CHANGES IN PERFORANT PATH-EVOKED POTENTIALS IN THE DENTATE GYRUS IN THE RAT DURING TRAINING IN THE MORRIS WATER TASK AND EXPERIENCE IN COMPLEX ENVIRONMENTS.

by

Janice M. Hoesing
B.Sc., University of Lethbridge, 1987

A Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Psychology

ACCEPTED
CULTY OF GRADUATE STUDIES
DEAN
TE 1991-05-13

We accept this thesis as conforming
to the required standard

Dr. R. W. Skelton, Supervisor (Department of Psychology)

Dr. M. E. Corcoran, Departmental Member (Department of Psychology)

Dr. G. H. Van Gyn, Outside Member (School of Physical Education)

Dr. D. H. Paul, External Examiner (Department of Biology)

© JANICE M. HOESING, 1991

University of Victoria

All rights reserved. Thesis may not be reproduced in whole or in part, by photocopy or other means, without the permission of the author.

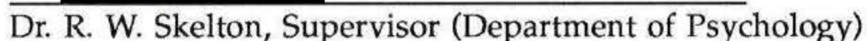
BF1169
H63

Supervisor: Dr. R. W. Skelton.

Abstract

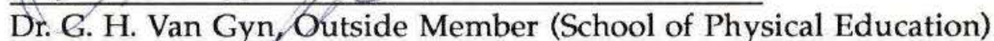
The hypothesis that long-term changes in synaptic patterns in the hippocampal formation represent the neural basis of spatial learning was examined. Perforant path evoked potentials in the dentate gyrus of rats were examined during spatial learning in the Morris water task. A small reliable increase in the population EPSP was observed, but no change occurred in the size of the population spike. In a second experiment, a robust, long-lasting increase in the size of the population spike and a less reliable increase in the population EPSP was observed when rats were housed in a novel complex environment. A third experiment demonstrated that the changes in evoked potentials are not dependent upon changes in the behaviour of the animal at the time of delivery of test pulses. These results provide limited support for the notion that LTP-like changes underlie spatial learning in the rat.

Examiners:

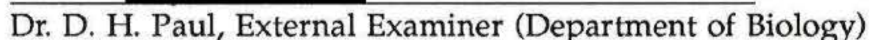


Dr. R. W. Skelton, Supervisor (Department of Psychology)

Dr. M. E. Corcoran, Departmental Member (Department of Psychology)



Dr. G. H. Van Gyn, Outside Member (School of Physical Education)



Dr. D. H. Paul, External Examiner (Department of Biology)

TABLE OF CONTENTS

ABSTRACT	ii
TABLE OF CONTENTS	iii
LIST OF FIGURES	v
ACKNOWLEDGEMENT & DEDICATION	vi
INTRODUCTION	1
GENERAL METHODS	11
Animals	11
Recording materials and apparatus	11
Surgery	12
Recording chamber	13
Training apparatus	13
Recording procedures	13
EXPERIMENT 1	15
Method	15
Behavioural procedures	15
Histological procedures	16
Results	17
Histological results	17
Behavioural results	20
Electrophysiological results	22
Discussion	28
EXPERIMENT 2	31
Method	31
Apparatus	31
Behavioural procedures	32
Results	32

Discussion 35

EXPERIMENT 3

Method 37

Results 38

Discussion 41

GENERAL DISCUSSION 42

REFERENCES 48

APPENDIX A 53

APPENDIX B 54

LIST OF FIGURES

Figure 1. Diagram of the hippocampal formation and representative evoked potential. 3

Figure 2. Electrode placements. 18

Figure 3. Performance in the Morris water task. 21

Figure 4. Effect of training on population spike. 24

Figure 5. Effect of training on population EPSP. 26

Figure 6. Effect of exposure to a complex environment on population spike and EPSP. 33

Figure 7. Effect of exposure to a complex environment on population spike and EPSP during movement vs. immobility. 39

ACKNOWLEDGEMENT & DEDICATION

I wish to acknowledge the contributions of Robert James Sutherland to this work and to dedicate the thesis to him.

INTRODUCTION

This thesis addresses a basic issue concerning our understanding of the neural basis of learning and memory. The issue is whether there is a relationship between spatial learning and long-term enhancement of hippocampal synaptic transmission.

Research using human and rodent subjects has shown that the ability to learn and remember the spatial layout of an environment and to navigate to locations in the environment depends upon hippocampal circuitry (Jarrard, 1986; Morris, Garrud, Rawlins, & O'Keefe, 1982; O'Keefe, Nadel, Keightly, & Kill, 1975; O'Keefe & Nadel, 1978; Smith & Milner, 1981; Sutherland, Whishaw, & Kolb, 1982; Sutherland & Rudy, 1989). These experiments used a variety of tasks that have different sensory-motor requirements (e.g., swimming or walking), different motivational stimuli (rewarding and aversive stimuli), and different apparatus (e.g., runway and open field). Perhaps the most important common element conceptually linking all of these experiments is that the animal must learn the topographical relationships among objects in the environment. The predominant conclusion is that animals with damage to the hippocampal formation exhibit an impaired ability to navigate to places or orient to locations using the spatial relationships among the objects in the environment.

One hypothesis on the relationship between hippocampal circuitry and spatial abilities is that during initial exposure to an environment the topographical relationships among the objects in the environment produce a long-lasting pattern of adjustments of synaptic strengths of connections within one or more subfields of the hippocampal formation and the pattern

of strengthened synapses are thereafter more readily activated (eg. McNaughton & Morris, 1987). It is suggested that this may be the method by which the animal learns and remembers the spatial layout of the environment.

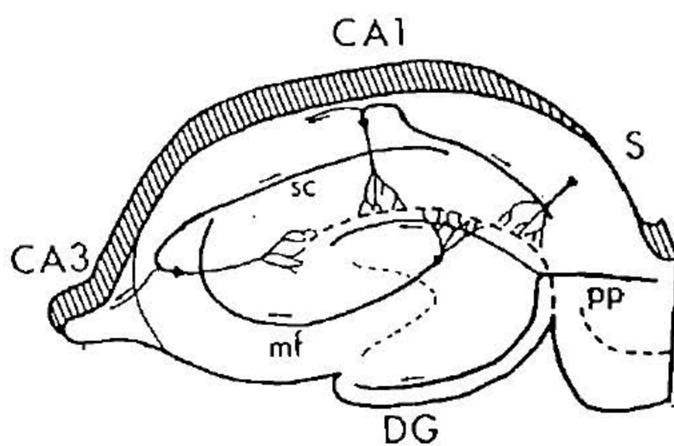
The hippocampal formation has a well defined laminar structure with essentially three serially connected subfields (Fig. 1A). Axons of entorhinal cortex neurons project primarily to the dendrites of the granule cells of the dentate gyrus (DG) via the perforant path (PP). Axons of the DG granule cells project to the dendrites of the pyramidal cells of the CA3 field via the mossy fibre system. Finally, the axons of the CA3 pyramidal cells project to dendrites of pyramidal cells of the CA1 field via the Schaffer collateral system (Amaral & Witter, 1989). The first subfield mentioned above, the DG, is easy to locate and record from, and its entorhinal afferents in the PP are easy to locate and stimulate.

The anatomical simplicity of the hippocampal formation and the important links with spatial learning, make the hippocampal formation well suited to the study of neural processes underlying learning and memory. What makes it an especially intriguing preparation is the recent discoveries concerning the properties of long-term potentiation (LTP) in the hippocampal formation.

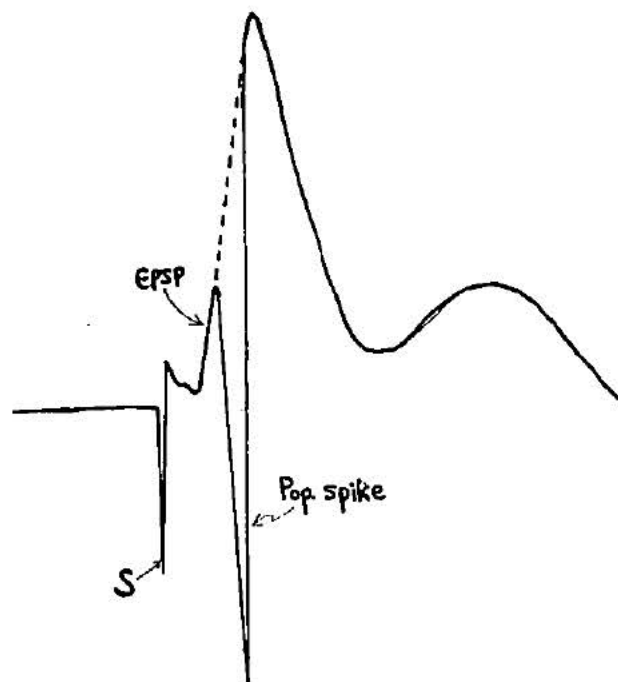
LTP is a phenomenon in which the strength of synapses is increased by applying high frequency stimulation to a sufficient number of presynaptic axons. In one common experimental arrangement a stimulating electrode is implanted into the PP and a recording electrode is implanted into the hilus of the DG. Stimulus pulses are delivered to the PP and the evoked field

Figure 1. **A**. Diagram of the hippocampal formation showing principal subfields and components of the trisynaptic circuit. Abbreviations: DG = dentate gyrus; pp = perforant path; mf = mossy fibre; sc = Schaffer collateral; S = subiculum (taken from Amaral, 1989). **B**. Example of perforant path-dentate evoked potential showing stimulation artifact (S), the population EPSP showing the proportion of the rising phase included in the slope calculation, and the population spike (Pop. spike) showing the tangent (dotted line) to the two positive peaks used in the measurement of the population spike area.

A.



B.



potential generated by the granule cells of the DG is recorded. The recorded waveform represents two distinct physiological events. The stimulus pulse excites a number of axons of the PP, which then fire, sending a synchronous volley of actions potentials to the DG. The PP terminals then release glutamate, causing an inward flow of current into the dendrites of the granule cells (the excitatory postsynaptic potential or EPSP). The second event, resulting from sufficient excitatory stimulation, is action potentials (the population spike) generated in the axon hillocks of the granule cells. The recorded waveform represents the summed, extracellular current of a population of DG granule cells. The smooth, gradual positive going, bell-shaped curve represents the sum of the EPSPs (and is called the population EPSP). The brief, deep, negative going trough represents the sum of the action potentials (called the population spike) and is superimposed near the middle of the EPSP (Fig. 1B). The width and depth of the trough is proportional to the number of action potentials that are firing (Lømo, 1971; Andersen, Bliss, & Skrede, 1971). The population EPSP and population spike can be measured systematically and are reliable representations of the strength of the PP synapses on cells in the vicinity of the recording electrode (Lømo, 1971; Andersen et al., 1971). High frequency stimulation of the PP produces a long term enhancement of transmission in the PP/DG which is characterised by: 1. an increase in the magnitude of the population EPSP, 2. an increase in the magnitude of the population spike, and 3. a reduction in the latency of the population spike (Bliss & Lømo, 1973). These changes can be very long-lasting -- up to three months in freely moving rats (Douglas & Goddard, 1975).

