident graphically (see Figure 8).

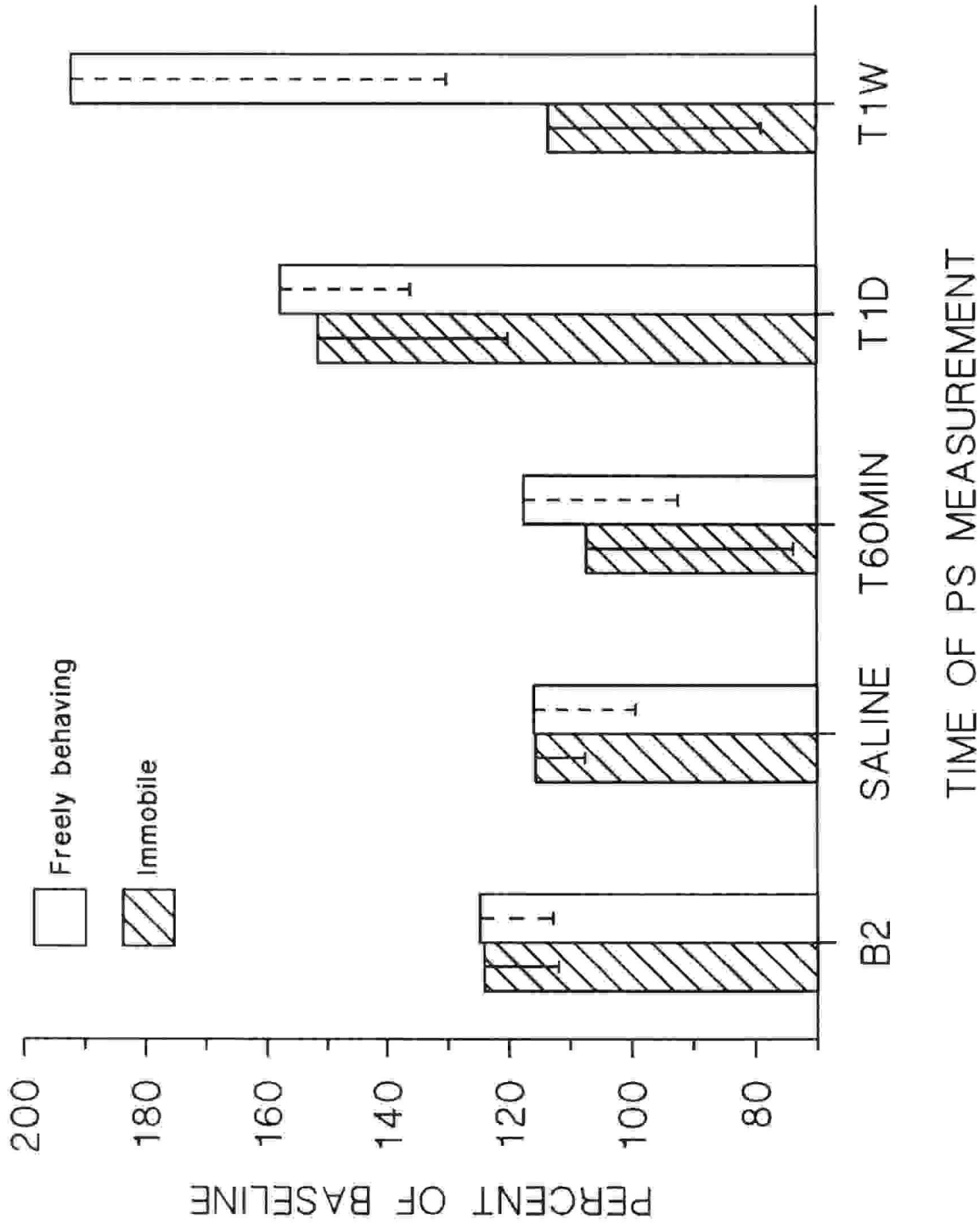
Histology

All DG electrodes were located in the granule cell layer of the DG. All PP electrodes were located in the area of the entorhinal cortex.

DISCUSSION

The purpose of Experiment 3 was to examine the effect of behavioural stateon the amount of LTP and on the variability of the evoked potentials. Behavioural state was found to be unrelated to either variable.

Figure 8. Population spike (PS) amplitude means (± SEM) plotted at baseline, immediately after saline injection and 3 times post-tetanization for immobile (n=6) and freely behaving (n=7) groups. PS amplitudes are expressed as a percentage of the first baseline measure. B2, SALINE, T60MIN, T1D and T1W represent the second baseline, post-injection, 60 minutes post-tetanization, 1 day post-tetanization, and 1 week post-tetanization.


Freely behaving
Immobile
200
180
160
140
120
100
80
PERCENT OF BASELINE
B2
SALINE
T60MIN
T1D
T1W
TIME OF PS MEASUREMENT

The lack of difference in variability between the freely behaving and immobile rats agrees with previous studies. Two groups of authors reported differences between evoked potentials measured when the animals were immobile vs engaged in forced walking (Racine et al, 1983; Hargreaves et al, 1990). However, the results of the 2 studies were opposite in direction, and neither reported differences between evoked potentials between the immobile and the freely behaving condition.

