THE EFFECTS OF LONG-TERM POTENTIATION OF
THE PERFORANT PATH-DENTATE GYRUS
SYNAPSES ON TONE EVOKED POTENTIALS IN
THE OUTER MOLECULAR LAYER
OF THE DENTATE GYRUS

CENTRE FOR NEWFOUNDLAND STUDIES

TOTAL OF 10 PAGES ONLY
MAY BE XEROXED

(Without Author's Permission)

KAREN-ANN MOORE
THE EFFECTS OF LONG-TERM POTENTIATION OF THE PERFORANT PATH-DENTATE GYRUS SYNAPSES ON TONE EVOKED POTENTIALS IN THE OUTER MOLECULAR LAYER OF THE DENTATE GYRUS

BY

Karen-Ann Moore, B.A.

A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Masters of Science

Department of Psychology
Memorial University of Newfoundland
August 1986

St. John's Newfoundland
Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilm cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-43343-4
Abstract

The effects of potentiation of the perforant path-dentate gyrus synapses on tone evoked potentials in the outer molecular layer of the dentate granule cells were examined in rats. 3 control groups and an experimental group participated in 2 testing sessions (M1 and M2), in which 300 tones (70dB, 10 msec in duration) were presented every 10 seconds against a background noise of 60 dB. Control Group 1 did not receive any experimental manipulations of the perforant path between the 2 sessions. The experimental group received high frequency stimulation of the perforant path (10 trains, 20 msec in duration at a frequency of 100 Hz, every 15 seconds) between the 2 tone presentation sessions. To control for perforant path stimulation, two further control groups were used. Control 2 received low frequency, low intensity stimulation of the perforant path and Control 3 underwent the same high frequency stimulation as the experimental group but did not exhibit EPSP potentiation of the perforant path evoked (PPEP).

Over time, from M1 to M2, the auditory evoked potential (AEP) increased in size. Potentiation inhibited the growth of the AEP during M2. The results suggested that a third variable may have a modulating influence over the AEP at the
level of the molecular layer. A theoretical model, implicating feed-forward and recurrent inhibitory interneurons is presented. This model suggests that presynaptic habituation and postsynaptic potentiation are the processes by which these interneurons exert an influence over the cellular response to a tone. It is further postulated that learning may not only involve direct activation of granule cells, but also active potentiation of inhibitory interneurons.
ACKNOWLEDGEMENTS

I would like to express my sincerest appreciation to my supervisor, Dr. Robert Adamec. His guidance, optimism, and infinite patience helped make my experience as a graduate student very rewarding and memorable. I would also like to thank Drs. Carolyn Harley and John Evans for their advice and constructive comments.

I also thank Memorial University of Newfoundland for providing financial support for me throughout my stay.

Finally, I am grateful for the unconditional support of my parents. Their understanding kept me going though the toughest of times and their confidence in me has helped me realize my goals.
## Table of Contents

<table>
<thead>
<tr>
<th>SECTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Methods</td>
<td>18</td>
</tr>
<tr>
<td>Subjects</td>
<td>18</td>
</tr>
<tr>
<td>Apparatus</td>
<td>18</td>
</tr>
<tr>
<td>Procedure</td>
<td>19</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>27</td>
</tr>
<tr>
<td>Potentiation Analysis</td>
<td>27</td>
</tr>
<tr>
<td>Auditory Evoked Potential Analysis</td>
<td>30</td>
</tr>
<tr>
<td>Results</td>
<td>39</td>
</tr>
<tr>
<td>Discussion</td>
<td>53</td>
</tr>
<tr>
<td>References</td>
<td>70</td>
</tr>
<tr>
<td>Appendix A</td>
<td>82</td>
</tr>
<tr>
<td>Appendix B</td>
<td>90</td>
</tr>
<tr>
<td>TABLE</td>
<td>DESCRIPTION</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Summary Table for the Means and Anova, Performed on the Area of the AEP for the PS Potentiated Group and PS Control group</td>
</tr>
<tr>
<td>2</td>
<td>Summary Table for the Means and ANOVA, Performed on the Peak Height of the AEP for PS Potentiated Group and PS Control Group</td>
</tr>
<tr>
<td>3</td>
<td>Summary Table for the Means and ANOVA, Performed on the Area of the AEP for the EPSP Potentiated Group and EPSP Control Group</td>
</tr>
<tr>
<td>4</td>
<td>SUMMARY Table for the Means and ANOVA, Performed on the Peak Height of the AEP for the EPSP Potentiated Group and EPSP Control Group</td>
</tr>
</tbody>
</table>
# LIST OF CHARTS

<table>
<thead>
<tr>
<th>CHART</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
</tr>
</tbody>
</table>

- 1. Experimental Design
<table>
<thead>
<tr>
<th>FIGURE</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The Efferent and Afferent Connections of the Hippocampus</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Electrical Circuitry Set-Up for Experiment</td>
<td>20</td>
</tr>
<tr>
<td>3a</td>
<td>Determining the Slope of the EPSP</td>
<td>28</td>
</tr>
<tr>
<td>3b</td>
<td>Determining the Amplitude of the PS</td>
<td>28</td>
</tr>
<tr>
<td>4a</td>
<td>Determining the Latency to Onset of the AEP Peak</td>
<td>32</td>
</tr>
<tr>
<td>4b</td>
<td>Determining the Amplitude of the AEP</td>
<td>32</td>
</tr>
<tr>
<td>4c</td>
<td>Determining the Slope of the Tangent of the AEP</td>
<td>32</td>
</tr>
<tr>
<td>4d</td>
<td>Determining the Area of the AEP</td>
<td>32</td>
</tr>
<tr>
<td>4e</td>
<td>Determining the Area/Height Ratio of the AEP</td>
<td>32</td>
</tr>
<tr>
<td>5a</td>
<td>Selection of the AEP based on Amplitude, Time, and Background Activity</td>
<td>35</td>
</tr>
<tr>
<td>5b</td>
<td>Elimination of the AEP from Selected Data based on Amplitude</td>
<td>35</td>
</tr>
<tr>
<td>5c</td>
<td>Elimination of AEP from Selected Data based on Background Activity</td>
<td>37</td>
</tr>
<tr>
<td>5d</td>
<td>Elimination of AEP from Selected Data based on Time Criterion</td>
<td>37</td>
</tr>
<tr>
<td>6a</td>
<td>Average Percent Change in EPSP Slope</td>
<td>41</td>
</tr>
<tr>
<td>6b</td>
<td>Average Percent Change in PS Amplitude</td>
<td>41</td>
</tr>
<tr>
<td>7a</td>
<td>Interaction of Group, Measurement, and Average (A1-A6) for the Area of the AEP</td>
<td>46</td>
</tr>
<tr>
<td>7b</td>
<td>Trend for an Interaction of Group, Measurement and Average for the Peak Height of the AEP</td>
<td>48</td>
</tr>
<tr>
<td>8a</td>
<td>Recording Electrode Placement in the Outer Molecular Layer of the Dentate Gyrus</td>
<td>51</td>
</tr>
<tr>
<td>8b</td>
<td>Stimulating Electrode Placement in the Perforant Pathway</td>
<td>52</td>
</tr>
<tr>
<td>9</td>
<td>The Role of Feed-forward Inhibitory Interneurons as a Modulating Influence over Changes in the AEP over Time</td>
<td>62</td>
</tr>
<tr>
<td>10</td>
<td>AEP, PPEP-1, and Histological Placement of the Recording Electrode of Rat-70 of Control-2</td>
<td>83</td>
</tr>
<tr>
<td>11</td>
<td>AEP, PPEP-1, and Histological Placement of the Recording Electrode of Rat-54 of Control-1</td>
<td>84</td>
</tr>
<tr>
<td>12</td>
<td>AEP, PPEP-1, and Histological Placement of the Recording Electrode of Rat-76 of Control-2</td>
<td>85</td>
</tr>
<tr>
<td>13</td>
<td>AEP, PPEP-1, and Histological Placement of the Recording Electrode of Rat-60 of PS Experimental Group</td>
<td>86</td>
</tr>
<tr>
<td>14</td>
<td>AEP, PPEP-1, and Histological Placement of the Recording Electrode of Rat-60 of PS Experimental Group</td>
<td>87</td>
</tr>
<tr>
<td>15</td>
<td>Comparison of Electrode Placements in Present Study to those of Deadwyler and Percent of Maximal AEP Observed</td>
<td>88</td>
</tr>
</tbody>
</table>
INTRODUCTION

Electrophysiological alterations in the nature of the central nervous systems of animals have been implicated in information processing and storage (Farley & Alkon, 1983; Teyler & DiCennna, 1984a; Thompson, 1983; Thompson, Berger, & Madden, 1983; Galey, Jeantet, Destrade & Jafford, 1983; Barnes, 1979; Thompson, Berger, Cegavske, Patterson, Rodman, Teyler & Young, 1976; Kandel & Spencer, 1968). The areas of the brain in which changes in synaptic efficiency have been observed are also the same areas in which lesions produce deficits in learning and memory (Teyler & DiCennna, 1984a; Thompson, 1983; Iversen, 1976; Isaacson & Pribram, 1975; Thompson & Langer, 1963). The limbic system, specifically, is one such area (Berry & Thompson, 1979; Thompson, 1983). The main structures which form the limbic system are the hypothalamus, the amygdala, the septum and the hippocampus. Lesions of some structures in this system produce deficits in learning and memory (Toga & Lothman, 1983; Thompson, 1983; Berry & Thompson, 1979; Niki, 1966; Thompson & Langer, 1963). Considerable attention has been focused on the hippocampus (HC) and its proposed involvement in learning. Studies have shown that the ability of an animal
to perform efficiently in a conditioning paradigm diminishes when the HC is lesioned (Collier & Routtenburg, 1984; Harris, Lasher & Steward, 1978; Kimble & Kimble, 1965). Functional changes of normal activity in the intact HC, or activity resulting from events which change the normal functioning of the HC, have also been studied in animals performing in conditioning tasks (Winocur & Gilbert, 1984; Thompson et al., 1983; Olton, 1978; Berger & Orr, 1982; Swanson, 1983; McNaughton, 1983; Jaffard & Jeantet, 1981; Barnes, 1979; Thompson et al., 1976; Izquierdo, 1975). Examination of hippocampal alterations in conditioning paradigms is aided by its relatively simple cellular organization. Because of the cytoarchitecture of the HC, it is possible to study the physiological, as well as the behavioural aspects of the stimulus-response relationship.

