MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1964-A
NAME OF AUTHOR: CAROL A. BARNES

TITLE OF THESIS: MEMORY DEFICITS ASSOCIATED WITH SENESCENCE: A NEUROPHYSIOLOGICAL AND BEHAVIORAL STUDY IN THE RAT

UNIVERSITY: CARLETON

DEGREE FOR WHICH THESIS WAS PRESENTED: PH.D

YEAR THIS DEGREE GRANTED: 1977

Permission is hereby granted to THE NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(Signed) C. A. Barnes

PERMANENT ADDRESS: Dept. Psychology
Dalhousie University
Halifax, N.S. B3H 4J1

DATE: AUGUST 24, 1977

SL-91 (10-68)

Supervisor: F.A. Fried
NOTICE

The quality of this microfiche is heavily dependent upon
the quality of the original thesis submitted for microfilm-
ing. Every effort has been made to ensure the highest
quality of reproduction possible.

If pages are missing, contact the university which
granted the degree.

Some pages may have indistinct print especially if
the original pages were typed with a poor typewriter
ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles,
published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed
by the Canadian Copyright Act, R.S.C. 1970, c. C-30.
Please read the authorization forms which accompany
this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche depend grandement de la
qualité de la thèse soumise au microfilmage. Nous avons
tout fait pour assurer une qualité supérieure de repro-
duction.

S'il manque des pages, veuillez communiquer avec
l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut
laisser à désirer, surtout si les pages originales ont été
dactylographiées à l'aide d'un ruban usé ou si l'université
nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'aute-
teur (articles de revue, examens publiés, etc.) ne sont pas
microfilmés.

La reproduction, même partielle, de ce microfilm est
soumise à la Loi canadienne sur le droit d'auteur. SRC
1970, c. C-30. Veuillez prendre connaissance des for-
mules d'autorisation qui accompagnent cette thèse.
MEMORY DEFICITS ASSOCIATED WITH SENESCENCE:

A NEUROPHYSIOLOGICAL AND BEHAVIORAL STUDY IN THE RAT

by

Carol A. Barnes

A Thesis
Submitted to the Faculty of Graduate Studies of Carleton University
in Partial Fulfillment of the Requirements for
the Degree of
Doctor of Philosophy

Department of Psychology
Carleton University
Ottawa, Ontario
August, 1977
The undersigned recommend to the Faculty of Graduate Studies acceptance of the thesis "Memory Deficits Associated with Senescence: A Neurophysiological and Behavioral Study in the Rat" submitted by Carol A. Barnes in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[Signature]
Thesis Supervisor