There are two approaches that have been used to investigate the

possibility that the processes that underlie learning and memory are the same processes that underlie LTP. These are: 1) the blockade/facilitation approach, and 2) the experiential modulation approach. The first approach is based on the idea that preventing LTP from occurring in the hippocampal formation should prevent certain kinds of learning from taking place. LTP has been prevented in two ways. One is the administration of a drug that blocks the induction of LTP by blocking N-methyl-D-aspartate (NMDA) receptors (Collingridge 1985). Morris, Anderson, Lynch & Baudry (1986) injected D,L-2-amino-5-phosphonovaleric acid (AP5, a competitive antagonist at NMDA receptors) into the lateral ventricles of rats and then tested their spatial learning ability in the Morris water task. In this task rats are required to swim to a small platform that is hidden just under the surface of the water in a large swimming poohe hidden platform. They also showed that AP5 treatment blocked LTP in the DG. Morris et al. (1986) concluded that AP5 impaired spatial learning by preventing hippocampal LTP.

A second way of preventing LTP from occurring during lel. Normal rats readily learn to use the spatial information about the layout of the room to locate the escape platform (Morris, 1981). Morris et al. (1986) found that AP5 impaired the rats' ability to learn to navigate directly to ttrning is by artificially inducing synaptic enhancement to its asymptotic level (LTP saturation) before behavioural training. The idea is that if synapses are enhanced to their maximum strength then no further enhancement can take place. If enhancement is required for learning, then no learning will occur. Using this method it has been shown that rats receiving bilateral LTP saturation of the PP/DG connections were unable to learn to find the hidden goal in the Morris

water task (Castro, Silbert, McNaughton & Barnes, 1989) or in the Barnes circular maze (McNaughton, Barnes, Rao, Baldwin & Rasmussen, 1986). In contrast, Berger (1984) found that induction of bilateral LTP in the PP/DG synapses enhanced rather than impaired performance in rabbits being trained on discriminative nictitating membrane response conditioning.

A second approach that has been used to discover whether the same processes underlie both learning and LTP is the experiential modulation method. The idea is to determine if long-lasting changes in synapse strength are correlated with learning. Barnes (1985) compared the time course of decay of LTP with the rate of learning and forgetting in the Barnes maze in middle-aged and old rats. After rats had learned to solve the Barnes task, LTP was induced by electrically stimulating the PP. There were two significant findings. The rats that showed the worst performance on the task showed the fastest decaying LTP, and the old rats showed faster decaying LTP than the younger rats. In another experiment Green & Greenough (1986) allowed rats to live in complex environments for several weeks. The rats were then sacrificed and slices of the hippocampal formation were investigated electrophysiologically. Rats that were exposed to the complex environments showed enhanced PP/DG evoked population spikes and EPSPs compared to rats living in standard wire-mesh homecages.

Skelton, Scarth, Wilkie, Miller & Phillips (1987) found reliable increases in PP/DG evoked population spike in rats trained to press a food hopper for food in response to an auditory stimulus. Similar increases were not found in a control group that was allowed free feeding in the same apparatus. Similarly, Weisz, Clark & Thompson (1984) found that classical conditioning

of the nictitating membrane response increased the amplitude of the PP/DG evoked population spike.

Sharp (1986) and Sharp, McNaughton & Barnes (1985) found increases in the PP/DG evoked potential recorded in chronically-implanted rats after exposure to a novel, complex environment. The amplitude of the population spike increased substantially over the first few days after the rats were introduced into the complex environment.

The results obtained from both of the approaches are consistent with the idea that the physiological processes that underlie LTP are the same as or very similar to the processes that underlie learning and memory. Preventing LTP from occurring, either pharmacologically or electrophysiologically, impaired learning in task requiring the hippocampal formation (Morris, Anderson, Lynch & Baudry, 1986; Castro, Silbert, McNaughton, & Barnes, 1990; McNaughton, Barnes, Rao, Baldwin, & Rasmussen, 1986), and slower LTP decay is associated with superior retention of spatial learning (McNaughton and Barnes, 1985). Additional, perhaps stronger, support comes from results of the experiments that have found naturally occurring increases in PP/DG synapses during learning (Skelton et al., 1987; Weisz, Clark, Thompson, 1984; Sharp et al., 1985; Sharp, Barnes & McNaughton, 1989). The experiments that employed tasks that do not require an intact hippocampal formation (e.g., Skelton et al., 1987; Weisz et al., 1984) are difficult to interpret since we are not sure how the tasks might interact with the PP/DG synapses. The novel, complex environment paradigm of Sharp (1986) provides the strongest support to date. However, it is difficult to discern which aspects of the complex environment cause the increases in PP/DG evoked potentials. Some

of these may not have anything to do with learning (eg., stress of moving to a new environment).

The literature is lacking an experiment that combines the following important elements: 1. measurement of changes in PP/DG evoked potentials during learning, 2. in a task which lends itself readily to experimental control and quantification, and 3. which requires an intact PP/DG system.

There were three objectives of the present experiments. The first was to determine if the magnitude of PP/DG evoked potentials increases with learning in the Morris water task. Experiments by others have shown that normal acquisition of this task is dependent on an intact entorhinal cortex (Schenck & Morris, 1985) and DG (Sutherland et al., 1982). If long lasting synaptic changes in the PP/DG underlie the acquisition of information, then it is obviously more likely that such changes would be detected as a result of training in a task for which the PP/DG connections are essential than in a task for which the PP/DG connections are not essential. The second was to determine if alterations in PP/DG evoked potentials occur during exploration of a complex, novel environment and to determine the time course of any changes. The complex environment treatment has been shown by others to have an important impact on PP/DG transmission (Green & Greenough, 1986; Sharp, 1986; Sharp et al., 1985) and may have larger effects, either because the exposure is continuous (whereas Morris water task training takes place in discrete sessions), or because the rats are learning a greater variety of new information (or both). The third was determine whether the PP/DG changes are dependend upon changes in behaviour of the rat at the time of recording. Hargreaves, Cain, & Vanderwolf (1990) have shown that the size of

the PP/DG evoked potentials is altered by the rat's behaviour at the time a stimulus pulse is delivered. They suggest that effects of experience on PP/DG transmission could be due to the experience altering the behaviour of the rat at the time of recording.

GENERAL METHOD

Animals

Long-Evans hooded, male rats were housed individually in hanging wire-mesh cages with continuous access to food and water. A 12:12 light-dark cycle was maintained throughout the experiment and all testing occurred in the light phase. All rats weighed approximately 300-450 g at the time of surgery. Sixteen rats were tested in two squads. The first squad contained 10 rats and the second squad contained 6 rats. Both squads were treated the same except as indicated.

Recording materials and apparatus

Recording electrodes for the first squad were made of nickel-chromium wire (270 µm O.D.), insulated except for the cross-section at the tips and teflon-coated stainless steel wire (114 µm) for the second squad. Stimulating electrodes were twisted bipolar, stainless-steel electrodes (Plastic Products MS-303/3, 114 µm electrodes) for the first squad, and monopolar teflon-coated stainless-steel wire (114 µm) for the second squad (see Appendix A). Ground and reference, (and a stimulation return electrode for the second squad) were uninsulated copper-tinned wire soldered to uninsulated jewellers' screws for both squads. Electrodes, ground, reference and return wires were all connected to Amphenol gold pins.

Signals from the recording electrode were amplified (gain = 100) and filtered (3 Hz - 3 kHz) with a Grass Model P15 AC preamplifier. The signals were further amplified 2-fold by a Neurolog (NL106) AC-DC amplifier. The amplified signal was monitored on a Nicolet 3091 oscilloscope and a Grass AM8 audio monitor. A microcomputer (386 Televideo) running "Brainwave"

software (Brainwave Systems Corporation, 1989) sampled and stored 40 ms "sweeps" of the recorded signal, triggered 5 ms prior to the stimulation pulse. Each sweep was sampled at 50 kHz. Constant current monophasic stimulus pulses were delivered by an AMPI Master-8 pulse generator and a AMPI Isoflex stimulus isolation/constant current stimulation unit.

Surgery

All rats received injections of atropine methyl nitrate (20 mg/kg, i.p.) and were anaesthetised with sodium pentobarbital (65 mg/kg, i.p.) prior to surgery. Sodium pentobarbital supplements were administered as necessary throughout surgery. During surgery burr holes were made in the clean dry skull 3.2 mm posterior to bregma and 1.6 mm left of the midline for the recording site, and 8.1 mm posterior to bregma and 4.3 mm left of the midline for the stimulation site. Ground screw and reference screws were placed in the skull approximately equidistant from the stimulation and recording electrodes. The stimulation return for the second squad of rats was placed on the side contralateral to the stimulation site. Two additional jewellers' screws were placed in the frontal bones to provide structural support for the electrode assembly. After the dura was punctured with a needle, the recording electrode was lowered through the cortex until the distinctive crackle of the CA1 field cells of the hippocampus could be heard on the audio monitor. The stimulation electrode was then lowered into the PP while 250 µA cathodal pulses (100 µs duration) were delivered every 30 seconds. When the evoked potential deflected from baseline, the recording electrode was lowered into the hilus and then the depth of both electrodes was adjusted so that the largest population spike was obtained. The arrangement was cemented into

place with clear dental acrylic. Penicillin (0.1 ml, intramuscularly) was injected into each thigh.

Recording chamber

The recording chamber was a small, rectangular, clear Plexiglas cage (29 x 22 x 20.5 cm) with a stainless-steel grid floor. The electrode leads ran through a commutator positioned 75 cm above the recording chamber and then to the stimulating and recording equipment, positioned approximately 1 m away.