The amount of LTP exhibited by the 2 groups of rats was not different. This lack of LTP in Experiments 2 and 3 is surprising compared to the relatively large potentiation within group 3 of Experiment 1. As in Experiment 2, one reason for this lack of LTP, particularly at 60 min, may be that the injection stressed the animal. It is possible that the injection itself causes some physiological reaction which somehow disrupts the ability of the synapses to react to the tetanizing stimulation. Another possibility is that LTP is not a reliable phenomenon in the awake, behaving rat when only one tetanizing stimulation is given. These possibilities are discussed in the General Discussion.

GENERAL DISCUSSION

The initial intent of this thesis was to study the effect of a β-adrenergic antagonist on induction of LTP. The subject of the actual thesis, however, could be described as a parametric study of factors affecting induction of LTP in the awake rat.

Experiment 1

In Experiment 1 I observed that rats with chronic chemitrodes exhibited a reduced amount of LTP when compared to rats with chronic electrodes. My data from the chemitrode group compare favourably with data from a published report of LTP induction in awake rats with chemitrodes (Cain et al, 1992). However, this thesis was intended to study the effects of β-adrenergic antagonists on induction of LTP. In order to do this, a significant amount of LTP must be observed in the control group. Therefore, I examined possible reasons for the reduced success rate of LTP induction in rats with chemitrodes.

One reason for the reduced success of LTP induction may be that tissue damage occurs with chemitrode implantation. Biochemical data from Errington's laboratory support this hypothesis. Histological observation of hippocampi from rats with chronic chemitrodes give visual evidence of damage to the tissue (see Figure 6). I speculate that this tissue damage disrupts normal synaptic plasticity. Discussion of the sequelae of events surrounding the implantation and subsequent

deterioration of the tissue is beyond the scope of this thesis. However, I cannot pass up the invitation to speculate. One scenario would involve proliferation of astrocytes in the damaged area that then disrupted glutamate transmission.

Therefore, 3 factors encouraged me to discontinue use of chemitrodes to study LTP: my low rate of success in inducing LTP; a review of the scanty literature using chemitrodes; and histological observation of tissue damage in hippocampi of rats with chemitrodes.

However, results from Experiment 1 also indicated that LTP was not induced significantly in rats with electrodes (although graphical presentation indicates a large amount of LTP). Again, in order to study drug effects on LTP induction, I wanted to induce statistically significant LTP in my control group. Therefore, I compared my data from the electrode group to published data of LTP in awake rats. The means and SEMs from my data are comparable to those in other reports (see Table 2). Therefore, I attempted to reduce the SEM in my next experiment in hopes of increasing statistical power. In expectation of a positive result, I included 3 drug groups in Experiment 2.

Experiment 2

The results of Experiment 2 were not those predicted. The most surprising finding of Experiment 2 was that the saline control group exhibited less LTP than the electrode group of Experiment 1. The only differences between these groups were that saline was injected and fewer test pulses were applied. The latter

difference suggests that perhaps the electrode group in Experiment 1 was potentiated as a result of continual test pulse application during the first hour post-tetanization. This seems unlikely since the frequency of test pulses was 0.03 Hz and Skelton et al (1983) found no potentiation from test pulse application at a frequency of 0.04 Hz.

Another possible reason for the lack of LTP is that the saline injection interfered with LTP. I speculate that the injection procedure, rather than the saline itself, stressed the rat, and initiated some physiological process that interfered with synaptic plasticity. This is a most tentative speculation. All rats were handled on a daily basis and showed relatively little reaction to the injection except for what might be described as momentary discomfort. The test of this hypothesis would be to repeat the experiment without the injection. Obviously this would obviate the possibility of studying drug effects on induction of LTP.

A second finding of Experiment 2 was that the SEMs were reduced. This may have occurred due to the change in experimental procedure in which several I/Os were eliminated. However, the decrease in SEMs may be an artifact of the decrease in mean values. Since none of the rats exhibited large amounts of LTP, a smaller SEM would be expected. In the group from Experiment 1, on the other hand, some of the rats exhibited large amounts of LTP while others exhibited none. Regardless, reduced SEMs can have little effect on statistical significance when the means are so low.

Finally, no effects of propranolol were observed. Graphical presentation

suggests that a small reduction may have occurred at 1 day post-tetanization. The expected effect of propranolol would have been a decrease in the amount of LTP. I chose not to pool data from the 3 drug groups because of the possibility of a non-linear dose-response curve. That is, the medium and high doses of propranolol both seemed to be associated with rates of LTP greater than the saline group at 60 minutes and 1 week post-tetanization, respectively. Therefore I could not assume that the drug groups were similar.

Experiment 2 indicated that I could not reliably obtain LTP in awake rats. Experiment 3 was designed to test another parameter that may have an effect on either LTP induction or on the variability of the evoked potentials, the behavioural state.

Experiment 3

Behavioural state was not found to be a factor affecting either the success rate of LTP or the variability of the evoked potentials. Data were very similar for groups of rats in either the immobile or freely behaving condition. Graphical representation indicates that some LTP occurred at 1 day post-tetanization in both groups, but no LTP was obvious 60 minutes post-tetanization. As in Experiment 2, the factor affecting the amount of LTP at 60 minutes may be the injection. Some other factors responsible for the lack of LTP are discussed in the following section.