[Signature]
Chairman, Department of Psychology

Carleton University
August 1977
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TABLE OF CONTENTS</td>
<td></td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>(i)</td>
</tr>
<tr>
<td>ABBREVIATIONS</td>
<td>(iv)</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>(v)</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Definition of Aging and Senescence</td>
<td>3</td>
</tr>
<tr>
<td>Problems of Control</td>
<td>4</td>
</tr>
<tr>
<td>Learning and Memory as Dependent Variables of Senescence</td>
<td>5</td>
</tr>
<tr>
<td>Experimental evidence of learning-memory deficits in aged humans.</td>
<td>7</td>
</tr>
<tr>
<td>Animal experiments</td>
<td>15</td>
</tr>
<tr>
<td>Conclusions</td>
<td>19</td>
</tr>
<tr>
<td>The Possible Role of the Hippocampus in Memory and the Aging Process</td>
<td>20</td>
</tr>
<tr>
<td>General Characteristics of Senescent Nervous Systems</td>
<td>29</td>
</tr>
<tr>
<td>Hippocampal Changes with Age</td>
<td>33</td>
</tr>
<tr>
<td>Long-Lasting Changes in Synaptic Efficacy as a Model of Learning-Memory Processes in the Nervous System</td>
<td>37</td>
</tr>
<tr>
<td>Hippocampal Connectivity</td>
<td>46</td>
</tr>
<tr>
<td>The Perforant Path - Granule Cell Evoked Response</td>
<td>49</td>
</tr>
<tr>
<td>Experimental Variables</td>
<td>55</td>
</tr>
<tr>
<td>GENERAL METHODS</td>
<td>57</td>
</tr>
<tr>
<td>Subjects</td>
<td>57</td>
</tr>
<tr>
<td>Random assignment to groups</td>
<td>57</td>
</tr>
<tr>
<td>Handling</td>
<td>58</td>
</tr>
<tr>
<td>Overall Design</td>
<td>59</td>
</tr>
<tr>
<td>Section Title</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>SECTION I: CIRCULAR MAZE (Procedure, Results and Discussion)</td>
<td>65</td>
</tr>
<tr>
<td>The Equipment and Maze</td>
<td>65</td>
</tr>
<tr>
<td>Summary Procedure</td>
<td>67</td>
</tr>
<tr>
<td>Data analysis:</td>
<td>71</td>
</tr>
<tr>
<td>General Description of the Results</td>
<td>72</td>
</tr>
<tr>
<td>Was this task measuring memory?</td>
<td>72</td>
</tr>
<tr>
<td>Motivation.</td>
<td>73</td>
</tr>
<tr>
<td>Patterns of behavior on the maze surface.</td>
<td>74</td>
</tr>
<tr>
<td>Cumulative Plots.</td>
<td>78</td>
</tr>
<tr>
<td>First hole investigated.</td>
<td>81</td>
</tr>
<tr>
<td>Percent correct responses.</td>
<td>84</td>
</tr>
<tr>
<td>Motor-Dependent Measures</td>
<td>85</td>
</tr>
<tr>
<td>Latency.</td>
<td>85</td>
</tr>
<tr>
<td>Speed.</td>
<td>85</td>
</tr>
<tr>
<td>Fixations.</td>
<td>88</td>
</tr>
<tr>
<td>Motor-Independent Measures</td>
<td>89</td>
</tr>
<tr>
<td>Total distance.</td>
<td>89</td>
</tr>
<tr>
<td>Errors.</td>
<td>90</td>
</tr>
<tr>
<td>Mean angle from the tunnel.</td>
<td>93</td>
</tr>
<tr>
<td>Time of Day</td>
<td>94</td>
</tr>
<tr>
<td>General Discussion of the Circular Maze Data</td>
<td>95</td>
</tr>
<tr>
<td>SECTION II: SURGERY</td>
<td>97</td>
</tr>
<tr>
<td>Electrodes and Equipment</td>
<td>97</td>
</tr>
<tr>
<td>Measurements Collected During Surgery</td>
<td>99</td>
</tr>
</tbody>
</table>
Skull dimensions. 99
Anesthetic rating. 100
Respiration. 101
Total amount of anesthetic. 102
Total time behaviorally anesthetized. 102
Measurement of peak evoked response. 103
Summary of Surgery Measurements 105

SECTION III: EEG 106

EEG Procedure 106
Description of the data matrix. 107
EEG analysis. 109
Results and Discussion 110
Average power spectra collapsed across time. 110
Average power at each frequency over time. 113

Summary of EEG data 125

SECTION IV: LOW-FREQUENCY STIMULATION 128

Procedure 128
Measurement of the Evoked Response 129
Results and Discussion 129
The population spike area. 130
The slope of the EPSP. 130
Time of day. 133
Summary. 134
SECTION V: HIGH-FREQUENCY STIMULATION

General Procedure 138

Phase I - Time Course of Decline After a Single HF Episode 138

Results and Discussion of Phase I 139

The EPSP slope. 139

Phase II - Repeated HF Stimulation Episodes 144

Results and Discussion of Phase II 153

The EPSP slope. 153

The population spike area. 153

Time of day. 154

Phase III - High-Intensity HF Stimulation 160

The EPSP slope. 160

Summary 163

SECTION VI: STIMULUS-RESPONSE RELATIONSHIPS 169

Rational for Determining Stimulus-Response Relationships 169

Systematic determination of stimulation parameters. 169

Advantage of low-intensity stimulation. 169

Stimulus-response determinations before and after HF stimulation. 170

Procedure 171

Results and Discussion 172

The fractional change after HF stimulation. 172

Time of day. 175

Initial stimulus-response determinations. 178

Summary. 182
SECTION VII: DOUBLE PULSE INHIBITION AND FACILITATION
- Procedure 184
- Results and Discussion 184
  The population spike. 187
  The EPSP. 187
- Summary 191

SECTION VIII: ANESTHETIC DOUBLE PULSE SERIES 193
- Procedure 194
- Results and Discussion 195
  Pulse 1. 195
  Pulse 2. 202
  Fractional change. 202
- Summary 203