Training apparatus

The swimming pool was circular (1.4 m dia. x 48 cm deep) and was filled with milky water (19° C) to a depth of 25 cm. The hidden platform was made of clear Plexiglas and its top surface (13 x 13 cm) was submerged 1.5 cm below the surface of the water. The visible platform (13 x 13 cm) was black and protruded 5 cm above the surface of the water. Several large objects (eg., counters, shelves, wall posters) were visible from the surface of the pool. A video camera mounted above the centre of the pool provided a signal to a VP112 target scanner (HVS Image Analysing). The sequence of positions of the rat's head was sampled at 20 Hz throughout each trial and stored by an APPLE II+ computer to be analysed later. The analysis of each trial included the latency to find the platform, swim path length, and proportion of swim path length in each of 4 quadrants of the pool. The swimming pool was located in a different room than the recording chamber.

Recording procedures

Beginning two weeks after surgery, each rat was placed into the recording chamber for 15 minutes on 5 consecutive days. On the 6th day an input/output (I/O) curve using stimulus intensities from 20 μA to 500 μA

was obtained for each rat. Then for the first squad of rats, the 7 stimulus intensities that best represented the individual rat's sigmoidal I/O curve were chosen to be the test stimuli for each rat. For the second squad, the 5 stimulus intensities that best represented the average sigmoidal shape of the I/O curve of all the rats were chosen to be the test stimuli for all the rats. All stimulus pulses were delivered with a 10 s interpulse interval for the first squad, and a 30 s interpulse interval for the second squad.

Analysis of the evoked potentials was conducted using the BrainWave System Experimenter's Workbench analysis programme version 2.1 (BrainWave Systems Corporation, 1989). Figure 1B shows an example of a typical waveform. Two measurements were made of each collected waveform: population spike area and EPSP slope. The slope of the EPSP was calculated as the rate of change in mV/ms in the middle 80% of the first positive-going portion of the evoked potential (occurring approximately 2 - 3 ms after stimulation). To calculate the area of the population spike, a tangent was calculated connecting the two positive peaks of the waveform and the area of the trough under the tangent was calculated. Both of these measures were calculated and stored using BrainWave Experimenter's Workbench software.

Each day the recording session with each rat yielded an I/O curve for population spike area and EPSP slope. Using a formula described and validated in a paper by Skelton et al. (1987), the area under each daily I/O curves was calculated (see Appendix B for representative I/O curves).

EXPERIMENT 1

The purpose of this experiment was to determine if changes in PP/DG evoked potentials are produced by training in the Morris water task. If LTP at PP/DG synapses underlies the acquisition of spatial information then evidence should be found for increases in evoked population spike and EPSP as a result of training in this task. Two versions of the Morris water task were used. One version uses a hidden platform in a fixed location (place condition). Previous work has demonstrated that this version requires an intact PP/DG system for successful performance. The second version uses a visible platform (cue condition) and does not require an intact hippocampal formation for successful performance (Morris et al., 1982). Training in the second version controls for the effects of variables such as cool water stress, swimming, and handling. The evoked potential recordings during training in these two conditions were compared with recordings from rats that received no water task training.

Method

Behavioural & electrophysiological procedures

Daily I/O curves were collected for 6 consecutive days and baseline was calculated as the average of the last 2 days. At the end of baseline collection the animals in the first squad were divided into 3 groups. The place-cue group (n = 7, composed of 4 rats from squad 1 and 3 rats from squad 2) was trained in the place condition in Phase 1 for 8 consecutive days and in the cue condition in Phase 2 for 5 consecutive days. The cue-place group (n = 3) was trained in the cue condition in Phase 1 for 8 consecutive days and the cue condition in Phase 2 for 5 consecutive days. Following training in the place-cue condition a

probe trial was conducted, consisting of a single 30 s trial with the platform removed from the pool. The control group (n = 6, composed of 3 rats from squad 1 and 3 rats from squad 2) did not receive any behavioural training. Daily I/O curves were collected from all rats in the experiment approximately 20 hr after the previous day's behavioural training (i.e., 4 hr before behavioural training on the following day).

In the analyses of behaviour and evoked potential data the results of these two squads were combined, despite the fact that the electrodes were made of different materials, stimulating electrodes were monopolar for one squad and bipolar for the other, and a different number of stimulus intensities was used for the two squads. This is justifiable because: 1) the evoked potentials recorded in the DG were very similar in amplitude and threshold between squads; 2) in respect to behavioural and general recording procedures they were treated identically; 3) rats from the two squads were assigned evenly to each of the treatment groups; 4) the behavioural and recording results were very similar between the two squads; and, 5) there was no *a priori* reason to suppose that any of the differences affect the way that PP/DG evoked potentials would respond to the experimental treatments.

After behavioural training was complete all rats in the first squad received tetanic stimulation consisting of 10 trains of 10 pulses (200 μA, 400 Hz, 20 s intertrain interval) and I/O curves were collected 24 hr later. Squad 2 did not receive tetanic stimulation.

Histological procedures

The brains were prepared using the Prussian Blue technique for identifying the position of the electrode tip. For each rat 25 μA DC was passed

for 25 s through both the stimulation electrode and the recording electrodes sequentially. The rats were then deeply anaesthetised and intracardially perfused with a solution of 0.9% NaCl followed by 10% formalin. The brains were removed from the skull and placed into 10% formalin with approximately 150 mg of potassium ferrocyanide. The brains were left in this solution for 24 hr and then the solution was replaced with 30% formol sucrose. The brains were cut into 40 µm sections, mounted, and stained with Cresyl violet.

Results

Histological results

Figure 2 shows the recording and stimulating electrode placements in the DG and PP of all rats. The electrode placements for 2 rats were insecure, such that for one rat the evoked potential had completely disappeared half way through the experiment and the evoked potential for the other rat changed so dramatically that it could no longer be compared to baseline recordings. The data for the two rats (one belonging to the cue-place group and the other belonging to the place-cue group of Experiment 1) were removed from the experiment.

The waveforms of three rats (one from each of the behavioural conditions in Experiment 1) did not have measurable population EPSPs, although they each had stable, measurable population spikes. We were unable to determine the cause of this from the histology. These rats were included in the experiment for their population spike measures, but were excluded from all analysis of population EPSP.

Figure 2. Recording (**A, B, & C**) and stimulating (**D, E, & F**) electrode tip placements in all rats. **A & D**: Placements in rats of squad 1; **B & E**: Placements in rats of squad 2; **C & F**: Placements in rats of Experiment 3. The recording sites are presented on a coronal section 3.3 mm posterior to the bregma and the stimulating sites are presented on a coronal section 7.04 mm posterior to the bregma. Placements in rats that were removed from Experiment 1 are indicated by "x". (Diagrams from Paxinos & Watson, 1986)

A.

B.

C.

D.

E.

F.

Behavioural results

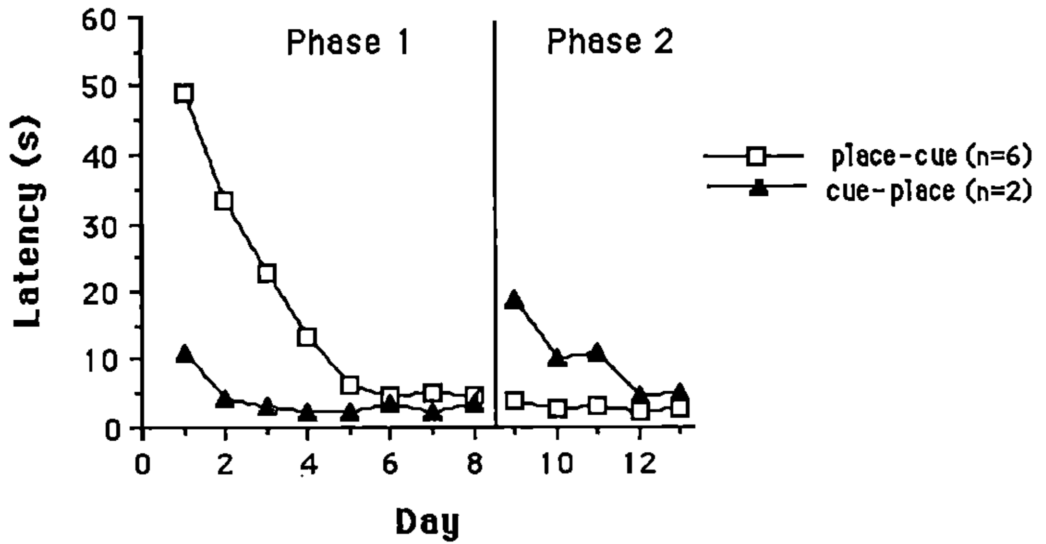
All rats learned to swim directly to the platform in both place and cue conditions of the Morris water task. Figure 3A shows the daily average latencies to find the platform for each group. By day 5 of training, all rats in the place-cue group swam directly to the hidden platform. The latency for the place-cue group did not change when the rats were switched to the cue condition in phase 2. The cue-place group, as expected, did show a substantial increase when switched to the place condition in phase 2. All rats in this group reached asymptote by the third day of training in Phase 2. Figure 3B provides the results of probe trials given to each group after training in the place version of the Morris water task. A paired t-test between the total swim distance in the quadrant where the platform had been and the average swim distance in the other 3 quadrants showed that the rats preferred swimming significantly more in the correct quadrant ($t(6) = -3.2$, $p < .01$). This indicates that the rats had learned the position of the hidden platform in relation to the extramaze cues.

Electrophysiological results

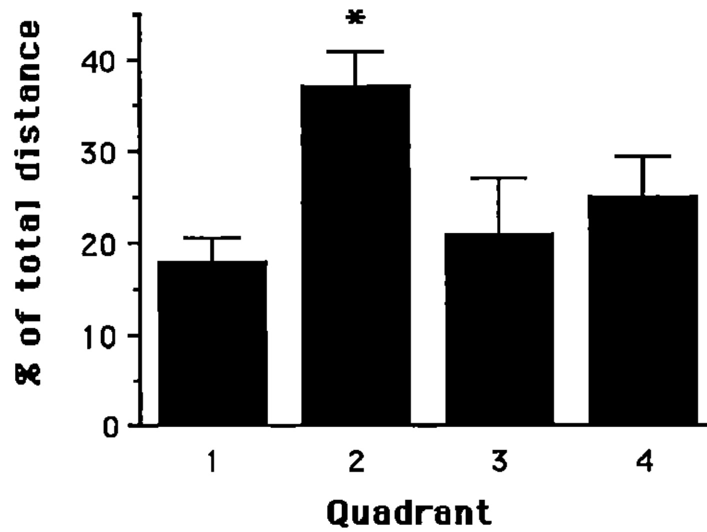
Population spike area did not change as a consequence of either place or cue learning. Figure 4A shows the daily percent change from baseline in area under the I/O curve for population spike for the three groups. A repeated-measures ANOVA revealed no significant group effect ($F(2,11) = 0.1$, $p > .9$), or day effect ($F(7,77) = 1.7$, $p > .1$), or group by day interaction ($F(14,77) = 0.6$, $p > .8$). Population spike area did not change significantly between Phase 1 and Phase 2 of behavioural training in the place-cue group ($F(5,6) = 0.6$, $p > .7$), or

Figure 3. Performance in the Morris water task. **A**. Phase 1: Latency to find the hidden platform for the place-cue group and latency to find the visible platform for the cue-place group; Phase 2: Latency to find the visible platform for the place-cue group and latency to find the hidden platform for the cue-place group. **B**. Percent of total swim distance in each quadrant of the pool during the probe trial in Experiment 1. The asterisk indicates quadrant with significantly greater percentage of swim distance than the remaining quadrants.