The hippocampus proper consists of four cell populations which lie medial and ventral to the rhinal fissure. Each of these are connected in such a way that a simple lamellar organization results (Swanson, 1983; 1982; Andersen, 1983; Breitenberg & Schuz, 1982; Kandel, Spencer & Beinley, 1978; Andersen et al., 1971). The cell populations which make up the HC are the subicular complex, Ammon's Horn (fields CA1 to CA3) and the dentate gyrus.
The dentate gyrus receives two of the major afferent pathways to the hippocampus. The entorhinal cortex projects fibres via the perforant pathway to synapse on the outer two-thirds of the dentate granule cell dendrites (Teyler & Dicenna, 1984a; Wyss, 1981; Steward & Scoville, 1976; Goldwitz, White, Steward, Lynch & Cotman, 1975; Hjorth-Simonsen, 1972; Andersen, Bliss & Skrede, 1971).

The medial septal projection forms the second afferent pathway to the HG. These fibres synapse on the inner most part of the molecular layer of the dentate gyrus (McNaughton & Miller, 1984; Steward & Scoville, 1976; Swanson, 1978; Berger & Thompson, 1977; Meibach & Siegal, 1977; Nafstad, 1967).

As information flows through the dentate gyrus-hippocampal formation, a trisynaptic circuit is formed in each of the lamellar divisions: Activation of the circuit originates in the entorhinal cortex. Fibres from the medial and lateral parts of the entorhinal cortex form the medial and lateral perforant path, respectively, and synapse on the outer molecular layer of the dentate granule cells. The dentate gyrus consists of an upper and lower blade of granule cells. The hilar region of the dentate is located between these two blades. The large polymorphic cells of the hilus are continuous with the CA3 layer of the HG. These cells provide afferents that form part of the commissural and ipsilateral association systems of the HG (Watson,

Much of the activity in the dentate gyrus is reflected in fields CA3 to CA1 of the HC. Activation of the dentate granule cells results in depolarization of the pyramidal neurons in field CA3. The axons of the granule cells form the mossy fibre system by which the CA3 region is activated. The CA3 sends efferents to the lateral septal nucleus via the fornix. However, within the HC, the axons of the CA3 form the Schaffer collateral system which activates field CA1. The axons of the CA1 project to the subiculum, lateral septal nuclei and the prefrontal cortex. Activation of the HC effects activity in the anterior and lateral thalamic nuclei, mammillary bodies and some cortical areas, via the subiculum (Irle & Markowitsch, 1982a, 1982b; Nautà, 1958). All the major connections within the HC are excitatory and the electrophysiology of the HC is such that activation of the perforant path fibres excites the granule cells which, in turn, excite the CA3 cells and thus discharge the pyramidal cells in area CA1. The trisynaptic circuit is then complete. Figure 1 illustrates these connections, using a flow chart.

Activation of the trisynaptic pathway can be artificially induced. Electrical stimulation of the entorhinal cortex or perforant path will produce excitatory post-synaptic evoked
Figure 1. The Efferent and Afferent Connections of the Hippocampus.
potentials (EPSP's) in dentate granule cells and hippocampal pyramidal cells ipsilateral and contralateral to the site of stimulation (Winson, 1982; Kandel & Spencer, 1978).

The EPSP in the dentate gyrus can be evoked by single pulse stimulation of either the entorhinal cortex or perforant path. This will induce dendritic depolarization of the granule cells. Ionic currents will then flow from polarized to non-polarized regions of the cell membranes resulting in a large extracellular post-synaptic potential. The shape of this evoked field potential or excitatory post-synaptic potential (EPSP) will depend on two aspects of the stimulation-recording situation. The first factor that influences the shape of the EPSP is the strength of the stimulus. This is reflected in the number of synapses activated. The lower the intensity of the stimulus, the smaller the number of synapses that will be activated, and therefore, the smaller the EPSP. The placement of the electrode relative to the area of synaptic termination of the perforant path is the other determining factor of the shape of the EPSP. If the electrode is placed above the sink into which the synaptic current is flowing, the current will be flowing away from the recording electrode tip. This will result in a negative going EPSP. However, if the electrode is placed below this sink, then the polarity of the EPSP changes to a positive-going potential. Perforant path stimulation produces an EPSP in the granule cells with a
latency to onset of 2.5 msec to 3 msec. If the stimulus intensity is sufficiently high, a group of cells may be depolarized to threshold causing them to fire in near synchrony. When this occurs, a population spike will be superimposed on the EPSP. Its polarity will be opposite to that of the EPSP.

It has been found that both the EPSP and the population spike reflect the synaptic events taking place. (McNaughton, Barnes & Andersen, 1981; Barnes, 1979; Bliss & Lomo, 1973; Bliss & Gardner-Medwin, 1973; 1971). Using relatively large, chronically implanted electrodes, the evoked field potentials have been shown to be stable for long periods of time in the trisynaptic pathway. This property allows hippocampal activity to be monitored in the freely moving animal. These aforementioned factors permit the recording of long lasting changes of hippocampal responses to excitatory input in chronic behavioural paradigms.

There are several processes which alter the synaptic efficiency of the connections of the HC. Facilitation is one such process. This is a growth in the EPSP during high frequency repetitive stimulation (Dunwiddie, 1980). A large enhancement of the EPSP as a result of high-frequency stimulation is referred to as post-tetanic potentiation (Bliss & Lomo, 1973). Although this begins after the termination of stimulation, it is not a long-lasting change in synaptic response. Long-term potentiation (LTP) is,
however. This process is the focal point of much of the research in synaptic plasticity.

LTP can be defined as a lasting increase in cellular response to a stimulus (Teyler, Goddard, Lynch & Andersen, 1982; Baudry & Lynch, 1980). This increased response is a result of high frequency stimulation of an afferent pathway of a specific cell population. LTP differs from the process of post-tetanic potentiation in that LTP begins later and lasts longer. Although LTP is usually induced by high frequency stimulation, in the range of 100 Hz to 400 Hz, it can also be produced by relatively low frequency stimulus trains (Skelton, Miller & Phillips, 1983; Teyler et al., 1982; Lea, 1980; Matthias, Ruehrich, Ott, Matthies & Matthies, 1986). Although there are many pathways in the limbic system which support LTP (Racine, Milgram & Hafner, 1983), the area exhibiting the largest degree of LTP is the HC. LTP in the HC has been proposed as a possible model for modulation of information processing in the central nervous system (Skelton, Miller & Phillips, 1985; Racine et al., 1983; Brown & McAfee, 1982; Swanson, 1983; Andersen et al., 1966).

Initial investigation of long-term potentiation focussed mainly on one of the afferents of the HC, the perforant pathway. When high frequency stimulus trains were used to activate the perforant pathway, the response of post-synaptic dentate granule cells to single pulse
activation was enhanced. The potentiated monosynaptic field potential of the granule cells of the dentate is characterized by several features. The latency to onset of the EPSP decreases and its slope increases. If the population spike is absent prior to potentiation, sometimes it will appear after potentiation. However, if the population spike is present prior to potentiation, the amplitude of the population spike increases after potentiation. The latency to onset of the population spike also decreases (deJonge & Racine, 1985; Skelton et al., 1983; Goddard, 1982; Thompson, 1982a; Andersen, 1980; Bliss, 1979; Douglas & Goddard, 1975; Bliss & Lomo, 1973; Bliss & Gardner-Medwin, 1973; 1971; Lomo, 1971a; 1971b; 1966; 1967).

Combining the recent research into the electrophysiology of learning and memory and that of long-term potentiation, several studies have been conducted drawing parallels between the two. They focus on the activity of the cells in the HC, with and without the induction of LTP, and their relation to rate and level of learning.

Classical conditioning of the nictitating membrane (NM) response is used by many investigators. This response is studied mainly in the rabbit, (Berger, 1984; 1982; Thompson, 1982b; Weisz, Clark, Wang & Thompson, 1982; Berger et al., 1980; Berry & Thompson, 1979; Berger &
Thompson, 1978a; 1978b; Disterhoft, Kwan & Lo, 1977; Berger et al., 1976; Cegavske, Thompson, Patterson & Gormazano, 1976; Gormazano, Kehoe & Marshall, 1962), but also, has been observed in the cat (Patterson, Berger & Thompson, 1979). Corneal stimulation causes the reflexive extension of the NM. By presenting the animal with a number of conditioned stimulus (CS)-unconditioned stimulus (UCS) pairings and then presenting the CS alone, the NM can be conditioned to respond prior to the onset of the UCS. More specifically, the tone CS is presented for 350 msec. The UCS is an airpuff to the cornea with a duration of 100 msec and follows the CS onset by 250 msec. As the rabbit associates the tone with the airpuff, NM response eventually begins to occur prior to the onset of the UCS, but after the CS.

By measuring the activity of specific cell populations in the HC during learning, several investigators have illustrated a positive relationship between the level of cell activity and the rate of learning (Segal, 1977; 1972; 1971; Black, 1975; Segal & Disterhoft, 1972). Berger, Alger & Thompson (1976), using post-stimulus histograms, showed that hippocampal unit activity increases during NM conditioning. Although this activity is not present initially, it begins to develop rapidly after the first few trials. As conditioning continues the activity in the HC not only increases but also its onset latency decreases. The pattern of hippocampal unit activity
temporally precedes the behaviourally conditioned NM response and yet parallels its development. Thompson (1982b), Berger, Laham and Thompson (1980) and Berger and Thompson (1978b) provide further support for this correlation. Thompson (1984) suggests that this activity forms a 'temporal model' of the learned behavioural response because hippocampal activity parallels, in amplitude and time, the NM behavioural response. This does not occur in animals given random stimulus CS presentations. Berry and Thompson (1978) found not only that the HC unit response correlates with rate of behavioural conditioning, but also, using specific features of the HC electroencephalogram (EEG), that the rate of learning could be predicted. Higher proportions of hippocampal slow, rhythmic activity (large amplitude almost sinusoidal waveforms of 3 Hz to 7 Hz) predicted faster rates of conditioning. EEG's with high proportions of frequencies of 8 Hz to 22 Hz predicted slower rates of learning.

Thompson (1982a), also using the rabbit NM response in a classical conditioning paradigm, demonstrated that the monosynaptic response of the dentate granule cells to electrical stimulation of the perforant path increased as a result of learning. This increase was positively related to the acquisition of the behavioural response.

Increased hippocampal activity during conditioning is, possibly, a naturally occurring form of long-term...
potentiation. The increase in the monosynaptic field response without prior tetanization of an afferent pathway (Thompson, 1982) and long lasting increases in cell activity (Berry & Thompson, 1979) provide support for this proposal. Berger (1984) potentiated the perforant path-dentate gyrus synapses prior to conditioning the HM response in rabbits. He found that the rate of acquisition of the behavioural task was increased. This was also accompanied by increased unit activity in the HC. From this, Berger suggested that the mechanisms which underlie increased hippocampal unit activity during normal learning are the same mechanisms that underlie LTP.