SECTION IX: BLOOD PRESSURE 207
- Procedure 208
- Results and Discussion 209

SECTION X: SPONTANEOUS ALTERNATION 213
- Apparatus and Procedure 214
- Results and Discussion 217
  Alternation behavior after surgery. 218
  Alternation behavior before and after high-frequency stimulation. 218
  Time of day. 219
- Summary. 219

SECTION XII: CORRELATIONS OF THE PHYSIOLOGY AND BEHAVIOR 223
SECTION XII: HISTOLOGY

DISCUSSION

General Neurophysiological Age Differences and Similarities

Circadian Effects

Learning-Memory Alterations with Age

REFERENCES

APPENDIX A: Reasons for Decreased Sample Size During the Course of the Experiment

APPENDIX B: Body Weights

APPENDIX C: Tumors

APPENDIX D: Pilot studies Completed for the Development of the Procedure in the Circular Maze Study

Odor Adaptation Trial

First Pilot Study Using the Circular Maze

Second Pilot Study Using the Circular Maze

The pilot results in relation to the major study.

APPENDIX E: Detailed Procedure for the Circular Maze

Day 1.

Day 2, 3, and 4.

Day 5 and 6.

APPENDIX F: Critical Dimensions of the Circular Maze

APPENDIX G: Diagram of the 8-Compartment Rat Cart Used During Electrophysiological Recording

APPENDIX H: Double Pulse "Facilitation"

Pilot Work

Tests for EPSP facilitation in chronic rats.

Tests for EPSP increases in acute rats.
ABSTRACT

Neurophysiology of the hippocampal formation and spatial behavior was observed in 32 mature adult (MA) and 32 senescent normal (SN) male Long Evans rats. The experiment extended over a six-month period so that the MA rats were tested between the ages of 10-16 months and the SN rats between 28-34 months. In addition to age, the time of day at which the rats were tested was included as an independent variable.

All rats were tested on a circular maze problem before neurophysiological testing. The SN rats did not perform as well on this spatial memory task as the MA rats. This deficit was independent of motor (and probably sensory) factors. On the first few trials the performance measures were essentially equivalent between age groups. On subsequent trials, however, the improvement in the number of errors and total distance traveled was significantly less in the SN than the MA animals. The results are interpreted in terms of a retention deficit in the SN rats.

Unilateral recording electrodes were implanted in the fascia dentata and stimulating electrodes in the ipsilateral angular bundle in all animals. The recording of the granule cells' responses to monosynaptic activation of perforant path fibers was done in the awake freely moving state. Evidence is presented that the SN rats' granule cells may have been more depolarized than the MA rats' granule cells (at least in the awake state) and that they may have more powerful
synapses than the MA rats. Furthermore, a striking difference in the maintenance of an elevated synaptic response, produced through high-frequency stimulation, was noted between age groups. After a single high-frequency stimulation session, the elevated synaptic response was observed to decay back towards baseline over 7 days. There were no differences in the initial fractional increase or the rate of decay following this first high-frequency stimulation.

After repeated daily stimulation, however, the retention of the elevated synaptic response improved in the MA rats, but not in the SN rats. Two weeks after the last high-frequency session, the synaptic response in the MA group remained 40% greater than baseline, whereas the response in the SN group no longer showed an elevation. The synaptic modifiability measured here was significantly correlated (within and between groups) with the ability to learn the spatial discrimination task discussed above.

Spontaneous alternation trails were given at a variety of time points through the study. Although the percent alternation behavior was equivalent between age groups before high-frequency stimulation, the SN rats' performance on this maze changed towards chance alternation after the high-frequency stimulation began. This occurred even though the elevated response was not maintained as well in the SN as in the MA rats.

The time course and extent of double pulse facilitation and inhibition of the perforant path - granule cell synaptic response were the same between age groups. It thus appears that aging selectively
effects the long rather than the short term synaptic modification processes, and that the properties of inhibitory interneurons do not change with age in this system.

Age differences were also found in the characteristics of EEG recorded from the hippocampus, and in the influence of a barbiturate anesthetic on the amplitude of the evoked response. Arterial blood pressure was not different between age groups.

Several lines of evidence suggest that circadian oscillations in neurophysiological and behavioral states were altered in the SN rats compared with the MA rats of this study. These data suggest the possibility that a wide variety of changes in circadian organization may accompany aging.