A.



B.

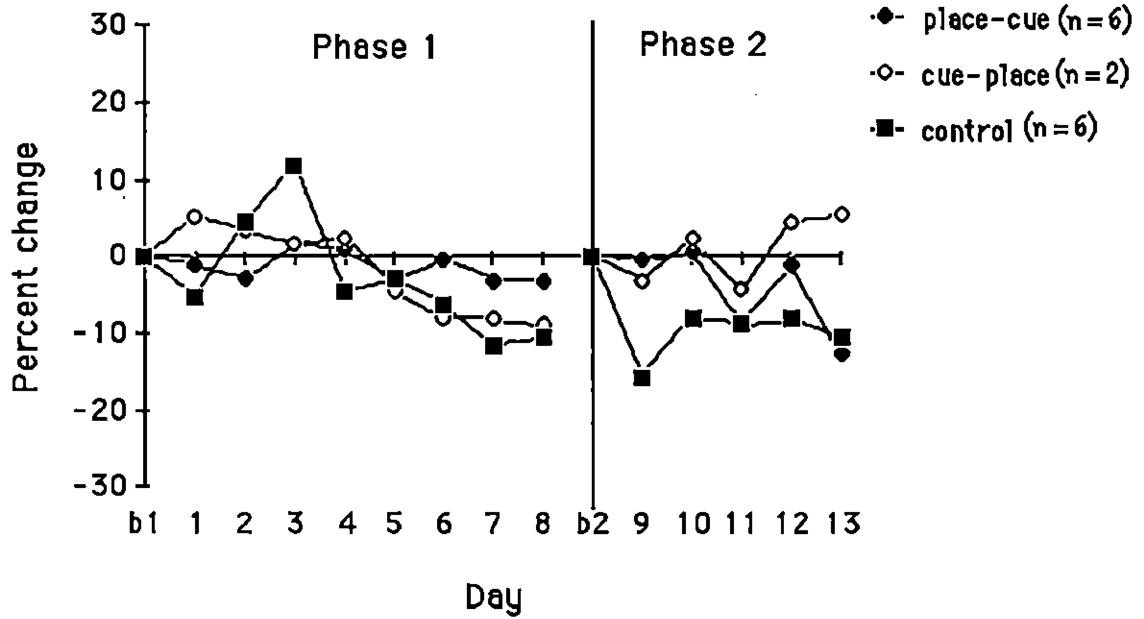


in the control group ($F(5,6) = 0.4$, $p > .8$). Even when the two groups were considered according to behavioural condition (place or cue, regardless of order) there was no significant change between conditions ($F(7,8) = 0.7$, $p > .6$). In order to assess the extent to which area under the I/O curves for population spike had changed relative to the pre-behavioural training level, daily percent changes were calculated using the first baseline measure (Figure 4B). The population spike for the place-cue group and the cue-place group showed a gradual decline in area relative to the baseline taken before behavioural training. The population spike for the control group shows a sharper decline following day 8. This decline was attributable to data from one rat who showed a precipitous drop following day 8.

Training in the Morris water task produced a small but reliable increase in the area under the EPSP slope I/O curve. Figure 5A presents the percent change from baseline in area under the I/O curve for population EPSP. Given the small number of rats in the cue-place group from which reliable EPSP's were recorded we could not include this group in the statistical tests. A repeated-measures ANOVA revealed a significant difference between groups ($F(1,7) = 10.7$, $p < .02$), though no significant day effect ($F(7,49) = 1.0$, $p > .4$), or group by day interaction ($F(7,49) = 1.3$, $p > .2$). There was no significant change in population EPSP between Phase 1 and Phase 2 in the place-cue group ($F(4,5) = 0.9$, $p > .5$), or in the control group ($F(3,4) = 5.2$, $p > .07$). Figure 5B shows the daily average percent changes in area under the I/O curve for EPSP calculated from the first baseline. The population EPSP for the place-cue group remained stable relative to the level before behavioural training. The EPSP slope for the control group showed a gradual decline from the initial

Figure 4. **A**. Average percent change from baseline in area under the population spike I/O curves for the place-cue group, cue-place group, and control group for phase 1 and 2 of behavioural training. Changes from baseline session in phase 2 were calculated from the second baseline session taken between days 8 and 9 of training. (Note: b1 = first baseline session; b2 = second baseline session) **B**. Average percent change in area under the population spike I/O curves calculated from the first baseline for the place-cue group, cue-place group, and the control group for phase 1 and 2 of behavioural training. Note: Unlike Figure 4A the changes in Phase 2 are calculated from the initial baseline only.

A.



B.

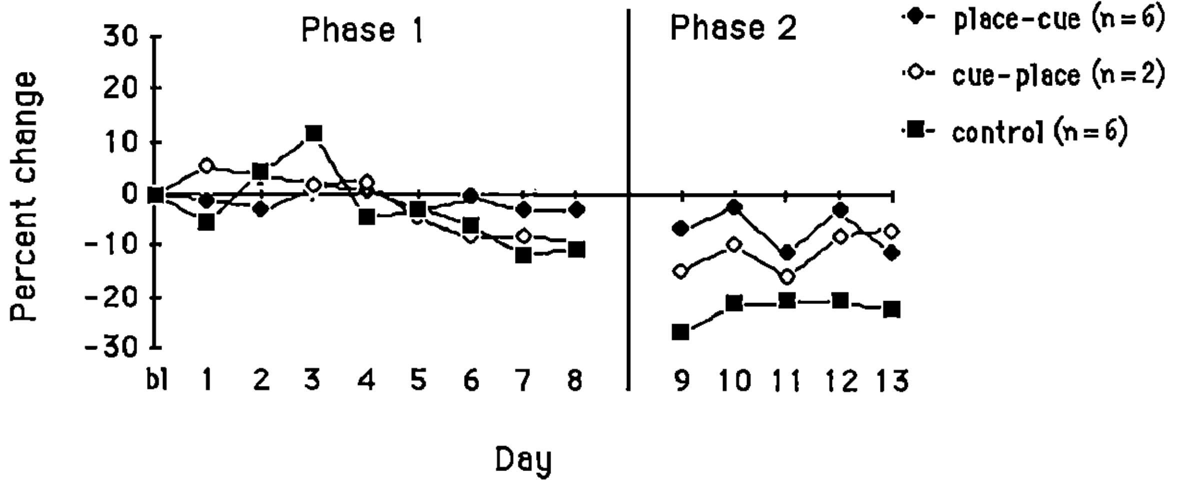
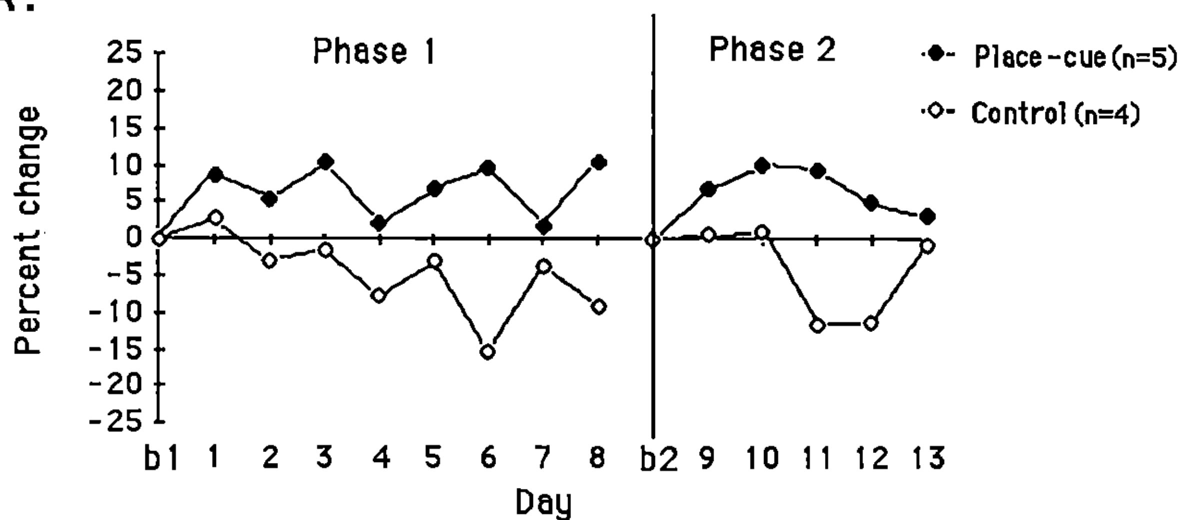
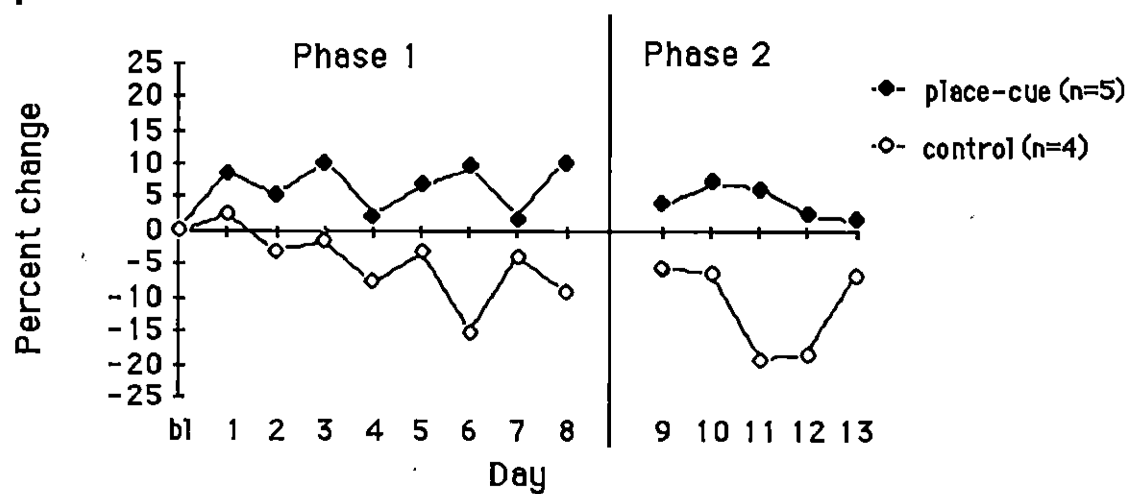


Figure 5. **A**. Average percent change from baseline in area under the EPSP I/O curve for the place-cue group, and the control group for phase 1 and 2 of behavioural training. Phase 2 was calculated from a second baseline taken between days 8 and 9 of behavioural training. Note: Reliable EPSPs were obtained from only one rat in the cue-place group, and so the data from that group are not presented. **B**. Average percent change from baseline in area under the EPSP I/O curve for the place-cue group, and the control group for phase 1 and 2 of behavioural training. Changes in Phase 2 were calculated from the initial baseline measure.