Another form of synaptic plasticity associated with learning has been observed by Deadwyler and his associates (Deadwyler, 1982; West, Christian, Robinson & Deadwyler, 1982; 1981; Deadwyler, West & Robinson, 1981a; 1981b; Deadwyler, West & Lynch, 1979a; 1979b). Alterations in an auditory evoked potential (AEP), appearing in the molecular layer of the dentate gyrus in rats, have been investigated during learning. As learning develops, the shape of the AEP changes. This AEP consists of two major negative components and a reciprocal relationship exists between their peak amplitudes and the level of learning the rat has attained.

Initially in Deadwyler's experiments, rats underwent operant conditioning. A nosepoke response to a tone was
reinforced with water reward. Following this, the rats were trained in an auditory discrimination task. It is within the discrimination conditioning paradigm that the AEP develops. During initial stimulus presentation (usually an auditory tone between 2.4 and 3.5 kHz), an early negative peak (N1) is evoked by the tone. It has a latency to onset and duration of 15 to 20 msecs. The N1 is largest during random tone presentation to naive animals and during the early stages of conditioning. The second negative peak (N2) of the AEP has a latency to onset of 45 to 55 msecs following stimulus presentation. It lasts between 60 to 80 msecs. The N2 is almost absent prior to conditioning but its amplitude increases as the conditioning process continues. It then stabilizes at a maximum amplitude when the discrimination task is learned to criterion level. As N2 grows during conditioning, the N1 amplitude decreases until it eventually disappears. During extinction, N1 reappears, whereas, the N2 decreases until it disappears.

Deadwyler suggests that the appearance of each component is related to the behavioural significance of the tone. During the early stages of conditioning and during random tone presentation, the tone is novel and unpredictable. The N1 appears at this point. If the stimulus is presented at regular intervals and is reinforced, the amplitude of N1 rapidly attenuates (Deadwyler, 1981). As conditioning continues, the tone is associated with water
reward and therefore takes on motivational significance. During the gradual increase in significance of the tone to the rat, the N1 disappears and the N2 grows larger in amplitude until it reaches a maximum at a criterion level of learning.

Depth profile studies (Deadwyler et al., 1981) reveal that the amplitude of the N1 component is maximal 0.15 mm above the granule cell layer. As the electrode is moved to more ventral positions in the granule cell layer and into the hilar region, the N1 amplitude gradually decreases until it inverts to a positive waveform. At its maximum, the amplitude of the N1 is approximately 300 microvolts. The maximum amplitude of the N2 component is also approximately 300 microvolts. The N2 maximum is located approximately 0.05 mm from the granule cell layer in a region of dense commissural-associational synaptic contacts. As the electrode is moved toward the granule cell layer, the N2 does not reverse. This suggests that the sources of the N1 and N2 components are different.

Granule cell discharges in response to the tone occur most consistently during the N2 component. During individual trials granule cells sometimes respond prior to the onset of the N2 component but their onset always follows the N1 peak (Deadwyler et al., 1979b). This suggests that increased granule cell activity is also related to the level of learning, which is reflected in the appearance of N2.
Lesion studies (Deadwyler et al., 1981a) suggested that the pathways into the HC which are involved in the production of the AEP are the perforant pathway and the medial septal pathway. Abolition of the N1 was produced in naive rats by lesioning the entorhinal cortex bilaterally. Within the tone discrimination paradigm, animals that learned to respond to criterion levels produced an N2 component after the lesioning, but never exhibited the N1 component of the AEP. Extinction produced a decrease in N2 and, still, N1 did not appear.

Lesioning the septum seemed to eliminate a modulating influence of the septum on the N2 component in the dentate. During random tone presentation to the lesioned naive animals, both the N1 and N2 components were observed in the molecular layer. However, the amplitude of the N2 was larger in the naive animals than in conditioned animals without lesions. As the lesioned naive rats learned to perform in the conditioning paradigm this difference disappeared. That is, the N2 of the lesioned rats decreased to the normal amplitude of a conditioned rat. The data suggest that N2 is not dependent on perforant path activation but is somehow modulated by the medial septal projection. Deadwyler suggests that the medial septum is involved in mediating the development of N2. In naive animals, the septum exerts a tonic suppression over the cell population in the hippocampus. During conditioning, however, this
suppression is released, leading to the appearance of N2. Following lesioning, the rat is able to learn the discrimination task however the effect of these lesions on the rate of learning is not mentioned.

The plasticity of the dentate granule cells in response to both sensory and electrical stimuli provides a basis for studying the processes of learning. Alterations in the shape of the AEP, examined by Deadwyler and colleagues, have been suggested as reflecting working memory during a discrimination task. Deadwyler suggests that the disappearance of N1 is an habituation response to a novel stimulus. The N1 has been shown to attenuate quite rapidly when the temporal distribution of the tones is constant (Deadwyler et al., 1981a). It has not been reported, however, whether the appearance and extinction or habituation of the N1 is dependent on the development of the N2 component.

To investigate the relationship between N1 and the processes of learning, an habituation paradigm was developed to investigate the N1 component of the AEP independent of the discrimination learning paradigm. This procedure eliminates the influences of the N2, since N2 appears only during conditioning. If the N1 component is primarily a function of tone novelty then, as the rat habituates to fixed interval tone presentation, the N1 should decrease in amplitude until it is not present.
Given that the N1 component of the AEP has been shown to originate over the perforant pathway, studies examining the effects of LTP at the perforant path-dentate gyrus synapses (Teyler, Goddard, Lynch & Andersen, 1982; Lee, 1983; Dunwiddie, 1980; Douglas, 1976; Lee, Schottler, Oliver, Lynch, 1980; Bliss & Lomo, 1973; Bliss & Gardner-Medwin, 1973; 1971) would suggest that the N1 should be altered by the induction of potentiation. The research showing that the rate of learning increases with potentiation and that learning induces potentiation would support the hypothesis that the N1 should attenuate faster following potentiation through increased granule cell activity. If this correlates with a diminished N1, as it does in the discrimination task, then it could be suggested that the habituation of N1 is dependent on increased cellular activity in the dentate gyrus. During the discrimination task increased cellular activity is usually associated with the appearance of N2. If increases in LTP induced granule cell activity do not affect the attenuation of N1, it may be concluded that the N1 is not affected by increased cellular activity.

The present study examines the normal course of change in the N1 component of the AEP in an habituation paradigm and investigates the influence of perforant path potentiation along the same pathways as those of the N1.
METHODS

Subjects:

Twenty-two female Sprague-Dawley rats (230-260 grams) from Charles River Canada were assigned to an experimental or one of two control groups. The experimental group contained 9 animals. The first control group (C1) and the second control group (C2) contained 7 and 5 rats, respectively. Prior to surgery, all animals were housed in polycarbonate cages in groups of 3 or 4. Following surgery, each rat was housed separately.

Apparatus:

Subjects were tested in a plexiglass box (23 cm x 20 cm x 53 cm) with a stainless steel removable floor. This testing box was placed inside an electrically shielded (copper-mesh) wooden cage, where both the surgery and testing procedures took place.

Presentation of tones was controlled by a Digital PDP11/03 computer which triggered a Hewlett-Packard 3310B function generator. The function generator produced a sine wave that was amplified through a Heathkit HIF1 Stereo amplifier. The amplifier output was fed to a loudspeaker, which was centered 5 cm directly below the testing cage floor. The position of the loudspeaker provided for a relatively even distribution of sound in the box. A sound level meter was positioned 4 cm above the testing cage floor in various places and it was determined that the sound was evenly...
distributed throughout the cage at the level of the ears of the rat.

Electrical stimulation of the perforant path (PP) was initiated by a W.P.I. (Model 831) stimulator, under the control of the PDP11/03. Stimulus pulses were biphasic constant current square waves delivered by a photon coupled constant current unit (W.P.I. Model 600).

The first stage of recording amplification was provided by Grass P15 AC amplifiers with low pass and high pass filters set at 10 Hz and 3 kHz, respectively. The P15 amplifiers were located inside the copper-meshed recording chamber. Tektronix AM 502 differential amplifiers provided the second stage of amplification. Signals were further amplified by Tektronix 5A18N dual trace oscilloscope amplifiers. The signal from the oscilloscope amplifiers was then passed through an analogue to digital converter. Digitized signals were averaged and stored by the PDP11/03. Data were also stored on an Ampex PR280 FM tape recorder. Figure 2 illustrates the electrical circuitry of the experimental set-up.

Procedure

Surgery:

Subjects were anaesthetized with a combination of sodium pentobarbital (Somnotol, 57.5 mg/kg) and ketamine hydrochloride (Ketalar, 65.13 mg/kg), intraperitoneally. Lidocaine hydrochloride (20 mg/mL) was infused around the incision area, both during and following surgery.
FIGURE 2
Electrical Circuitry Set-Up for Experiment
The heads of the rats were positioned in the stereotaxic apparatus using a nose clip. The nose clip was used, rather than the conventional ear bars, to avoid the possibility of breaking an eardrum. Head position was adjusted in a horizontal plane with the aid of a level.

A bipolar Plastic Products (Model MS303/2) stainless steel electrode was placed in the perforant path. The coordinates for the stimulating electrode were 7.2 mm posterior to bregma, 4.1 mm lateral to midline, and 3.5 mm below dura. This electrode served three purposes. These were: i) to stimulate the perforant path during surgery, in order to establish the placement of the recording electrode (ii) to stimulate the perforant path with single pulses, so that perforant path evoked potentials (PPEP's) could be sampled at various times throughout the experiment and (iii) to potentiate the perforant path-dentate gyrus synapses.

A monopolar teflon-coated tungsten electrode was placed in the molecular layer of the dentate gyrus of the dorsal hippocampus. Each recording electrode had a tip diameter of 10-20 um and an impedance of 1-3 megohms. The electrode was placed 3.5 mm posterior to bregma and 2.0 mm lateral to midline. Earlier pilot studies, placing the electrode at a depth where Deadwyler reported a maximum N1 produced variable EPSP's. Therefore, the location of the electrode in the dorsoventral axis was fixed when a stable
PSEP was evoked indicating an electrode position just below both the PP synapses and the point of maximum M1 depth.

Ground and reference electrodes were attached to two skull screws located contralaterally to the dentate and perforant path electrodes. Ten subjects had teflon-coated stainless steel EMG electrodes placed in the muscle located directly behind one of the ears.