The results of most importance to aging research and neuropsychology include the fact that individual performance on the spatial maze was significantly correlated with the amount of elevation in the synaptic response of cells thought to be involved in processing spatial information. The implications of these findings to theories of memory and aging are discussed.
ABBREVIATIONS

ANS: autonomic nervous system
CA 1, 2, 3, 4: Cornu Ammonis 1-4
DNA: deoxyribonucleic acid
EEG: electroencephalogram
EPSP: excitatory post-synaptic potential
GABA: \( \gamma \)-aminobutyric acid
HF: high frequency
IPI: interpulse interval
IPSP: inhibitory post-synaptic potential
MA: mature adult
RNA: ribonucleic acid
rRNA: ribosomal ribonucleic acid
SN: senescent normal
\( V(t) \): extracellular voltage at time \( t \)
\( V_0 \): extracellular voltage at \( t = 0 \)
\( \alpha \): value of exponential function at \( t = 0 \)
\( \lambda \): exponential rate constant
\( \tau \): \( 1/\lambda \)
ACKNOWLEDGEMENTS

While it is impossible to thank everyone who has contributed to this thesis, the following people deserve special mention.

Dr. Peter Fried, my supervisor, has been extremely patient in encouraging me to pursue interests other than those in his own area of specialization. Our correspondence during the preliminary writing of this thesis was invaluable.

Dr. Graham Goddard, provided the laboratory facilities, the support for this project, and useful critiques of the manuscript. Without his faith in the impossible, and his ability to help one focus ideas without hindering, this thesis would have been different indeed.

Robert Douglas wrote the elegant computer programs for collecting, analyzing, and plotting the electrophysiological data. Special thanks are due for his help in setting up the equipment to monitor behavior on the circular maze.

I would also like to acknowledge my appreciation to Dr. Robert Adamec for his instruction in surgical and electrophysiological techniques, and for hours of useful discussions; Georgia Cottrell for encouragement and thinking of me while reviewing the current literature; Mai Riives for her expert drafting and assistance with histology; Lorraine White for her patience and excellent typing skills; and to my committee members for reviewing the manuscript.

Finally, I would like to express my indebtedness to Bruce McNaughton, whose profound influence pervades all aspects of this thesis. Although his rigorous scientific standards led us to dozens of heated debates, his creative scientific mind was always inspiring.
INTRODUCTION

Age related memory deficits in senescent organisms are typically conceptualized as due to wear and tear of the nervous system, or to an accumulation of errors throughout life. Implicit in recent theoretical models of memory, however, is the possibility of saturating the nervous system with information. Indeed, this is necessarily true of any finite system. Two contrasting theoretical positions emerge: one asserting that memory changes with age are a result of some form of pathology, the other considering memory deficits as a natural consequence of the mechanism by which the brain stores information. This research is directed toward distinguishing between these hypotheses, or developing a reasonable composit model.

Since the emergence of the neuron doctrine, the principle mechanisms that have been postulated for behavioral plasticity, in one form or another, are alterations of synaptic efficacy and rearrangement of morphological connections. The former is of particular interest here. Although direct evidence is lacking, it is generally assumed that an increased efficiency of cell to cell communication would facilitate the performance of a particular behavioral sequence. The indirect studies which provide some support for this notion will be discussed in a later section; it is assumed here that this hypothesis is reasonable. It is further assumed that particular brain areas are more important than other structures in organizing or processing the input to be learned or remembered. For reasons
to be outlined, the hippocampus and related structures have been implicated in such memory function. Moreover, these structures have been found to be particularly vulnerable to morphological changes which accompany senescence. Hence, it is felt that a study of the modifiability of particular synapses in the hippocampal formation, in relation to the age of the organism, will provide clues to the mechanism of the memory deficits which accompany advanced age.

A number of factors will be discussed in some detail in the following sections. First of all, a working definition of senescence will be formulated, and problems of experimental control arising from differential environmental experience between age groups will be considered. It will be established that there are real learning-memory deficits which accompany normal aging. 'Normal' is stressed here, because this work is attempting to study neuro-physiological correlates of memory deficits which occur in the life span of even the healthiest organism, not deficits due to some specific pathological cause (e.g., tumor, anoxia, circulatory defect). Evidence will be presented that the hippocampus may be involved in certain aspects of learning-memory operations. The general characteristics of a senescent nervous system will be outlined, and specific changes in hippocampal metabolism and ultrastructure which appear with advanced age will be discussed. Finally, the phenomenon of long-lasting potentiation in the hippocampus will be discussed as a mechanism for memory.
Definition of Aging and Senescence

Although the term 'senescence' is commonly used to describe a pathological state in old organisms, this word is not used in the aging research literature to specifically connote deterioration. In this thesis a 'senescent' organism will imply an organism of advanced age. If there is, for instance, dementia or other illness associated with the old organism, it will be specified.