A.



B.



pretraining baseline except for the 11th and 12th day, when a drop from baseline occurred. This drop is attributable to one rat that showed a sharp decline for two days and then recovery. The cause of the sharp drop followed by recovery of the evoked potential is unknown.

Tetanic stimulation increased the area under the population spike I/O curves in all rats in the first squad when measured 24 hr after stimulation. The place-cue group showed a 57% increase over baseline, the cue-place group increased 22%, and the control group showed a 44% increase.

Discussion

The behavioural results indicate that all rats trained in the Morris water task learned to navigate to the platform. Training increased the PP/DG population EPSP but did not affect the population spike. If training had increased the efficacy of PP/DG synapses one would have expected that the population spike would have increased. The reason for the dissociation between the effect on population EPSP and spike is not clear. Since the EPSP is generated in the middle third of the molecular layer, but the recording electrode is positioned in the hilus, the recorded EPSP current may be influenced by factors other than the strength of the perforant path synapses. Factors such as tonic dendritic and somatic hyperpolarization or depolarization of the granule cells or activity of other granule cell inputs can influence the size of the EPSP when recorded so far from the synaptic current sink. The absence of an effect on population spike in the present results does not provide much support for the hypothesis that LTP at PP/DG synapses underlies acquisition of spatial information in a task that is known to depend upon the integrity of PP/DG circuitry.

There are several reasons why training might not affect the size of the PP/DG population spike. 1) The efficacy of the stimulated axons in the PP cannot be increased. 2) The magnitude of increases in PP/DG synapses produced by behavioural experience is too small to be detected by our measures. 3) Training in the Morris water task does not increase PP/DG synaptic strength. 4) PP/DG synaptic strength is not increased by *any* behavioural experience.

The first possibility is ruled out by our demonstration of a long-lasting increase in the PP/DG evoked potentials produced by high-frequency electrical stimulation. Using *present* techniques the second and third possibilities cannot be ruled out. If more sensitive measures of PP/DG synapse strength, such as single neuron recording in conjunction with electrical stimulation of PP, were used it would be possible to detect much smaller increases or inceases in a very small percentage of synapses.

The fourth possible reason for why population spike was unaffected by training in the Morris water task is that PP/DG synapse strength is not modified by any behavioural experience. However, work by others using similar electrophysiological techniques has demonstrated that continuous exposure to a novel, complex environment causes a long-lasting increase in the PP/DG evoked population spike (Green & Greenough, 1986; Sharp, 1986; Sharp et al., 1985). Although rats may be affected by a multitude of aspects of this situation, through exploration rats do learn the spatial layout of the environment (O'Keefe & Nadel, 1978; Sutherland, 1985). Other work has shown that exploration is impaired by hippocampal damage (O'Keefe & Nadel, 1978; Sutherland, 1985). Further, the opportunity for continuous

exploration of complex environments produces long-lasting increases in dendritic branching of DG granule cells (Juraska, Fitch, Henderson, & Rivers, 1985). Based upon these considerations, at least some aspect of experience in complex environments must have an important bearing upon hippocampal circuitry. Thus, examining the effects of continuous exposure to a novel, complex environment on PP/DG evoked potentials in the present preparation may allow testing of the fourth alternative in the list above.

EXPERIMENT 2

The second experiment was conducted to see if the present electrophysiological techniques and measurement procedures reveal an effect of continuous exposure to a novel, complex environment similar to the one reported by Sharp (1986) and Sharp et al. (1985). One testable interpretation of the lack of effect of Morris water task training on the PP/DG population spike reported in Experiment 1 is that behavioural experience does not alter PP/DG evoked potentials. This hypothesis would be disconfirmed if Sharp's effect could be replicated using our preparation.

Method

Apparatus

The novel, complex environment consisted of a large box (1.5 m x 1 m x 1 m) that had three levels of interconnected living space. One wall contained a large window through which people and objects in the surrounding laboratory could be seen. A diverse array of objects (e.g., beverage cans, cups, beakers, mirrors) were strewn throughout the living space. Various odours (e.g., vanilla, deodorant, aftershave, cinnamon) were present in cotton balls secured in pill bottles. Various flavours and foods (e.g., sweet water, rat chow, fruit-flavoured cereal, chocolate chip cookies, sunflower seeds, apple pieces, potatoes) were available. Many tunnels, hidden spaces, and nesting areas and nesting materials (e.g., newspaper, foam chips, sawdust, paper towel, cotton batting) were present. Many textural surfaces (e.g., plastic grate, sawdust, foam chips, mirror) made up the floors and walls. Various types of music, human voices, and other noise were present through the days and during some nights. The arrangement of features inside the box (e.g.,

layout of the floor space, types of toys, smells, foods, textures, and visual cues) was changed daily.

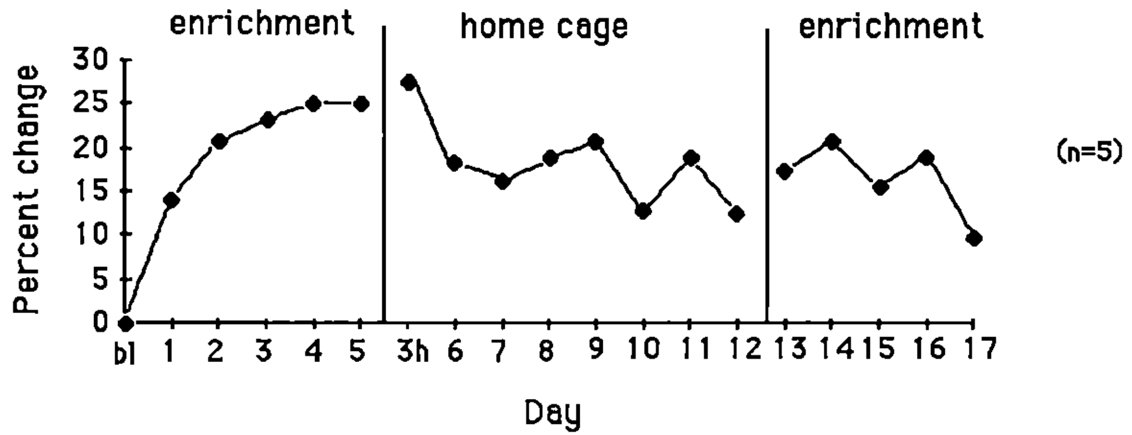
Behavioural procedures

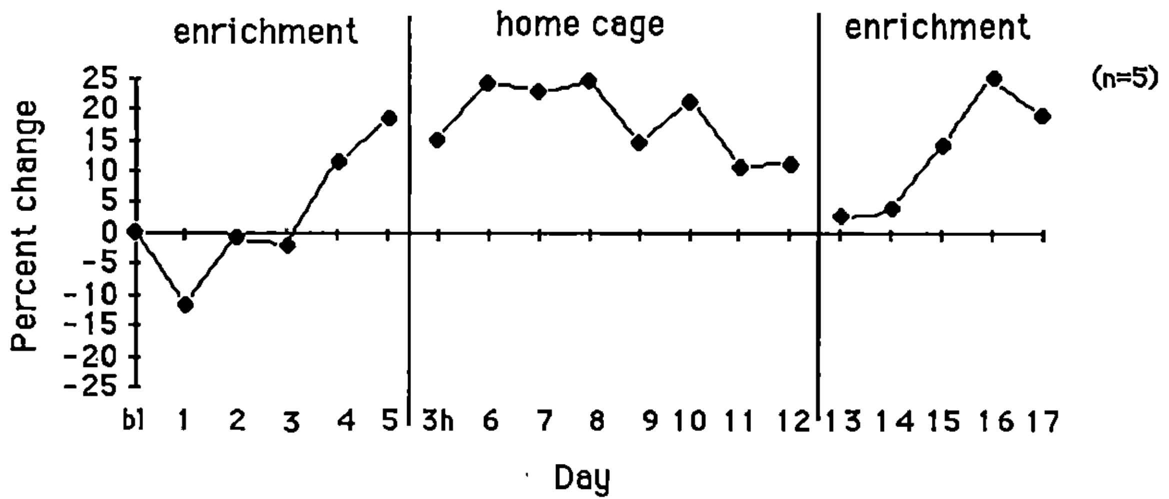
The rats of the second squad (n = 5) in Experiment 1 were used. Daily I/O curves using the same range of intensities as in Experiment 1 was obtained from each rat for 6 days. Baseline was calculated as the average of the last 2 days. The rats then were placed in the novel, complex environment for 5 days and were returned to their home cages for 7 days to assess the persistence of observed changes. The rats were replaced in the complex environment for a final 5 days.

Results

Substantial increases in PP/DG evoked potentials were observed as a result of exposure to the complex environment. The population spike measures are presented in Figure 6A. Exposure to the complex environment significantly increased the population spike ($F(4,20) = 163.1$, $p < .0001$). The increase developed rapidly over the first 2 days, reaching an asymptote of 25% on days 3 and 4. In all rats, the population spike increased over baseline (2 rats showed an increase of 60%, one rat showed an intermediate increase of 20%, and the final 2 rats showed an increase of 10% over baseline during the exposure period). When the rats were returned to their homecages, the increase in population spike decayed slowly over the next 7 days to 12% above baseline. An exponential curve ($y = 89.179 * 10^{(-3.1568e-2x)}$, $R^2 = .47$) calculated by best fit to a scatterplot of the group means for days 5-12 (using the exponential curve fitting procedure in Cricket Graph v. 2.1 for the Macintosh) and a time constant for decay was estimated to be 12 days (i.e.,

Figure 6. A. Average daily percent change from baseline in area under the population spike I/O curve during exposure to a complex, novel environment (enrichment), placement into the original home cages (home cage), and return to the complex environment (enrichment). Note: Data at 3h were recorded three hours after returning to the homecages from the complex environment. B. Average daily percent change from baseline in area under the EPSP I/O curve during exposure to a complex, novel environment (enrichment), placement in to the original home cages (home cage), and return to the complex environment (enrichment).