When all electrodes had been properly placed, the skull openings were filled with bone wax. An acrylic cement cap was prepared to fix the electrodes. Furacin antibacterial ointment was applied around the cap after it had hardened.

Testing Procedure:

Animals were allowed no less than one week to recover. Three days prior to the first testing session, animals were habituated to the testing box. Pilot studies showed that M1 was largest when the rat was quiet. Therefore, M1 and M2 began only when the rat was quiet.

Measurement I (M1): All groups participated in M1. One-half hour prior to recording M1, the subject was placed in the testing box, with leads attached for the first time. This time allowed the animal to explore and then settle down.

A 4 kHz, 70 dB tone, 10 msec in duration, was presented every 10 seconds against a background noise of 60 dB. Tone evoked activity in the dentate molecular
layer and muscle (where appropriate) was sampled
beginning 20 msec prior to and for 131 msec following
tone onset for 300 trials.

Measurement Two (M2): After completion of M1, no
less than 24 hours and no more than 48 hours elapsed be-
tween the M1 and the second measurement (M2). M2 was
simply a repetition of M1.

Experimental Group:

During the approximate 24 hour interval between M1
and M2, the PP of animals in the experimental group received
high frequency trains to potentiate the dentate field
potential response to single PP pulses. Prior to these
trains an input/output (I/O) curve was established for each
subject.

A baseline PPEP measurement (PPEP-1) was recorded
prior to the I/O or potentiation procedures to establish the
state of the PPEP prior to manipulation of the perforant
path-dentate gyrus synapses. The PPEP measurement recorded
at this point and at all other times during the experiment,
consisted of delivering 5 single pulses with a 200 usec
pulse width to the perforant path once every 10 seconds.
PPEP's were sampled beginning 1 msec prior to and for
72 msec following stimulus onset. The 5 samples were
averaged and stored by the PDP11/03.

1) Input/Output Curve: A PPEP measurement was
recorded from each subject at each of the following
In intensities: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 mA, peak current. The intensity producing a PPEP with a population spike amplitude that was 75% of the maximum amplitude that could be produced was the intensity used to potentiate the perforant path. If a population spike could not be evoked, the intensity which produced 75% of the maximum EPSP was used.

ii) Potentiation: Experimental subjects were given 10 trains, 20 msec in duration. Frequency of stimulation during a train was 100 Hz. Trains were delivered every 15 sec at the intensity indicated above. Three minutes following the trains, a PPEP measurement was recorded. Three minutes following the PPEP measurement, the potentiation procedure was repeated. This procedure continued until the potentiated PPEP reached a plateau. That is, neither the EPSP slope nor the population spike amplitude increased further. Fifteen minutes prior to M2, PPEP-2 was recorded. At the end of M2, a final PPEP measurement (PPEP-S) was recorded.

Control Groups (C1 and C2):

Prior to M2, animals in C1 remained in the cage for the same amount of time as the experimental group. The I/O curve procedure was not undertaken. Perforant path stimulation was not administered either. A PPEP measurement was taken prior to M1 (PPEP-1) and M2 (PPEP-2).
A second control group, C2, underwent the I/O curve procedure and low frequency, low intensity PP stimulation. This form of stimulation controlled for the effects of the I/O curve procedure and PP stimulation received by the experimental group. The number of stimuli received by the animals in C2 was determined by randomly yoking them to 8 of the 11 animals in the experimental group. Each animal received the same number of stimuli as its yoked experimental group subject. Perforant path stimulation was presented in single, 250 uamp pulses with a 200 usec pulse width. Pulses were presented once every 5 seconds. This did not produce potentiation. The intensity which produced 75% of the maximum PPEP was not used because this frequency of presentation in combination with a higher intensity has been shown to induce potentiation in other studies. (Matthies, et al., 1986; Skelton et al., 1983). Although the frequency used has produced potentiation in other experiments, the intensity of the stimulation was sufficiently low not to potentiate the perforant path. Following this procedure, a PPEP measurement was recorded. A final PPEP measurement followed MQ. Chart 1 outlines the basic design of the experiment.

Histology:

All rats were lesioned by passing a 5.0 mamp DC current through the dentate and perforant path electrodes.
<table>
<thead>
<tr>
<th>GROUP</th>
<th>M1 (N=300)</th>
<th>PPEP-1</th>
<th>I/O CURVE</th>
<th>LOW FREQUENCY STIMULATION</th>
<th>HIGH FREQUENCY STIMULATION</th>
<th>PPEP-2</th>
<th>M2 (same as M1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL 1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N=6(4)**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL 2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N=5(2)**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL 3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N=4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EXPERIMENTAL</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N=5(7)**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

"+": participated in the procedure; "-": did not participate in the procedure; "+": Animals in Control group 3 were originally in the Experimental Group but showed less than 115% potentiation of the EPSP. All but one showed potentiated PS, however, and were considered in the PS potentiated group; "**": numbers within brackets represent the number of subjects in the groups used during the analysis of PS potentiation. There were no subjects in Control Group 3 for this analysis; Numbers not starred indicate rats used in EPSP analysis.

**CHART 1**

EXPERIMENTAL DESIGN
for 2.5 seconds. Intracardial perfusion with 10% formal saline followed perfusion with saline. Brains were removed, and left in 10% formal saline until they were sectioned. Tissue was sectioned with a cryostat in 38μm thick sections and stained using a metachromatic cresyl violet procedure. Stained sections were examined to verify the location of perforant path and dentate gyrus electrodes.

**Statistical Analysis**

**I. Potentiation Analysis**

The slope of the EPSP and the amplitude of the population spike (PS) were analyzed to compare changes in each measurement of the perforant path evoked field potential (PPEP). The slope of the EPSP was measured by subtracting the point of initial rise of the EPSP from the point prior to PS contamination of the EPSP and dividing by the number of sampled points between them. The onset of the PS contamination of the EPSP was determined using methods outlined by Adarac and Douglas (1976). The point at which the standard deviation curve of the PPEP average increased above baseline was considered a conservative measure of the beginning of population spike contamination of the EPSP.

The amplitude or peak height of the population spike was determined by the PDPII computer. The method involved drawing a tangent from the onset of the population spike to its offset. The amplitude of the PS is represented by the height from the point of maximum negativity of the PS to the
Figure 3a illustrates the points, along the initial rise of the EPSP, used to determine the slope of the EPSP: "a": the point of initial rise; "b": a point prior to contamination by the population spike; "c": averaged PPEP; "d": standard deviation curve of the PPEP.

Figure 3b illustrates how the amplitude of the population spike was determined. "a": the tangent drawn from population spike onset to population spike offset; "b": the amplitude of the population spike; "c": averaged PPEP.
FIGURE 3A
Determining the Slope of the EPSP

FIGURE 3B
Determining the Amplitude of the PS
Each parameter value was expressed as a percentage of the initial value obtained in PPEP-1. All PPEP analyses were performed on percentage data and not on raw data values. Subjects were considered in the experimental group if the degree of potentiation in PPEP-2 was at least 115% of the initial value in PPEP-1. Subjects were divided into groups to explore any changes specifically related to PS or EPSP potentiation. Preliminary analyses indicated that four rats in the experimental group did not show potentiation of the EPSP. Those animals were then considered as a third control group during comparison of the AEP of the EPSP potentiated rats and the control groups. Control group three, therefore, received the same potentiation procedure as the experimental group, but did not exhibit EPSP potentiation of the PPEP. A third control group was not selected for the analysis of the PS because all experimental animals exhibited population spikes showing a 15% or more increase from PPEP-1. The control groups showed no changes from PPEP-1 to PPEP-2.

II. Auditory Evoked Potential (AEP)

a) Unselected Data:

Each measurement of evoked cell response to the 300 tone presentations was averaged over 50 consecutive trials.
blocks. Six averages (Al'-A6'), therefore, represented the AEP over time for each measurement. Each average was analyzed using five parameters of the AEP. i) Peak latency (PL) was determined as the point in time, after the tone onset, when the AEP reached its maximum amplitude. ii) Peak height (PH) represented the amplitude of the AEP. This was measured using the same methods as those used to determine PS amplitude. iii) The slope (SL) measured the rate of change of the tangent used to determine the PH measurement. This parameter indicated any change in averaged activity prior to or following the appearance of the AEP. iv) Area (AR) was used as an indication of AEP size and was represented by the area below the tangent. v) The Area divided by Height ratio (A/H) indicated any changes in the relative shape of the AEP. Figures 4a to 4e illustrate how each parameter of the AEP was determined.

Each average for both measurements, (M1)' and (M2), was expressed as a percentage of the initial average value (Al) of M1. A three-way ANOVA was conducted on each of the five parameters, comparing changes in the AEP both within and between each group.

b) Selected Data:

It was noted that the N1 did not appear in every trial. Therefore, although the five parameters may not have indicated any change as a result of potentiation, there may have been a change in the frequency at which the N1
Figure 4a: "a": (PL), the latency to the peak of the AEP is determined along the time base from the beginning of the averaged sweep to the point of maximum negativity of the AEP.

Figure 4b: "a": (PH), the amplitude of the AEP is determined by using the slope of the tangent, as in Figure 4c, and dropping a perpendicular line from the tangent to the point of maximum negativity of the AEP.

Figure 4c: "a": (SL), the slope of the tangent is determined by establishing \( \frac{\Delta y}{\Delta x} \), where \( \Delta y \) is the change in height from the onset of the AEP to the offset of the AEP, and \( \Delta x \) is the change in time from the onset of the AEP to the offset of the AEP.

Figure 4d: "a": (AR), the area of the AEP is represented by the area below the tangent, as determined by the SL parameter.

Figure 4e: The Area/Height (AH) ratio is determined by dividing the AR parameter by "a".
FIGURE 4A
Determining the Latency to Onset of the AEP Peak

FIGURE 4B
Determining the Amplitude of the AEP

FIGURE 4C
Determining the Slope of the Tangent of the AEP

FIGURE 4D
Determining the Area of the AEP

FIGURE 4E
Determining the Area/Height Ratio of the AEP
appeared. Based on this premise, AEPs were selected from each block of 50 trials. Only the trials where N1 appeared were considered within a specified average.

The criteria used to determine whether or not an N1 appeared during a specific trial were: i) The N1 must fall within or below the range of the 95% confidence limit of the original 50 block average. If the N1 fell above this confidence limit, it was not selected. ii) The amplitude of the AEP must be larger than the normal background activity. If the N1 could not be distinguished from background levels of activity, it was not selected. iii) The N1 must fall within the same time range, specified by the 95% confidence limit of the original 50 trial average. Potentials appearing prior to or after this time range were not considered for the selected data. Figures 5a to 5d illustrate these three criteria.