While the relatively fixed life span of an organism suggests genetically determined mechanisms controlling the rate of aging, the variability in the decline of certain physiological functions, as well as the variability of absolute life span, suggests modifying effects of exogenous factors (Gutmann and Hanzlikova, 1972). Hence it is not surprising the both genetic or 'programmed' and stochastic or 'error-accumulation' models of aging have received experimental support (Griffiths, 1973; Lewis, 1972; Lewis & Holliday, 1970; Orgel, 1963, 1970; Rose & Bell, 1971; Von Hahn, 1973). Obviously both types of analysis fit into the complex process of aging. Strehler (1960) proposed some general criteria which any hypothetical mechanism of aging must incorporate; it must be universal, with all the population undergoing the observed changes; it must be deleterious, with age marking a general decline of capacity; it must be progressive, with the deleterious effects being cumulative; and it must be intrinsic, the decisive events ultimately residing within the individual cell. Even these broad guidelines are too restrictive if one wishes to consider aging as a developmental process beginning
at or before birth and continuing until death.

Simple definitions which claim universal applicability are probably inappropriate at this stage, and no attempt will be made here to utilize such concepts. Satisfactory operational definitions of aging are numerous, each seeming to suit the particular approach of the investigator. A definition relevant to the experimental approach in this thesis may be formulated as follows. Developmental differentiation continues into advanced years, the process speeded or delayed depending upon individual genetic and environmental endowments. It is a multi-dimensional process, involving changes at various levels of organization which alter the organism's adaptive functional potential; the period of senescence is marked by decline in a variety of such adaptive functions. Hence the view here is that throughout the life of the organism, differentiation of neuronal connection takes place as the animal adjusts to and manipulates its environment. Eventually, as the system approaches the limits of its capacity, the potential for adaptive change declines.

Problems of Control

In experiments where subjects of different ages are compared, the experimenter is confronted with the possible influence of differential experience interacting with other aspects of the aging process. The measures must reflect the influence of a longer life, but not certain specific events occurring unequally between subject populations. This situation may be closely approximated if the subjects are housed in a restrictive but standard fashion, although this is not a complete
guarantee of equivalent experiences. Also, it should be noted that
in keeping a colony of rats over some years, many in the colony die,
and those which are used as experimental old subjects are in some
respects selected for being hardy. These problems are unavoidable
because of the nature of the experimental question, and the con-
straints thereby imposed on the interpretation of the results must
be acknowledged.

Learning and Memory as Dependent Variables of Senescence

Anecdotal evidence that memory deficits accompany senes-
cence has accumulated at least since Aristotle's treatise "On Memory
and Recollection" (303 B.C.) where he states:

The process of movement involved in the act of
perception stamps in, as it were, a sort of
impression of the percept, just as persons do
who make an impression with a seal. This explains
why, in those who are strongly moved owing to
passion, or time of life, no mnemonic impression
is formed; just as no impression would be formed
if the movement of the seal were to impinge on
running water; while there are others in whom,
owing to the receiving surface being frayed, as
happens to old walls, or owing to the hardness of
the receiving surface, the requisite impression
is not implanted at all. Hence both very young
and very old persons are defective in memory; they
are in a state of flux, the former because of
their growth, the latter, owing to their decay.
(from Dennis, 1948, pp. 2-3).

As will be seen, the above is not unlike modern views of age and
memory formation.

Although it is generally agreed that such a memory deficit
exists, it is difficult to decide what is meant by such terms as
learning or memory, and therefore to locate this deficit on a continuum
such as aquisition-registration, consolidation-storage, or retrieval. Botwinick (1967) aptly discusses the processes of learning and memory as "so interrelated and so interdependent that it is often difficult to determine whether or not they are distinct...if a man does not learn, he cannot remember. Conversely, if he cannot remember, there is no sign of his having learned".

The term learning covers a heterogeneous collection of phenomena which are often unobservable. This includes the concepts that learning is a hypothetical construct, or that it is an intervening variable which links some external conditions to observed behavioral changes. This by no means approaches an exhaustive explanation, but it is difficult to arrive at a definition that is not too specific or on the other hand not too general to be useful.