A.
enrichment
home cage
enrichment
Percent change
30
25
20
15
10
5
0
bl 1 2 3 4 5 3h 6 7 8 9 10 11 12 13 14 15 16 17
Day
(n=5)


B.
enrichment
home cage
enrichment
(n=5)
Percent change
25
20
15
10
5
0
-5
-10
-15
-20
-25
bl 1 2 3 4 5 3h 6 7 8 9 10 11 12 13 14 15 16 17
Day

time required for the population spike to decline to 37% of its maximum).

Figure 6B shows the daily percent changes in area under the I/O curve for EPSP slope. There was an initial drop below baseline on the first day of exposure to the complex environment. Thereafter, EPSP slope increased to 19% above baseline. The increase in population EPSP over the 5 day treatment period was statistically significant ($\underline{F}(4,20) = 4.7$, $\underline{p} < .008$). However, it should be noted that only 2 rats showed a consistent increase (one of these rats increased steadily over days and reached 120% above baseline). The other 3 rats showed a slow, steady decrease from baseline. When the rats were returned to their home cages, the increases decayed to 10% above baseline with a decay constant of about 9 days ($y = 127.49 * 10^{(-6.062e-2x)}$, $R^2 = .7$). The pattern of effect on the population EPSP, but not on the population spike, was reproduced when the rats were returned to the complex environment the second time (Figure 6B). That is, the population EPSP declined slightly on the first two days of return to the complex environment and then increased to approximately 20% above baseline, but the population spike did not change.

Discussion

The results show that behavioural experience can produce long-lasting increases in the PP/DG evoked population spike in the present preparation. Thus, the data disconfirm the hypothesis that the apparent lack of effect of Morris water task training on PP/DG synaptic efficacy is due to an inability of any behavioural experience to modify PP/DG evoked potentials in our preparation.

It is not possible to identify which aspects of the experience in the novel, complex environment caused the changes in PP/DG evoked potentials. Sharp

(1986) and Sharp et al. (1985) hypothesize that it is the acquisition of information about the spatial layout of the environment that modifies PP/DG synaptic efficacy. Although this hypothesis has not been refuted, Hargreaves, Cain, & Vanderwolf (1990) have suggested an alternate, nonmnemonic interpretation. They showed that the size of the PP/DG population spike and EPSP is modulated reliably by the specific behaviour emitted by the rat at the time of delivery of a stimulus pulse. They propose that effects of prior experience on the PP/DG may not be due to altered synaptic efficacy, but rather may be due to an indirect effect of altering the rat's behaviour at the time of evoked potential testing.

EXPERIMENT 3

A third experiment was conducted to investigate a possible cause of the increases in the strength of PP/DG evoked potentials observed in Experiment 2. Two experiments have shown that the size of PP/DG evoked potentials is affected by the behaviour of the rat at the time of delivery of the stimulus pulse. One experiment found that if the rat is walking, the population spike is larger and the EPSP is smaller (Green, McNaughton, & Barnes, 1990) than if the rat is immobile. A second experiment found that if the rat is walking, the EPSP and population spikes are both smaller than if the rat is immobile (Hargreaves et al., 1990). An interpretation of the effects of exposure to a novel, complex environment on evoked potentials is that the rat's behaviour during recording is modified by the experience in the complex environment such that the recorded evoked potentials are larger (Hargreaves et al., 1990). This hypothesis predicts that if behaviour is held constant at the time of the stimulus pulse, then exposure to the complex environment will not increase EPSP or population spike area.

Method

Surgical materials and procedures were the same as those used for the second squad of rats in Experiment 1. The apparatus and general behavioural procedures were the same as Experiment 2.

Seven naive rats were implanted with chronic stimulating and recording electrodes and allowed 2 wk for recovery. Evoked potentials were obtained daily from each rat until the recordings were stable. Only one stimulus intensity was used for the test pulses. The intensity selected was one that produced approximately a half-maximal population spike area in order

to increase the likelihood of detecting both increases and decreases in the evoked potential. Baseline was calculated as the average of the last two days prior to exposure to the complex environment. The rats were placed in the novel, complex environment for 9 days. They were removed briefly (approximately 20 min) from the complex environment for recording sessions on days 1, 2, 3, 5, 7 and 9. They were returned to the complex environment immediately after each recording session.

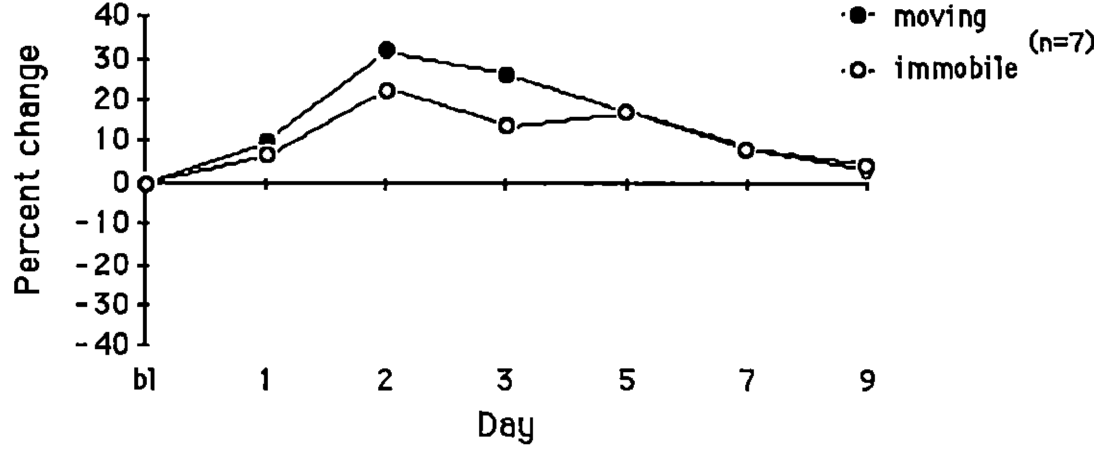
In each recording session two sets of 10 pulses were delivered when the rat was moving (walking, running, or in the active process of rearing). Another two sets of 10 pulses were delivered when the rat was immobile with eyes open. These behaviours correspond to the movement and immobile conditions of Hargreaves et al., (1990). The sets of pulses were alternated within each session such that if the first set was delivered when the rat was immobile then the second was delivered when the rat was moving, the third set was delivered when the rat was immobile, and the fourth set was delivered when the rat was moving. This sequence was counter-balanced over the testing days such that one session began with an immobile set and the next session began with a moving set. The stimulator was triggered by the experimenter so that the stimulus pulses were delivered when the rat was engaged in the appropriate behaviour. The PP/DG evoked potentials collected during the moving and immobile conditions were analysed separately and then compared.

Results

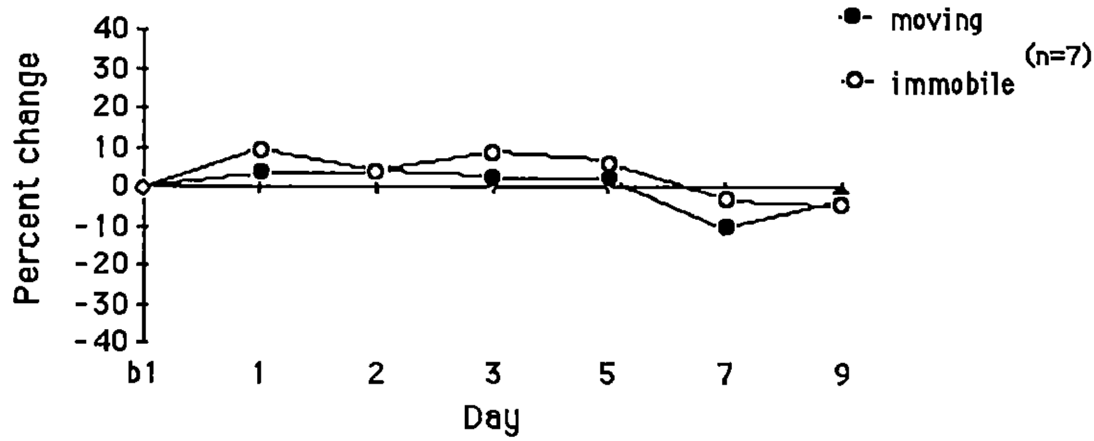
The amplitude of the population spike increased by 32% during exposure to a complex, novel environment (Fig. 7A). All rats showed an increase

Figure 7. **A**. Average percent change in population spike amplitude produced by exposure to a complex, novel environment. Stimulus pulses were delivered when the rats were moving or immobile. **B**. Average percent change in EPSP slope produced by exposure to a complex environment. Stimulus pulses delivered when the rats were moving or immobile.

A.



B.



during both the moving and immobile conditions. One rat's population spike increased by 60-63%, 3 rats' increased by 30-35%, and 1 rat's increased by 25-28%, and the last rat's increased by 10-17% in both behavioural conditions. The increase in amplitude of the population spike produced by exposure to the complex environment was significant, both when the rats were moving ($F(6,42) = 20.1$, $p < .0001$), and when they were immobile ($F(6,42) = 16.4$, $p < .0001$). The population EPSP slope (Fig. 7B) increased by 10% over baseline during exposure to the complex environment. The EPSP for two rats either decreased slightly or remained the same as baseline when they were moving or immobile, in one rat the EPSP increased by 12% and 4%, in the fourth rat the EPSP increased by 28% and 17%, in the fifth rat the EPSP increased by 32% and 35%, and in the final rat the EPSP increased the most, by 62% and 47%, in the moving and immobile conditions. The population EPSP increased significantly over the treatment days when the rats were moving ($F(6,42) = 35.6$, $p < .0001$), and when they were immobile ($F(6,42) = 42.8$, $p < .0001$).

The population spike amplitudes were significantly larger when the rats were moving than when they were immobile ($F(6,7) = 25.1$, $p < .0002$) (Fig. 7A). In contrast, the EPSP slope was significantly lower when the rats were moving than when they were immobile ($F(6,7) = 23.6$, $p < .0003$) (Fig. 7B).