The total number of N1's for each block of 50 trials was recorded. ANOVAs were performed on the frequency of occurrence of the N1 during each block of 50 trials over M1 and M2 to determine if the frequency with which the N1 appeared was altered between or within the four groups.

The five parameters were redetermined for the selected averages (Al-A6) for M1 and M2 and data were expressed as a percentage of the initial value found for average Al from M1. As with the unselected averages, a 3-way ANOVA was performed for each of the five parameters of the
Figure 5a: Illustrates an individual AEP trial that would be selected to be averaged based on the criteria of amplitude, background activity and time base.

Figure 5b: Illustrates an individual AEP trial that would be eliminated when selecting averages based on the amplitude criterion.

"a": 95% confidence limit of a specified average
"b": Specific trial which was considered in the original unselected average.
Figure 5c: Illustrates an individual AEP trial that would be eliminated when selecting averages based on the background activity criterion.

Figure 5d: Illustrates an individual AEP trial that would be eliminated when averages based on the time base criterion.

"a": 95% confidence limit of specified average.
"b": Specific trial which was considered in the original unslected average.
FIGURE 5C
Elimination of AEP from Selected Data based on Background Activity

FIGURE 5D
Elimination of AEP from Selected Data based on Time Criterion
AEP to compare any changes in the selected AEP averages between or within groups.

c) Correlating EMG Evoked Activity with the AEP:

There was a possibility that muscle activity influenced the size, shape, or appearance of the N1. In those rats in which movement related activity was recorded, EMG during a trial was correlated with the corresponding AEP trial of selected data only. The influence of motor movement was covaried out of the AEP. An ANOVA was performed on the AEP selected averages with the EMG influence eliminated. If the results of this ANOVA were different from the ANOVA results of the original selected data, then it could be stated that motor activity during tone presentation did influence the N1.

RESULTS

I. Perforant Path Evoked Potential (PPEP) Data

Subjects in the experimental group were divided into those showing EPSP potentiation and those showing PS potentiation. They were considered for the experimental group only if the degree of potentiation from PPEP-1 to PPEP-2 of the groups was 115% or greater than the initial value of PPEP-1. The EPSP potentiated group contained five rats whereas the PS potentiated group contained seven. Three rats showed both EPSP and PS potentiation and were included in both groups. The number of subjects in the control groups and the experimental groups are given in Chart 1.
The number of rats in the control groups for the EPSP and the PS analyses varied. Only the control rats showing a PS were included in the analysis involving PS potentiation. However, since all PS control rats exhibited EPSPs, they were included in the EPSP control groups during the analysis involving EPSP potentiation. Figure 6a illustrates the change in the slope of the EPSP in each of the groups. Differences were not found between PPEP-1 and PPEP-2 in any of the control groups. There was a 19% average increase in the slope of the EPSP in the experimental group. Figure 6b illustrates the change in amplitude of the population spike from PPEP-1 to PPEP-2 in the experimental PS potentiated group and the two PS control groups. The PS experimental group showed a 25% average increase in the amplitude of the population spike (PS). The PPEP measurement in the potentiated groups remained stable from the point in time immediately following the potentiation procedure until the PPEP-S was taken.

II. Auditory Evoked Potential (AEP) Data

A 3-way ANOVA comparing control groups with measurement (M1 and M2) and time (A1 to A6) was performed on the unselected and selected AEP data comparing the control groups only. There were no significant differences between the control groups. Therefore, the data for each of the control groups were combined to increase the number of
Figure 6a: Illustrates the change in the EPSP from PPEP-1 to PPEP-2 in the control groups and experimental group.

Figure 6b: Illustrates the change in the PS amplitude from PPEP-1 to PPEP-2 over all groups.

"a": Control Group 1 underwent no manipulations between PPEP-1 and PPEP-2.

"b": Control Group 2 underwent the I/O procedure and low frequency stimulation between PPEP-1 and PPEP-2.

"c": Control Group 3 underwent the potentiation procedure but did not show potentiation from PPEP-1 to PPEP-2.

"d": Experimental Group underwent the potentiation procedure and showed at least 115% potentiation of the EPSP (Fig. 7a) or the PS (Fig. 7b).
Figure 6A
Average Change of EPSP Slope

Figure 6B
Average Change in PS Amplitude
subjects in the control group. This merging procedure was conducted on both unselected and selected AEP data.

a) Unselected AEP Data

i) Comparing the Control group with the EPSP potentiated group: The 3-way ANOVA contrasting groups, measurement and time performed on each of the five parameters produced only one main effect. The peak height of the AEP increased with repeated tone presentations in both groups. Although A1 to A3 were the same statistically, these three averages differed significantly (p<.05) from A4 to A6, which also were the same statistically. The other four parameters did not change with time or with measurement.

ii) Comparing the PS Control group with the PS potentiated group: When the five parameters were compared between the control and experimental groups, no significant differences were found.

b) Selected Data

i) Frequency of occurrence of the AEP: The AEP occurred, on average, 50% of the time in both M1 and M2. An analysis of variance was performed on the frequency of occurrence of the AEP over each 50 trial block. No significant differences were found between or within groups. This made it possible ANOVAs on the selected AEP data using the same five AEP parameters as those analyzed in the unselected data.

ii) Comparing the EPSP Control group with the EPSP potentiated group: Peak height showed a significant main effect.
over time (F(5,80)=2.840, p<.05) and over each measurement 
(F(5,80)=5.109; p<.05). The main effects of time and 
measurement indicated that the peak height of the AEP 
increased over repeated presentation of the tone. Also, the 
peak height is significantly larger in the second 
measurement (M2) of the AEP in response to 300 tone 
presentations. However, an interaction between time and 
measurement was not observed. Table 4 in Appendix B ill-
ustrates the means and the summary table for the ANOVA 
performed on the peak height parameter for the EPSP groups 
comparing the three EPSP control groups and the EPSP experi-
mental group.

iii) Comparing the PS Control group with the PS poten-
tiated group: When comparing the PS potentiated group with 
the control group, a significant group by measurement by 
time interaction was found with the area of the AEP 
(F(5,45)=2.535, p<.05). Table 1 in Appendix B illustrates 
the means and results of the 3-way ANOVA comparing the 
area of the AEP of the PS control and PS experimental 
groups. Figure 7a shows the significant interaction 
between groups, measurement and time of the area of the 
AEP for the PS potentiated group and its comparative 
control group. In the control group, the area of the AEP 
did not change over repeated tone presentation during M1. 
However, during M2 the area significantly increased over M2 
levels from A2 to A6 (t(45)=3.675, p<.05). Although the area
of the AEP in the PS potentiated group was statistically similar to the control group during M1, following potentiation, the area of the AEP did not change. The analysis of the AEP associated with the EPSP groups did not show a similar interaction to that of the PS group analysis. The EPSP potentiated group data are also plotted in Figure 7A for comparison of the changes in the AEP of the EPSP potentiated group to those of the PS groups. The AEP of the EPSP potentiated group changed similarly to the control group. However, the increase in the area of the AEP in the EPSP potentiated rats is somewhat depressed relative to the control group because of the influence of the 3 PS potentiated rats included in the EPSP potentiated group.

If the area of the AEP was getting larger, it was suspected that the trend in the peak height parameter (F(5, 45) = 1.550, p = .194) would also show a similar pattern. Figure 7b illustrates a similar pattern of the PH parameter of the AEP as that found in the AR parameter. Although this resembles the AR pattern there was no significant interaction found when the peak height was analyzed. Again, the changes in the AEP of the EPSP potentiated group are plotted against those of the PS potentiated group and its comparative control. This graph further exemplifies the measurement and time main effects observed in the PH parameter of the EPSP potentiated group. The changes of the AEP of the EPSP potentiated group are similar to the PS control.
Figure 7a: Illustrates the significant interaction of group by measurement by average (time) for the area of the AEP in the PS potentiation analysis.

"A--A": Control (M1); "A--A": Control (M2);
"o--o": PS Potentiated (M1); "o--o": PS Potentiated (M2);
"o--o": EPSP Potentiated (M1); "o--o": EPSP Potentiated (M2).
FIGURE 7A

Interaction of Group, Measurement, and Average (A1-A6) for the Area of the AEP.
Figure 7b: Illustrates a similar trend in the interaction between groups, measurement and average for the amplitude (PH) of the AEP.

"- - -": Control (M1); "- - -": Control (M2);
"- - -": PS Potentiataed (M1); "- - -": PS Potentiataed (M2);
"- - -": EPSP Potentiataed (M1); "- - -": EPSP Potentiataed (M2).
FIGURE 7B
Trend for an Interaction of Group, Measurement, and Average for the Peak Height of the AEP
group. Tables 2 and 3 in Appendix B present the means and summary table for the FH and AR parameters for the FS and EPSP analysis, respectively.

c) EMG Influence on the AEP:

When motor activity was covaried out of the AEP, the results did not differ from those found in the analyses performed on the selected AEP data. This indicated that the EMG activity did not influence the appearance of the AEP.

IV. Histology:

Recording electrode tips, placed in the dendritic layer of the dentate gyrus, were located in an approximate 125 um range, 100 um above the cell layer. They were located in a 400 um range, 3.5 mm posterior to bregma, and a 225 um range, 2.0 mm lateral to the midline.

The stimulating electrodes were located in a range of 526 um, 7.2 mm posterior to bregma and 4.1 mm lateral to the midline in a 150 um range. Figures 8a and 8b illustrate the positions of the recording and stimulating electrodes respectively.

Figures 10 to 14 in Appendix A illustrate more specifically alterations of the AEP over time for five rats in the three testing groups, their relative PPEP-1's and specific histological placements of the recording electrode.

Figure 15 in Appendix A illustrates similar changes in the AEP with respect to the level of the recording.
Shaded area represents the general placement of the recording electrode within the molecular layer of the DC.

**Figure 2A**

Recording Electrode Placement in the Outer Molecular Layer of the Dentate Gyrus.
electrode above the cell layer in this study as those seen in Deadwyler's depth profile. A "quasi"-depth profile was constructed using the histological placements of all the rats that participated in the experiment. The amplitudes of the AEP's for the rats with similar electrode placements were averaged and plotted against Deadwyler's reported depth profile (Deadwyler et al., 1981). This depth profile is not as extensive as Deadwyler's since placements were restricted to the molecular layer. The PPEP for each of the lesioned areas in the present study is also shown. Although the amplitude of the AEP is, on average, smaller than that reported by Deadwyler, the size of the AEP with respect to the depth of the recording electrode in this study are consistent with those of Deadwyler's.