Factors affecting success rate of LTP

The general conclusion from the 3 experiments is that induction of LTP with only one tetanizing stimulation in awake rats may not be a reliable phenomenon. Reasons for this conclusion come from the data collected here and from published data in awake rats. I examined some of the possibilities for my apparent lack of success in inducing LTP consistently.

I begin this discussion with a caveat that the I/O at 1 week is not a particularly strong indicator of success of LTP induction. The half-life of LTP of the PS is 6.6 days, as determined from decay curves (Racine, Milgram & Hafner, 1983). Therefore, it would be expected that substantial decay in LTP would have occurred in some rats by 1 week post-tetanization. This could also account for the larger variability seen in all groups 1 week post-tetanization. Data collected at 1 week were thus not included in statistical analyses in Experiments 2 and 3.

One possible explanation for my reduced success rate in Experiments 2 and 3 may be the diameter of the electrode I used as compared to those used in other laboratories. To determine whether my electrodes were larger than those used elsewhere, I examined all the publications included in Tables 1 and 2. Of those reporting electrode diameter, all used electrodes of a similar diameter, with one exception. Another reason to rule out this factor is that the electrode group in Experiment 1 exhibited large amounts of LTP with electrodes identical to those used in Experiments 2 and 3. Thus the size of the electrode cannot explain my low rate of induction of LTP in Experiments 2 and 3.

Circadian rhythm was shown by West and Deadwyler (1980) to result in variation in PS amplitudes. This could not be a factor in my experiments as the evoked potentials at 1 day and 1 week post-tetanization were collected at similar times of the day.

Conclusions

Across the 3 experiments, the amount of LTP obtained from rats with electrodes was not reliable. The decrease in success rate in Experiments 2 and 3 may have been due to the stressful effects of the injection of saline, to inadvertent induction of LTP in Experiment 1 by more frequent sampling of evoked potentials, or to a chance result in Experiment 1.

The results of my experiments and an examination of these results in light of published research suggest that LTP induction in awake rats with **one** tetanizing stimulation is not a statistically reliable phenomenon. This suggests that drug effects on induction of LTP cannot be studied using this procedure. One test to determine the role of the injection procedure in disruption of LTP would be to repeat Experiment 2, eliminating the injection procedure. Another approach would be to assess drug effects on daily tetanizing stimulations, so that LTP is saturated.

Sara (1985) suggests that: "...application of NA to target structures...will probably prove to be the best method with which to test the hypothesis that the observed NA modulation of signal/noise in the forebrain is related to...learning and memory." In conclusion, I hope this will soon be possible.

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APPENDIX A: RATIONALE FOR VARIOUS TETANIZATION AND I/O SAMPLING PROTOCOLS

Pattern of tetanizing pulses

Douglas (1977) initiated the use of tetanizing stimulation parameters that mimic normal discharge characteristics of hippocampal cells in freely behaving rat. The pattern was developed from recordings of spontaneous hippocampal neuronal discharges by Ranck (1973). Specifically, Ranck (1973) recorded spontaneous bursts of 2-7 action potentials with 1.5-6 msec interpulse intervals; these bursts appeared at a rate not less than 2/sec. Douglas translated this natural pattern of activity into the following stimulation pattern: 10 trains of 8 pulses with an interpulse interval of 2.4 msec, each train separated by 10 seconds. Douglas observed potentiation in 21 of 24 rats tested, although no statistical tests are reported. Potentiation occurred across a range of number of trains, from 8 to 20, and across a range of pulses, from 4 to 10. Small and unreliable potentiation was observed with single burst, high frequency stimulation. Douglas' stimulation pattern has been used in numerous studies using awake rats.

Tetanization frequency

Skelton, Miller, and Phillips (1983) added to Douglas's study (1977) of stimulation parameters by determining the lowest frequency stimulation at which potentiation is observed. Rats were stimulated with test pulses at frequencies of

0.2, 0.1, and 0.04 Hz. LTP was observed at all but the lowest frequency. They concluded that studies of hippocampal plasticity in vivo should be concerned with frequency of test pulses and should not apply test pulses more frequently than 1/25 seconds.

Sampling intensities and I/O's

Racine, Milgram, and Hafner (1983) sampled with 20 test pulses at an intensity that produced responses at 80 percent of maximum. Samples were taken at 15, 30, 60 minutes, 4, 8, and 24 hours post-tetanization. Cain, Hargreaves, Boon, and Dennison (1993) sampled across three intensities: those eliciting threshold, medium, and maximum PS amplitude; 10 test pulses were applied at each intensity. Skelton, Miller, and Phillips (1983) sampled using 3 test pulses at 10 intensities. Jeffrey and Morris (1993) applied 10 test pulses at an intensity that gave 1-3 mV PS amplitude. I sampled 5 evoked potentials at 5 intensities: maximum, and 80 percent, 60 percent, 40 percent, and 20 percent of maximum PS ampitude.

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