Discussion

The results indicate that exposure to a novel, complex environment produces long-lasting increases in PP/DG population spike and EPSP. This confirms the results of Experiment 2 and the results of Sharp (1986) and Sharp et al. (1985). In addition, we found that the behaviour at the time that a stimulus pulse is delivered to the PP significantly modulates the size of the

evoked potential recorded in the DG. The pattern of the changes confirms observations by Green et al. (1990) that the population spike is larger and the EPSP slope lower when rats are moving than when they are immobile. These observations are not in line with Hargreaves et al. (1990) who reported that the population spike is decreased when rats are moving. The reason for this discrepancy is not clear.

The results disconfirm Hargreaves et al.'s (1990) hypothesis that increases in PP/DG evoked potentials produced by prior experience are indirectly caused by changes in the rat's behaviour at the time of recording. The size of evoked potentials increased due to exposure to the complex environment when the rats were moving or immobile. In addition the magnitude of the difference in evoked potentials recorded when the rat is moving vs. immobile is not as large as the increases, measured in the same preparation, caused by exposure to a complex environment. These results clearly show that the effects of exposure to a complex environment cannot be attributed to a change in behaviour at the time of electrophysiological testing. The results are therefore consistent with suggestions by Sharp (1986) and Sharp et al. (1985) that PP/DG connections are strengthened by the acquisition of information obtained during exploration in the complex environment.

GENERAL DISCUSSION

The results of the three experiments only partially support the hypothesis that LTP in the PP/DG synapses underlies spatial learning. It is clear from the behavioural results of Morris water task training that all the rats learned to navigate directly to the hidden platform. Training produced a small, but statistically significant, increase in EPSP slope. However, there was

no change in the population spike area following training. If the PP synapses had been strengthened, then one should find that accompanying the increase in EPSP there should be a corresponding increase in the population spike, since more granule cells would reach threshold for discharge. Because the population spike was not affected by training, our results are not consistent with the idea that PP synapses were strengthened. Even though the PP/DG connections are necessary for normal performance in the Morris water task, consistent increases in the ability of PP/DG synapses to drive activity in DG granule cells as a result of learning to solve this task were not observed. In the same rats, we were able to detect increases in PP/DG evoked potentials 22 hr after tetanic electrical stimulation was administered. This result demonstrates that the PP synapses in the DG activated by our stimulus pulses were modifiable and that our recording procedures could detect increases in synaptic efficacy when they occurred.

There are at least three other reasons why increases in PP/DG evoked population spike might not have been observed following place learning in the Morris water task. The first reason is that spatial learning in the Morris water task might not depend on changes in synaptic efficacy in the hippocampal formation. The second reason is that PP synapses were enhanced but the total change was too small to detect, either because the size of the change in each element was very small or because the number of elements changed was very small. Morris and Baker (1984) presented this possibility when discussing the inherent weakness of negative results in the context of training-induced potentiation studies. The increase in EPSP slope that we observed during learning in the Morris water task may indicate that

small increases were occurring. However, it is worth noting that the population spike size, which is a better index of granule cell through-put processing, tended to decrease rather than increase.

A third reason why changes in PP/DG evoked potentials during learning in the Morris water task were not observed may be that enhancement did occur but at some other set of PP synapses outside the DG but within the hippocampal formation. The DG receives the largest projection from the entorhinal cortex, however there are additional, direct projections of the PP to both CA3 and CA1 (Amaral & Witter, 1989). Perhaps these other synapses of the PP are strengthened as a result of spatial learning in the Morris water task. It may be that the spatial learning deficits observed in experiments in which hippocampal LTP was pharmacologically blocked or in which PP LTP was saturated are caused by effects on direct inputs to CA3 or CA1. It is also important to note that synaptic enhancement in response to spatial learning could occur at other hippocampal synapses such as those of the mossy fibre and/or Schaffer collateral systems.

None of these three possibilities can be ruled out by our data, but the latter two options are most compatible with previous research. These options are that LTP-like processes do occur during learning and are necessary for spatial learning in the Morris water task but these changes are either too small to detect or occur in an area of the hippocampal formation other than the PP/DG connection. These two possibilities, and not the first, are more consistent with the obervations that elimination of hippocampal LTP by means of NMDA receptor blockade or by means of LTP saturation blocks spatial learning (Castro et al., 1990; McNaughton et al., 1986; Morris et al.,

1986.

Changes in PP/DG evoked potentials which could reflect an LTP-like process were observed in Experiment 2. Reliable increases were observed in population spike area during exposure to a complex, novel environment. Substantial increases were found in the population EPSP slope during enrichment treatment but the effect was far less reliable from animal to animal. In the second experiment only 2 of the 5 rats showed increases, and in the third experiment 4 out of the 6 rats showed increases, whereas all of these rats showed increases in population spike in both experiments. These results partially confirm Sharp's (1986) findings on the effect of environmental enrichment on PP/DG evoked potentials. She found a consistent increase in the population spike measure, but the changes in EPSP were less consistent between rats. It is important to note that with recording sites in the hilus, LTP is sometimes characterised by an increase in the population spike without an accompanying increase in the EPSP (Bliss & Gardner-Medwin, 1973; Bliss & Lømo,1973). The variability in the EPSP increases between rats in the present experiments may reflect the fact that hilar recording sites are less than optimal for recording synaptic current generated by PP synapses on the granule cell dendrites in the molecular layer. A more reliable pattern of EPSP change may be detected with recording electrodes positioned in the middle third of the DG molecular layer, close to the synaptic current sink of the perforant path inputs (Lømo, 1971).

Increases in population spike and EPSP did not occur simultaneously. The population spike grew from the first day, and reached asymptote after 3-4 days. In contrast, the population EPSP remained at or below baseline for the

first 3-4 days and increased only after the population spike had reached asymptote. This result along with the fact that several rats showed substantial increases in the size of the population spike without an increase in EPSP slope indicates that the EPSP and population spike are dissociable.

The increases in evoked potentials during exposure to a novel, complex environment persisted long after the rats were taken out of the complex environment and returned to their home cages. The magnitude of the increases and the slow rate of decay have clear similarities with LTP induced by electrical stimulation of the PP. This result indicates that the increases were probably not due to some general effect of the experience in the complex environment on behaviour (e.g., increased arousal). It is also important to note that no aspect of the behavioural experience in Experiment 1 in the Morris water task, for example, stress of cold water, the act of swimming, transportation to and from colony to testing rooms, handling or other types of stressors enhanced the PP/DG evoked population spike. One possible interpretation of the present results is that the increases in population spike and EPSP reflect an LTP-like process in PP/DG connections due to learning while in the complex environment.

The results from Experiment 3 indicate that the behaviour of the rat at the time of the test pulse does have a small, consistent effect on the size of the PP/DG evoked potential. The population spike was on average 8% larger when test pulses are given when the rat is walking, running or in the active process of rearing than when the rat is immobile. In contrast, the EPSP was consistently smaller (4%) when test pulses were delivered when the rat was moving than when the rat was immobile. This data supports the idea put

forth by Hargreaves et al. (1990) that the behaviour of the animal during evoked potential recording effects the size of the evoked potential. However, our results disconfirm the Hargreaves et al. (1990) hypothesis that increases in evoked potentials caused by experience in novel, complex environments are due to the behaviour of the animal during evoked potential recording. We observed significant increases in both population spike amplitude and population EPSP slope even when we controlled for behaviour during evoked potential recording.

The principal findings of the present experiments are that: 1) LTP-like increases in PP/DG evoked potentials are not produced as a consequence of learning in the Morris water task; 2) LTP-like changes do result from exposure to a novel, complex environment, and 3) these changes are not dependent upon altering the behaviour of the rats during recordings. The results imply that when an animal learns place navigation, LTP at PP/DG synapses either does not occur or the changes in synapse strength are so small that they are not detectable using the present field recording techniques. The results of exposure to a novel, complex environment support the idea that long-lasting increases in PP/DG synapse strength underlie certain kinds of learning.

REFERENCES

Amaral, D. G., & Witter, M. P. (1989). The three-dimensional organisation of the hippocampal formation: A review of anatomical data. Neuroscience, 31, 571-591.

Anderson, P., Bliss, T. V. P., & Skrede, K. K. (1971). Unit analysis of hippocampal population spikes. Experimental Brain Research, 13, 208-221.

Barnes, C. A., & McNaughton, B. L. (1985). An age comparison of the rates of acquisition and forgetting of spatial information in relation to long-term enhancement of hippocampal synapses. Behavioural Neuroscience, 99, 1040-1048.

Berger, T. W. (1984). Long-term potentiation of hippocampal synaptic transmission affects rate of behavioural learning. Science, 224, 627-630.

Bliss, T. V. P., & Gardner-Medwin, A. R. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the unanaesthetized rabbit following stimulation of the perforant path. Journal of Physiology, 232, 357-374.

Bliss, T. V. P., & Lømo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetised rabbit following stimulation of the perforant path. Journal of Physiology, 232, 331-356.

Byrne, J. H. (1984). Cellular analysis of associative learning. Physiological Reviews, 67, 329-439.

Castro, C. A., Silbert, L. H., McNaughton, B. L., & Barnes, C. A. (1990). Recovery of spatial learning deficits after decay of electrically induced synaptic enhancement in the hippocampus. Nature, 342, 545-548.

Collingridge, G. L. (1985). Long term potentiation in the hippocampus: mechanisms of initiation and modulation by neurotransmitters. Trends in Pharmacological Sciences, 6, 407-411.

Douglas, R. M., & Goddard, G. V. (1975). Long-term potentiation of the perforant path-granule cell synapse in the rat hippocampus. Brain Research, 86, 205-215.

Green, E. J., & Greenough, W. T. (1986). Altered synaptic transmission in dentate DG of rats reared in complex environments: Evidence from hippocampal slices maintained in vitro. Journal of Neurophysiology, 55, 739-750.

Green, E. J., McNaughton, B. L., & Barnes, C. A. (1990). Exploration-dependent modulation of evoked responses in fascia dentata: dissociation of motor, EEG, and sensory factors and evidence for a synaptic efficacy change. The Journal of Neuroscience, 10, 1455-1471.

Hargreaves, E. L., Cain, D. P., & Vanderwolf, C. H. (1990). Learning and behavioural-long-term potentiation: Importance of controlling for motor activity. The Journal of Neuroscience, 10, 1472-1478.

Jarrard, L. E. (1986). Selective hippocampal lesions and behaviour: implications for current research and theorising. In R.L. Isaacson & Karl Pribram (Eds.), Hippocampus, Volume 4 (pp. 93-126). New York: Academic Press.