DISCUSSION

I. Selected versus Unselected Averaging

The results of this study indicate that the appearance of the AEP is often a variable phenomenon. Similar analyses performed on unselected and selected AEP data produced quite different results. When the data were not selected, significant interactions were not observed. In the analysis of unselected data, contrasting EPSP potentiated animals with controls, only the overall amplitude of the AEP was altered. Although this result indicated that the AEP did not habituate over time, it did not suggest that
potentiation affected the AEP. However, analysis of the selected AEP data, comparing the population spike potentiated animals with controls, indicated a significant interaction between groups, measurement and time. While the size of the AEP increased over time in the controls, induction of potentiation inhibited this increase. Since the frequency of occurrence of the N1 did not change during the experiment, the effects observed using selected AEP data are due to changes in size of the N1 when it occurs. Such variability of the AEP requires that analysis of the N1 be performed on selected data.

II. Habituation of the AEP
a) Measurement One (N1):

One of the most interesting observations of this study was that the AEP did not habituate. In fact, with further repetition of tone presentations during M2, the neuronal response to the tone seemed to sensitize at the level of the dentate gyrus. This was reflected in the gradual increase in the area of the AEP from A2 to A6 of M2 in the EPSP controls and the PS controls. This observation conflicts with that made by Deadwyler et al. (1982; 1981a; 1981b). The biphasic aspect of the curves (Fig. 7a & 7b) cannot be explained with the present data. It is suspected that the N1 might continue to grow in such a pattern until it
reached a plateau. The most interesting observation, however, is that the N1 did not habituate.

A possible reason for this discrepancy is that the AEP in the present study is not the N1 recorded by Deadwyler. The electrodes, although in the molecular layer, in most cases were placed closer to the cell layer than the point where Deadwyler reported a maximum N1 amplitude (150 um above the granule cell layer). Most electrodes in the present study were located 25 to 150 um above the cell layer. The lesions allowed only 25 um resolution. On the other hand, with electrodes placed 100 to 150 um above the granule cell layer, 3 control group rats showed increases in the size of the AEP during M2. Thus the precise location of the recording electrode in the molecular layer does not seem to be a determinant of the results found in the present study.

It also seems unlikely that the AEP in this study is not the same potential recorded by Deadwyler. Deadwyler reported an N1 in the area recorded from in this study. Moreover, the similar latency and duration of the tone evoked potentials in both of the studies and the quasi-depth profile constructed from the lesioned rats suggests that the AEP in this study is the same as the N1 reported by Deadwyler.

The fact that the N1 did not habituate in this experiment might be explained by the Waston and Abzug (1978) "effect". This suggests that the changes observed in this experiment might only reflect behavioural changes during the period of
data collection. The differences between groups could simply reflect a change in arousal level due to LTP procedures or their absence. However, it was found in pilot studies, that the NL was usually only seen above background EEG when the animal was not moving and in a relaxed posture. Every effort was made throughout the experiment to make sure that the animal was quiet and still.

Differences in frequency of tone presentation, tone duration and the context in which the tone was presented may also provide an explanation for the discrepancy between the results of this study and Deadwyler's reports on the habituation of NL. Although the tone intensity and frequency were comparable to those employed by Deadwyler, tone presentation was fixed in this study, rather than variable, as in Deadwyler's discrimination conditioning paradigm. The tone duration was also significantly shorter than that used by Deadwyler. Furthermore, in the present study, the tone was not associated with a reward, the animal was not required to respond to the tone in any way, nor was the rat water deprived. In essence, the tone did not take on the same behavioural significance as it did in Deadwyler's studies. In Deadwyler's studies, tone presentation had a significant behavioural impact. This factor may account for the conflicting observations of the changes of the AEP over time in these two studies.
A second possible explanation for not observing AEP habituation may be that the N1 only habituates with the development of the second negative component (N2). Since the N2 was not present in this study but is present in Deadwyler's studies of N1 habituation, it is possible that the habituation of N1 is dependent on the development of N2. This line of thinking suggests that habituation of the N1 in Deadwyler's studies is linked to the conditioning paradigm. Details regarding the observation that the N1 habituates when the tone is presented in fixed temporal intervals without conditioning are unclear.

It is clear, however, that if the AEP in this study is representative of the N1 observed by Deadwyler, then habituation does not occur.

b) Measurement Two (M2):

During M2, the area of the AEP increased above baseline levels following A1 in the controls. This observation is somewhat more difficult to understand given the present state of research into tone evoked potentials in the hippocampus. There does seem to be either a sensitization or disinhibition process occurring. Although neurons have been shown to sensitize in response to cutaneous stimulation (Frank & Fuortes, 1956; Wall, 1959) such sensitization has not been observed in the auditory system. The fact that this increase in the area of the AEP does not occur in the potentiated group, suggests that potentiation of the
perforant path-dentate gyrus synapses somehow inhibits this growth of the AEP.

There is a similarity between the behaviour of the AEP of M2 in the Control group and the Experimental group of this study and that of the N1 during Deadwyler's discrimination conditioning, only if M2 is considered. Naive animals in Deadwyler's studies exhibit large N1's prior to learning the tone discrimination. As these animals learn, the N1 decreases in size. Control animals in this study also exhibit large N1's. In the potentiated animals, the N1 is much smaller than that of the control animals. If the N1 decreases in size as learning continues, and if potentiation increases the rate of learning, as has been suggested, then it might be concluded that the AEP in this study should be smaller in the potentiated animals. Potentiation, in this case may have mimicked a similar process which occurs over the perforant path during Deadwyler's discrimination conditioning. With this interpretation, however, the increase in the N1 over time in the controls is left unexplained.

An alternate explanation for the increased size of the AEP over repeated tone presentations is considered at a later point in the discussion.

II. Changes in the AEP in the PS Potentiated Group versus the EPSP potentiated Group: Analyses of rats showing EPSP potentiation versus those exhibiting PS potentiation suggested
that the inhibition of the growth of the AEP is correlated with those mechanisms underlying PS potentiation. The fact that changes in the AEP are associated with the potentiation of the PS and not the EPSP is curious. Deadwyler has shown in lesion studies that the perfromant path synapses carry the auditory information to the dentate gyrus. Christian, West, and Deadwyler (1985) also have shown that the administration of opiates increases the NI and the EPSP, providing further support that the NI is carried over the perfromant pathway.

In this study, however, the NI is dissociated from the EPSP. That is, the AEP is unaffected when the degree of potentiation of the EPSP is considered.

The conflicting observations between Deadwyler's studies and the present study suggest that different processes might be altering the behaviour of the AEP in this study.

If it is assumed that the AEP observed in the present study is Deadwyler's NI, then a third variable, other than those already mentioned, may be influencing the changes observed. That variable may be feed-forward or recurrent inhibition. Interneurons have been found to project aspinous dendrites to the region where the perfromant path synapses on the dendrites of the dentate gyrus (Ribak & Seress, 1983; Seress & Pokorny, 1981; Amaral, 1978; Andersen, Eccles, Loyning, 1964). They have also been shown to be GABA-ergic (Seress & Pokorny, 1983; Goldwitz, Vincent, Wu & Hokfelt, 1982; Storm-Mathisen, 1978) implying that the role of the
interneurons are inhibitory in nature. The latency of response of these interneurons to afferent stimulation is shorter and the firing threshold is lower than that needed to evoke a population spike in the granule cells via perforant path stimulation (Fox & Ranck, 1981; Knowles & Schartzkroin, 1981). These two characteristics of the interneuron in the molecular layer cannot be justified by the process of recurrent inhibition and therefore, it is suspected that their role is to produce mainly feed-forward inhibition. Some of these interneurons have been associated with both recurrent and feed-forward inhibition (Buzaki & Eidelberg, 1984).

Several authors have used inhibitory interneurons in the molecular layer to explain several aspects of LTP in the HC. Wilson, Levy and Steward (1981) used the presence of the feed-forward inhibitory interneurons to explain the dissociation of the EPSP and FS during LTP. Also, blockade of GABA-mediated inhibition (presumably of interneurons) has been found to facilitate the induction of long-term potentiation and produce larger population spikes lasting longer than those evoked by potentiation induced without GABA inhibition (Wigstrom & Gustafsson, 1985(a), (b); 1983(a), (b); Wigstrom, Gustafsson & Hirany, 1985).

Based on these observations, Buzaki (1984) presents a "double effect" hypothesis of afferent activation at the level of the HC. This hypothesis considers both cellular
discharges through direct excitation of some principal neurons and also the reduction of background discharging of adjacent groups of principal cells via feed-forward inhibition. This effect would increase the signal to noise ratio and would result in a higher probability of signal detection during sensory or electrically evoked afferent stimulation. The overall response of a group of principal cells would depend on the ratio of the strength of direct afferent stimulation and the degree of feed-forward inhibition exerted over the principal cells. In the present study, the recording electrode was placed just below the perforant path-dentate gyrus synapses, and possibly just below the level of the interneurons in the molecular layer. It is possible that the activity recorded at this level in response to afferent stimulation is reflective of the activity of both direct perforant path activation of the granule cells and feed-forward inhibition from the interneurons. These two inputs may modulate the changes observed in the AEP with and without the induction of LTP.

The model, illustrated in Figure 9, considers the role of inhibitory interneurons in the changes of the AEP observed in the present study. Because these interneurons have a lower threshold for firing, afferent stimulation produced by the tone can activate the feed-forward inhibitory interneurons more easily than the granule cells of the dentate. According to the model, the AEP recorded
Figure 9: Illustrates the possible role of feed-forward inhibitory interneurons in modulating changes in the AEP observed in this experiment.

Repeated stimulation of these interneurons produces presynaptic habituation at "A". This decreases the inhibitory influence at synapses "D" and "E", resulting in a larger AEP over time.

Following potentiation, even though presynaptic habituation occurs, (A), increased efficiency of the post-synaptic membrane of the interneurons would be strong enough to counterbalance the disinhibition produced by repeated tone presentations. This would result in little change in the AEP.
FIGURE 9

The Role of Feed-Forward Inhibitory Interneurons As A Modulating Influence over AEP's Multi-Unit Tone Evoked Activity
during M1 is reflective of the depolarization of granule cell dendrites by perforant path-dentate gyrus synaptic activation and hyperpolarization of the same or adjacent dendrites induced by interneuronal firing. It is assumed that the inhibitory interneurons habituate to the repetitive afferent stimulation produced by the tone. Indirect evidence for this assumption is shown in other interneuronal responses in the HC (Misgeld, Sarvey & Klü, 1979; Yamamoto & Chujo, 1978). Also, McCarron and Alger (1985) observed IPSP depression in the CA1 pyramidal cells following a single conditioning stimulus in in vitro preparations. Kandel (1985) also suggests that habituation processes are mediated presynaptically. Therefore, further repetitive stimulation during M2 would cause the interneuronal inhibitory influence to decrease presynaptically. Subsequently, in the control group, the increase in the size of the AEP observed during M2 would reflect this disinhibition process.

Following high frequency stimulation in the experimental group, if both the perforant path-dentate gyrus synapses and the interneuron synapses are potentiated postsynaptically, the normal habituation of the interneurons would be counterbalanced by the increased postsynaptic inhibition exerted by the feed-forward and recurrent interneurons. Little change in the size of the AEP would be recorded during M2. This adapts Buzaki's model (1984) which
suggests that the same interneurons mediate feed-forward and recurrent inhibition.

The two assumptions made at this point in this model are supported, again, by indirect evidence. The first assumption made is that the interneurons have been potentiated. Buzaki and Eidelberg (1982) have found long-lasting increases in interneuronal activity produced by potentiation of direct afferents. The second assumption that the process of potentiation is postsynaptically mediated has been suggested by Scharfman and Sarvary (1985) and Wigstrom and Gustafsson (1985; 1984).

LTP affected AEP growth only in the PS potentiated group. This can be explained by the possible varying degrees of potentiation of the inhibitory interneurons. If PS potentiated rats produce more LTP in the inhibitory interneurons over the recurrent pathway, this would result in heterosynaptic potentiation of the interneuron, which would enhance the potentiation induced over the feed-forward pathway. Therefore, even though the feed-forward synapse onto the interneuron is habituating, the increased excitability of the postsynaptic interneuronal membrane due to heterosynaptic potentiation would counterbalance the normal growth of the AEP due to disinhibition. However, the EFS potentiated group may not have very strong recurrent inhibitory potentiation in the interneurons and therefore insufficient potentiation of
inhibition would not be generated by the feed-forward pathway to counteract the growth of the AEP.

In summary, there are several assumptions made in this model. However, there is indirect evidence to support each of these. The first assumption is that a population of feed-forward inhibitory interneurons is the third variable influencing the AEP. Although direct manipulation of these interneurons has not been tested with respect to the AEP, Christian, West, and Deadwyler (1985) have shown that administration of opiates modify the N1 and N2 components of the AEP. Opiates increased the amplitude of the N1. It is suggested that opioids block inhibitory interneurons causing increased excitability in the hippocampus (Lee, Dunwiddie & Hoffer, 1980). disinhibition, in the model presented, produced by presynaptic habituation of the interneurons would cause the N1 to increase. There is also histological, as well as electrophysiological evidence that implicate the feed-forward inhibitory interneurons in a modulating role in the alterations of the PPEP. Further assumptions are that these feed-forward inhibitory interneurons habituate with repeated stimulation and also potentiate following induction of high frequency trains. Again, there is indirect evidence that lends support to these assumptions (Alger & Nicoll, 1982; Buzaki & Eidelberg, 1982). Finally it is assumed that the process of potentiation is mediated postsynaptically. This assumption allows for the normal habituation of the
feed-forward inhibitory interneurons to occur simultaneously with increased postsynaptic efficiency, leading to increased inhibition following potentiation, which counterbalances habituation.

This theoretical model, although based on indirect evidence, can be tested. It would be necessary to test whether or not inhibition was involved in changes in the AEP. It would be expected that if the processes of recurrent or feed-forward inhibition mediated the changes in the AEP, introduction of GABA agonists or antagonists would alter this inhibition. In the control group, GABA antagonists would increase the size of the AEP because of the blockade of the inhibitory influence. GABA agonists would be expected to decrease the size of the AEP, since increased GABA at the synapses would increase the inhibition exerted by these inhibitory interneurons. In the potentiated rats, GABA antagonists should eliminate the LTP induced suppression of the growth of the AEP during M2. GABA agonists should increase the suppression exerted over the AEP in M2, unless LTP saturates the GABA receptors.

If GABA manipulations alter the AEP, it would then be necessary to establish whether or not these changes in the AEP were mediated by feed-forward or recurrent inhibition. Orthodromic paired-pulse stimulation of the perforant path activates both feed-forward and recurrent inhibitory interneurons (Alger & Nicoll, 1982). Measuring
PS suppression during orthodromic stimulation would indicate the strength of these two forms of inhibition; in combination, paired-pulse antidromic stimulation of the mossy fibres on the other hand, would eliminate the influence of the feed-forward inhibitory interneurons (Buzaki, 1984) The difference between the PS suppression recorded during orthodromic and antidromic stimulation would give the relative degree of feed-forward inhibition involved in the production of the PPEP population spike suppression. By observing the alterations in the AEP during both these procedures, it could be established whether or not feed-forward inhibition played a significant role in the modulation of neural responses to tone presentation at the level of the dentate gyrus.

There are several discrepancies when comparing the AEP in this study to that of the NI in Deadwyler's studies. Firstly, the AEP does not habituate in the present study. In fact, the size of the AEP increased during a second testing session. This observation introduced some doubt as to whether or not the AEP in this study was evoked by the same processes as the NI in Deadwyler's studies. Similar latency and duration parameters and histological evidence presented in this study suggests clearly that the AEP is sufficiently similar to Deadwyler's NI to assume that it is more likely the NI reported by Deadwyler than an AEP from some other source. One way to resolve this conflict would be to replicate the present
study and following to the testing sessions, lesion the entorhinal cortex. If the AEP disappeared, this would provide more conclusive evidence that the AEP in this study was the NI reported by Deadwyler.

Two other possible explanations for the discrepancies are:
i) the context in which the tone was presented (i.e. habituation vs. absolute rate of repetition) and ii) feed-forward inhibitory influences at the level of the dentate gyrus. Habituation of these interneurons accounts for the increase in the size of the AEP in controls. Potentiation of these interneurons accounts for the suppression over the growth of the AEP seen in the experimental group.

The theoretical model presented is supported by studies of these interneurons and their influence on PPEPs. If the model presented is considered as a valid explanation for the behaviour of the AEP observed in this study, a very interesting suggestion with respect to learning can be made, when the animal learns to associate the tone with a behaviourally relevant stimulus, as is the case in Deadwyler's studies, the NI attenuates. It could be implied that NI attenuation during learning reflects the potentiation of interneuronal inhibitory processes. Learning, therefore, may involve not only activation of the granule cells but also the active potentiation of inhibitory interneurons.
References


Barnes, C.A. Memory deficits associated with senescence: A neurophysiological and behavioural study in the rat. Journal of Comparative and Physiological Psychology, 1979, 93, 74-104.


Bliss, T.V.P. Synaptic plasticity in the hippocampus. *Trends in Neuroscience*, 1979, 2, 42-45.


Hjorth-Simonsen, A. Projection of the lateral part of the entorhinal area to the hippocampus and the fascia dentata. Journal of Comparative Neurology, 1972, 146, 219-232.


Irle, E. and Markowitsch, H.J. Connections of the hippocampal formation, mammillary bodies, anterior thalamus and cingulate cortex. Experimental Brain Research, 1982b, 47, 79-94.


Lee, N.S., Schottler, F., Oliver, M., and Lynch, G. Brief bursts of high frequency stimulation produce two types of structural change in the rat hippocampus. Journal of Neurophysiology, 1980, 44, 247-258.

Levy, W.B. and Steward, O. Synapses as associative memory elements in the hippocampal formation. Brain Research, 1979, 175, 233-245.


Lomo, T. Patterns of activation in a monosynaptic cortical pathway: The perforant path input to the dentate area of the hippocampal formation. Experimental Brain Research, 1971a, 112, 18-45.
Lomo, T. Potentiation of monosynaptic EPSPs in the perforant path-dentate granule cell synapse. Experimental Brain Research, 1971b, 12, 46-63.


Patterson, M.M., Berger, T.W. and Thompson, R.F. Neuronal plasticity recorded from the cat hippocampus during classical conditioning. Brain Research, 1979, 163, 339-343.


Scharfman, H.E. and Sarvey, J.M. Postsynaptic firing during repetitive stimulation is required for long-term potentiation in the hippocampus. Brain Research, 1985, 331, 267-274.


Appendix A

Figures 10a-c to 14a-c illustrate in a more condensed fashion the changes in the AEP for specific rats in the control and experimental groups. The PPEP and recording electrode placement in relation to the AEP are also shown.

Rat 70 of Control Group 1 (Figure 10) exhibited the growth in the AEP during M2 from A1 to A6. This was associated with a positive EPSP at approximately 50 μm above the cell layer.

Rat 54 of Control 2 (Figure 11) also shows an increased AEP, however, its electrode placement was located 150 μm above the cell layer of the dentate gyrus. This was associated with a negative EPSP with a positive FS superimposed.

Rat 76, also of Control 2, shows a very small AEP during M1:A1 and yet its size is enhanced by M2:A6. The electrode placement was 75 μm above the cell layer. (Figure 12).

Rat 60, of the experimental FS Group shows little growth in the AEP. It is associated with a positive EPSP and its electrode placement was 75 μm above the granule cell layer. (Figure 13)

Rat 34, also of the experimental FS group, does not show any change in the AEP either and its electrode is 25 μm closer to the cell layer than the placement in Rat 60. (Figure 14)
FIGURE 10: AEP, PPEP-1, AND HISTOLOGICAL PLACEMENT OF THE RECORDING ELECTRODE OF RAT-70 OF CONTROL-2
Figure 12: AEP, PEP-1, and histological placement of the recording electrode of rat 75 of control 2.
FIGURE 13: AEP, PEPP-1, AND HISTOLOGICAL PLACEMENT OF THE RECORDING ELECTRODE

C. RECORDING ELECTRODE PLACEMENT

500 mm

A. AUDITORY EVOKED POTENTIAL (OVER TIME)

B. PEPP-1
Figure 15: Illustrates the depth profile of Deadwyler et al. (1981) study (A—A) compared to the "Quasi" depth profile constructed from the lesion data obtained in the present study ( ). PPEP's are also shown which correspond to each lesion level observed. The scale for each PPEP illustration is 1 mv and 5 msec.
FIGURE 15
Comparison of Electrode Placements in Present Study to those of Deadwyler and Percent of Maximal AEP Observed
Appendix B

Tables 1 and 2 illustrate the means and summary F-tables for the AR and PH parameters, respectively, for the PS analysis. Tables 3 and 4 illustrate the means and summary F-tables for the AR and PH parameters, respectively, for the EPSP analysis.
<table>
<thead>
<tr>
<th>GROUP</th>
<th>MEASUREMENT</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL M1</td>
<td>1.000</td>
<td>1.074</td>
<td>1.091</td>
<td>1.117</td>
<td>0.937</td>
<td>1.020</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>1.224</td>
<td>1.702</td>
<td>1.668</td>
<td>1.455</td>
<td>1.814</td>
<td>1.899</td>
</tr>
</tbody>
</table>
| PS
| Potentiated M1 | 1.000       | 1.087    | 1.152    | 1.165    | 1.067    | 1.097    |
|           | M2          | 1.194    | 1.099    | 1.193    | 1.161    | 1.089    | 1.113    |