Juraska, J. M., Fitch, J., Henderson, C., & Rivers, N. (1985). Sex differences in dendritic branching of dentate granule cells following differential experience. Brain Research, 333, 73-80.

Lømo, T. (1971). Patterns of activation in a monosynaptic cortical pathway: the

perforant path input to the dentate area of the hippocampal formation. Experimental Brain Research, 121, 18-45.

McNaughton, B. L., Barnes, C. A., Rao, G., Baldwin, F., & Rasmussen, M. (1986). Long-term enhancement of hippocampal synaptic transmission and the acquisition of spatial information. Journal of Neuroscience, 6, 563-571.

McNaughton, B. L., & Morris, R. G. M. (1987). Hippocampal synaptic enhancement and information storage within a distributed memory system. Trends in Neuroscience, 10, 408-416.

Morris, R. G. M. (1981). Spatial localisation does not require the presence of local cues. Learning and Motivation, 12, 239-260.

Morris, R. G. M., Anderson, E., Lynch, G. S., & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by N-methyl-D-aspartate receptor antagonist. Nature, 319, 774-776.

Morris, R. G. M., & Baker, M. (1984). Does long-term potentiation/synaptic enhancement have anything to do with learning or memory. In L. R. Squire & N. Butters, (Eds.), Neuropsychology of memory (pp. 521-535). New York: Guilford Press.

Morris, R. G. M., Garrud, P., Rawlins, J. N. P., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. Nature, 297, 681-683.

O'Keefe, J., & Nadel, L. (1978). The hippocampus as a cognitive map. Oxford, England: Clarendon Press.

O'Keefe, J., Nadel, L., Keightly, S., & Kill, D. (1975). Fornix lesions selectively abolish place learning in the rat. Experimental Neurology, 48, 152-166.

Schenk, F., & Morris, R. G. M. (1985) Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesion. Experimental Brain Research, 58, 11-28.

Sharp, P. (1986). Effects of environmental manipulations on PP path-evoked dentate granule cell population responses in the rat. Doctoral dissertation, University of Colorado.

Sharp, P., Barnes, C. A., & McNaughton, B. L. (1989). Exploration dependent modulation of evoked responses in fascia dentata: fundamental observations and time course. Psychobiology, 17, 257-269.

Sharp, P., McNaughton, B. L. , & Barnes, C. A. (1985). Enhancement of hippocampal field potentials in rats exposed to a novel, complex environment. Brain Research, 339, 361-365.

Skelton, R., Scarth, A. S., Wilkie, D. M., Miller, J. J., & Phillips, A. G. (1987). Long-term increases in dentate granule cell responsivity accompany operant conditioning. Journal of Neuroscience, 7, 3081-3087.

Smith, M., & Milner, B. (1981). The role of the right hippocampus in the recall of spatial location. Neuropsychology, 19, 781-793.

Sutherland, R. J. (1985). The navigating hippocampus: An individual medley of space, memory and movement. In G. Buzsaki & C. H. Vanderwolf (Eds.), Electrical activity of the archicortex (pp. 255-279). Budapest: Akademiai Kiado.

Sutherland, R. J., Kolb, B., & Whishaw, I. Q. (1982). Spatial mapping: definitive disruption by hippocampal or medial frontal cortical damage. Neuroscience Letters, 31, 271-276.

Sutherland, R. J., & Rudy, J. W. (1989). Configural association theory: the role

of the hippocampal formation in learning, memory and amnesia. Psychobiology, 17, 129-140.

Weisz, D. J., Clark, G. A., & Thompson, R. F. (1984). Increased responsivity of dentate granule cells during nictitating membrane response conditioning in rabbit. Behavioural Brain Research, 12, 145-154.

Whishaw, I. Q. (1987). Hippocampal granule cells and CA3-4 lesions impairs the formation of place learning-set in rats and induce reflex epilepsy. Behavioural Brain Research, 24, 59-72.

Yeomans, J. S. (1990). Principles of brain stimulation. New York: Oxford University Press.

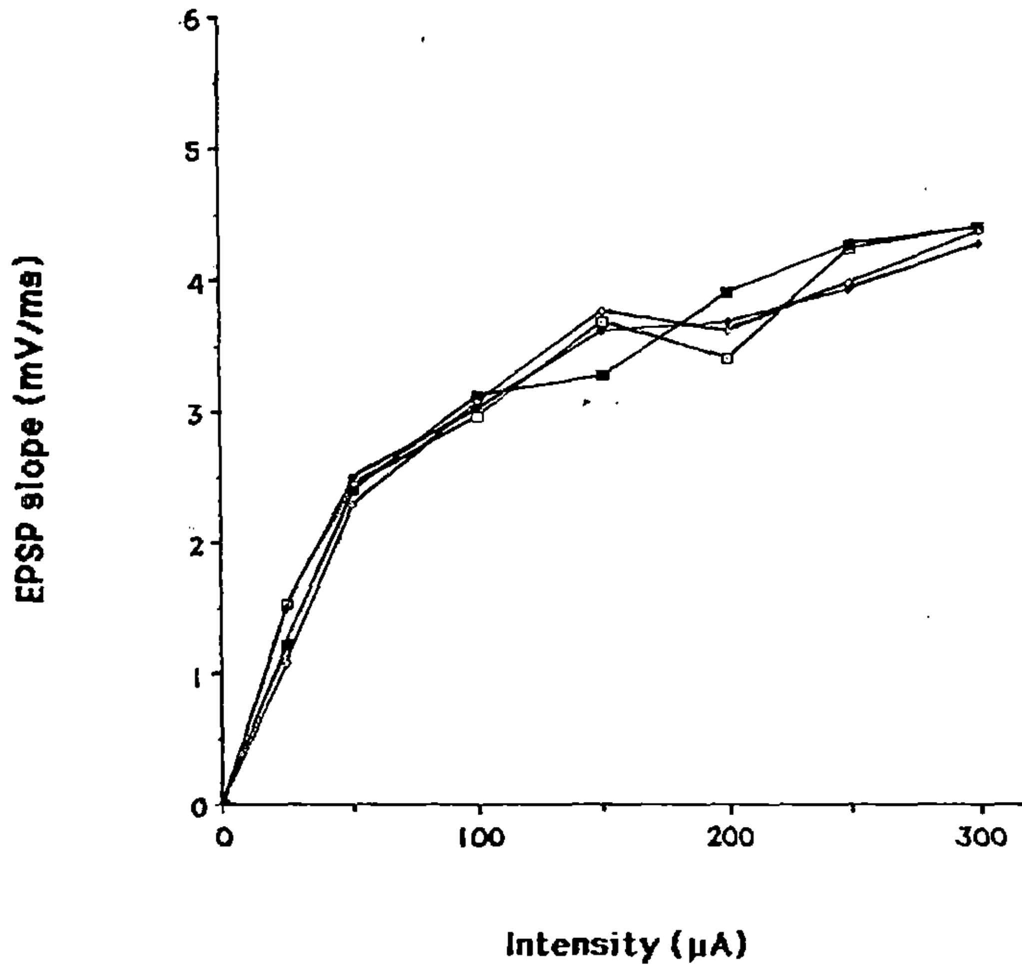
APPENDIX A

Bipolar vs. Monopolar Stimulation Electrodes.

In the present bipolar technique only the cross-section of the electrode tips was uninsulated and the tips were aligned in the same coronal plane (perpendicular to the trajectory of the PP). With the present monopolar technique only the cross-section of the depth electrode was uninsulated and the stimulation return, a stainless-steel screw tapped into the skull, was completely uninsulated. The properties of bipolar stimulation are less clearly understood than monopolar, primarily because one of the bipolar tips provides cathodal stimulation while the other tip provides anodal stimulation. The characteristics of the electric field surrounding the tips are therefore difficult to determine (Yeomans, 1990). An advantage of bipolar stimulation is that during simultaneous stimulation and electrical recording there is usually a smaller recording artifact associated with delivery of each stimulus pulse. In monopolar stimulation with the tip of the depth electrode being the cathode and the skull screw the anode, only the depth tip stimulates the brain (Yeomans, 1990). This is due to two factors: the uninsulated surface of the screw is very larger, thus the effective current density is low around the screw, and the screw is outside the pia mater, which has a high resistance (Yeomans, 1990). The monopolar technique probably produces less damage for two reasons: 1. the diameter is less than the twisted, bipolar arrangement, and 2. the skull screw (rather than a depth electrode) serves as the anode (this obviously reduces damage to the brain produced by anodal metal ion deposition) (Yeomans, 1990). There has been no *definitive* advantage established for either bipolar or monopolar stimulation techniques.

APPENDIX B

Representative EPSP Slope I/O Curves Recorded on Four Consecutive Days During the Pretraining Phase of Experiment 1.



6
5
4
3
2
1
0
0
100
200
300
EPSP slope (mV/ms)
Intensity (µA)

VITA

Surname: Hoesing Given Names: Janice Mary

Place of Birth: Salem, Oregon, U.S.A. Date of Birth: March 13, 1961

Educational Institutions Attended:

University of Victoria 1989 to 1991

University of Lethbridge 1978 to 1980, 1985 to 1987

Degrees Awarded:

B.Sc. University of Lethbridge 1987

Publications:

Sutherland, R. J., Hoesing, J. M., & Whishaw, I. Q. (1990). Domoic acid, and environmental toxin, produces hippocampal damage and severe memory impairment. Neuroscience Letters, **120**, 221-223.

Abstracts:

Sutherland, R. J., Rice, H. N., & Hoesing, J. M. (1990). Diencephalic antrograde amnesia for spatial information in the rat. Society for Neuroscience Abstracts, **16**.

Sutherland, R. J., McDonald, R. J., Hoesing, J. M., & Rudy, J. W. (1989). Hippocampal formation and configural learning and memory. Society for Neuroscience Abstracts, **15**.

Hoesing, J., Sutherland, R. J., & Arnold, K. A. (1988). Retrograde amnesia for spatial information following hippocampal, entorhinal, or cingulate cortex damage. Annual Research Conference, Alberta Heritage Foundation for Medical Research, **9**.

PARTIAL COPYRIGHT LICENCE

I hereby grant the right to lend my thesis to users of the University of Victoria Library, and to make single copies only for such users or in response to a request from the library of any other university, or similar institution, on its behalf or for one of its users. I further agree that permission for extensive copying of this thesis for scholarly purposes my be granted by me or a member of the University designated by me. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Title of thesis: Changes in perforant path-evoked potentials in the dentate gyrus in the rat during training in the Morris water task and experience in complex environments.

Author

Janice M. Hoesing

April 16, 1991