<table>
<thead>
<tr>
<th>BETWEEN S</th>
<th>SS</th>
<th>MS</th>
<th>DF</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOT</td>
<td>34.1</td>
<td>3.41</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>GROUP</td>
<td>1.52</td>
<td>1.52</td>
<td>1</td>
<td>0.419</td>
</tr>
<tr>
<td>SWGEP</td>
<td>32.6</td>
<td>3.62</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>WITHIN S</td>
<td>SS</td>
<td>MS</td>
<td>DF</td>
<td>F</td>
</tr>
<tr>
<td>TOT</td>
<td>30.6</td>
<td>0.253</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>MEASUREMENT</td>
<td>2.82</td>
<td>2.82</td>
<td>1</td>
<td>1.475</td>
</tr>
<tr>
<td>GROUP X</td>
<td>2.39</td>
<td>2.39</td>
<td>1</td>
<td>1.249</td>
</tr>
<tr>
<td>MEASUREMENT X SUBJECTS (W)</td>
<td>17.2</td>
<td>1.91</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>AVERAGE</td>
<td>0.391</td>
<td>0.078</td>
<td>5</td>
<td>0.895</td>
</tr>
<tr>
<td>GROUP X</td>
<td>0.443</td>
<td>0.089</td>
<td>5</td>
<td>1.014</td>
</tr>
<tr>
<td>AVERAGE X SUBJECTS (W)</td>
<td>3.93</td>
<td>0.087</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>MEASUREMENT X AVERAGE</td>
<td>0.299</td>
<td>0.060</td>
<td>5</td>
<td>1.094</td>
</tr>
<tr>
<td>GROUP X</td>
<td>0.693</td>
<td>1.39</td>
<td>5</td>
<td>2.535 * (p&lt;0.05)</td>
</tr>
<tr>
<td>MEASUREMENT / AVERAGE X SUBJECTS (W)</td>
<td>2.46</td>
<td>0.053</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 1**

Summary Table for the Means and ANOVA Performed on the Area of the AEP for the PS Potentiated Group and PS Control Group
<table>
<thead>
<tr>
<th>GROUP</th>
<th>MEASUREMENT</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>MEANS (X)</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>M1</td>
<td>1.000</td>
<td>1.045</td>
<td>1.037</td>
<td>1.063</td>
<td>0.958</td>
<td>1.008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>1.287</td>
<td>1.494</td>
<td>1.415</td>
<td>1.367</td>
<td>1.678</td>
<td>1.691</td>
<td></td>
</tr>
<tr>
<td>PS POTENTIATED</td>
<td>M1</td>
<td>1.000</td>
<td>1.085</td>
<td>1.148</td>
<td>1.126</td>
<td>1.010</td>
<td>1.036</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>1.139</td>
<td>1.115</td>
<td>1.195</td>
<td>1.182</td>
<td>1.124</td>
<td>1.124</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BETWEEN S</th>
<th>SS</th>
<th>MS</th>
<th>DF</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOT GROUP</td>
<td>28.5</td>
<td>2.85</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>SWGRP</td>
<td>0.073</td>
<td>0.073</td>
<td>1</td>
<td>0.223</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>WITHIN S</th>
<th>SS</th>
<th>MS</th>
<th>DF</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOT MEASUREMENT GROUP X</td>
<td>22.0</td>
<td>0.182</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>MEASUREMENT GROUP X MEASUREMENT</td>
<td>2.18</td>
<td>2.18</td>
<td>1</td>
<td>1.405</td>
</tr>
<tr>
<td>MEASUREMENT X SUBJECTS (W)</td>
<td>1.25</td>
<td>1.25</td>
<td>1</td>
<td>0.810</td>
</tr>
<tr>
<td>13.0</td>
<td>1.55</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AVERAGE</th>
<th>SS</th>
<th>MS</th>
<th>DF</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVERAGE GROUP X</td>
<td>0.142</td>
<td>0.028</td>
<td>5</td>
<td>0.534</td>
</tr>
<tr>
<td>AVERAGE X</td>
<td>0.260</td>
<td>0.052</td>
<td>5</td>
<td>0.982</td>
</tr>
<tr>
<td>AVERAGE X SUBJECTS (W)</td>
<td>2.39</td>
<td>0.053</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MEASUREMENT X AVERAGE</th>
<th>SS</th>
<th>MS</th>
<th>DF</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVERAGE GROUP X</td>
<td>0.237</td>
<td>0.073</td>
<td>5</td>
<td>1.065</td>
</tr>
<tr>
<td>MEASUREMENT X AVERAGE</td>
<td>0.234</td>
<td>0.047</td>
<td>5</td>
<td>1.550 (p=0.194)</td>
</tr>
<tr>
<td>MEASUREMENT/ AVERAGE X SUBJECTS (W)</td>
<td>1.36</td>
<td>0.030</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2**

Summary Table for the Means and ANOVA Performed on the Peak Height of the AEP for PS Potentiated Group and PS Controls
## Table 3

Summary Table for the Means and ANOVA Performed on the Area of the AEP for the EPSP Potentiated Group and the EPSP Control Group

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MEASUREMENT</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>M1</td>
<td>1.000</td>
<td>1.038</td>
<td>1.029</td>
<td>1.087</td>
<td>0.987</td>
<td>1.141</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>1.128</td>
<td>1.308</td>
<td>1.440</td>
<td>1.360</td>
<td>1.470</td>
<td>1.517</td>
</tr>
<tr>
<td>EPSF POTENTIATED</td>
<td>M1</td>
<td>1.000</td>
<td>1.092</td>
<td>1.192</td>
<td>1.152</td>
<td>1.087</td>
<td>1.101</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>1.394</td>
<td>1.198</td>
<td>1.368</td>
<td>1.305</td>
<td>1.186</td>
<td>1.214</td>
</tr>
</tbody>
</table>

### BETWEEN S

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOT</td>
<td>5.14</td>
<td>17</td>
<td>0.302</td>
<td></td>
</tr>
<tr>
<td>GROUP</td>
<td>0.046</td>
<td>3</td>
<td>0.015</td>
<td>0.042</td>
</tr>
<tr>
<td>SWGRP</td>
<td>5.09</td>
<td>14</td>
<td>0.364</td>
<td></td>
</tr>
</tbody>
</table>

### WITHIN S

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOT</td>
<td>4.50</td>
<td>198</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>MEASUREMENT</td>
<td>0.150</td>
<td>1</td>
<td>0.150</td>
<td>0.950</td>
</tr>
<tr>
<td>GROUP X MEASUREMENT</td>
<td>0.452</td>
<td>3</td>
<td>0.151</td>
<td>0.954</td>
</tr>
<tr>
<td>SUBJECTS (W)</td>
<td>2.21</td>
<td>14</td>
<td>0.158</td>
<td></td>
</tr>
</tbody>
</table>

### AVERAGE

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVERAGE</td>
<td>0.096</td>
<td>5</td>
<td>0.019</td>
<td>1.962</td>
</tr>
<tr>
<td>GROUP X AVERAGE</td>
<td>0.123</td>
<td>15</td>
<td>0.008</td>
<td>0.084</td>
</tr>
<tr>
<td>AVERAGE X SUBJECTS (W)</td>
<td>0.683</td>
<td>70</td>
<td>0.010</td>
<td></td>
</tr>
</tbody>
</table>

### MEASUREMENT X AVERAGE

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEASUREMENT X AVERAGE</td>
<td>0.042</td>
<td>5</td>
<td>0.008</td>
<td>0.913</td>
</tr>
<tr>
<td>GROUP X MEASUREMENT X AVERAGE</td>
<td>0.098</td>
<td>15</td>
<td>0.007</td>
<td>0.713</td>
</tr>
<tr>
<td>MEASUREMENT/ AVERAGE X SUBJECTS (W)</td>
<td>0.639</td>
<td>70</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>GROUP</td>
<td>MEASUREMENT</td>
<td>MEANS (X)</td>
<td>A1</td>
<td>A2</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------</td>
<td>-----------</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>CONTROL</td>
<td>M1</td>
<td>1.000</td>
<td>1.015</td>
<td>1.026</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>1.254</td>
<td>1.328</td>
<td>1.472</td>
</tr>
<tr>
<td>EPSP POTENTIATED</td>
<td>M1</td>
<td>1.000</td>
<td>1.120</td>
<td>1.217</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>1.381</td>
<td>1.309</td>
<td>1.448</td>
</tr>
</tbody>
</table>

**BETWEEN S**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>MS</th>
<th>DF</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOT</td>
<td>44.2</td>
<td>2.60</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>GROUP</td>
<td>0.002</td>
<td>0.002</td>
<td>1</td>
<td>0.001</td>
</tr>
<tr>
<td>SWGRP</td>
<td>44.2</td>
<td>2.76</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

**WITHIN S**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>MS</th>
<th>DF</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOT</td>
<td>40.4</td>
<td>0.204</td>
<td>198</td>
<td></td>
</tr>
<tr>
<td>MEASUREMENT</td>
<td>7.38</td>
<td>7.38</td>
<td>1</td>
<td>5.109*(p&lt;.05)</td>
</tr>
<tr>
<td>GROUP X</td>
<td>0.137</td>
<td>0.137</td>
<td>1</td>
<td>0.094</td>
</tr>
<tr>
<td>MEASUREMENT X</td>
<td>23.1</td>
<td>1.45</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

**AVERAGE**

<table>
<thead>
<tr>
<th></th>
<th>0.792</th>
<th>0.158</th>
<th>5</th>
<th>2.840*(p&lt;.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP X</td>
<td>0.619</td>
<td>0.064</td>
<td>5</td>
<td>1.142</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>0.46</td>
<td>0.056</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

**MEASUREMENT X AVERAGE**

<table>
<thead>
<tr>
<th></th>
<th>0.278</th>
<th>0.056</th>
<th>5</th>
<th>1.200</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP X</td>
<td>0.150</td>
<td>0.030</td>
<td>5</td>
<td>0.647</td>
</tr>
<tr>
<td>MEASUREMENT</td>
<td>3.71</td>
<td>0.046</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4**

Summary Table for the Means and ANOVA Performed on the Peak Height of the AEP for the EPSP potentiated Group and the EPSP Control Group.