To further complicate matters, the learning-memory concept is divided into subprocesses such as short- and long-term storage systems. The definitions as well as the actual existence of these storage systems are also a matter of controversy. The reported duration of the short-term memory stage varies from seconds (Chorover & Schiller, 1965) to days (Flexner, Flexner, De La Haba, & Roberts, 1964; Deutsch, 1969) so that time constants of this process have little absolute rigidity across experimental situations. It seems that the same event may require different time intervals in short-term memory depending upon internal and/or external factors. Further, tasks of varying complexity may remain varying amounts of time in short-term stores (Vinogradova, 1970).
The lack of clarity with regard to the short-term system makes the exact nature and time course of the long-term storage system difficult to discern. Hence experimenters working in the field of learning-memory processes tend to define such concepts in a language relevant to their particular theoretical or experimental needs; so when discussing topics in this field, an attempt will be made to operationally clarify such terminology.

The purpose of this section is to establish the simple fact that there are learning-memory differences between old organisms and mature or middle-aged organisms. Further, a description of the types of experimental situations in which such differences have arisen will be given.

**Experimental evidence of learning-memory deficits in aged humans.** Moenster (1972) has operationally separated 'learning' and 'memory' variables in attempt to determine whether cognitive declines in later years are more a function of one than the other variable. In this study, meaningful paragraphs of reading material were utilized which were typical of ones commonly encountered in daily living. Memory differences for learned materials between young and older (noninstitutionalized) adults became non-significant statistically when memory performance was adjusted for how well the material had initially been learned. Learning was defined as immediate performance on a questionnaire for the paragraph, and memory as performance on the questionnaire ten minutes following an interpolated task. Whether immediate recall is any more 'valid' a measure
of learning than recall after ten minutes is not clear, and it seems that Moenster could be comparing two types of memory storage systems rather than learning versus memory processes.

One of the major sources of evidence that a short-term memory exists comes from human verbal memory experiments. Notably, Broadbent (1957), in his dichotic listening experiments, found that items presented to one ear, but not permitted to be recalled until after the items presented to the other ear had been recited, were only available for a number of seconds. By this method, the time constants of decay of information from these short-term stores can be measured. With the use of these dichotic stimulation techniques, a number of investigators have demonstrated such short-term memory deficits in aged adults. The general finding is of an age-related loss in the ability to recall the material from whichever ear is recalled second in non-institutionalized adults (Inglis & Ankuš, 1965; Inglis & Caird, 1963; Inglis & Tansey, 1967). This, as described earlier, is the material which must be held in the short-term store. Hence the deficit on dichotic recall tasks have been interpreted as an overall short-term deficit.

Schonfield, Trueman, and Kline (1972) recently examined the age effect (in healthy non-institutionalized adults) in a dichotic listening situation when recognition tests were used rather than recall tests. A recognition task was used in an attempt to determine whether the problem existed in the retrieval of the information or in the actual availability of it in the memory store. Since the people were pre-
sented with a list which included the correct choice, this would be expected to facilitate retrieval. Unlike the recall data for dichotic experiments, the old adults in this study showed no greater loss in the ability to retain information presented to the first ear (which had to be held in the short-term store) on the recognition tests. However, both first and second ear recognition scores were lower in the older than in the younger adults. Schonfield contends that the age associated recognition deficit of both first and second ear material in his experiment is due to an acquisition deficit, whereas in the dichotic recall experiments of other laboratories the deficit on the first ear material is in fact a retrieval deficit. A more parsimonious interpretation, however, would be that acquisition difficulties underlie poorer performance on both the recall and recognition dichotic tests. Moreover, this issue is complicated by a recent study of dichotic recall which was unable to replicate the earlier recall experiments. Clark and Knowles (1973) observed a decrement of performance in recall of material from both ears (rather than just the second ear material) using both written and vocal responses. This, by the same logic which Schonfield used for his recognition task, suggests a deficit in the initial registration of the material. It is not clear, however, what percentage of their older adults were institutionalized, which may account for a portion of the observed difference.

Several lines of evidence indicate that retrieval difficulties may exist with advanced age. Most of these studies use the
technique of free recall or recognition, where learning new information in the form of word lists or paragraphs is required. This information is then either recalled (free recall) or recognized among information not seen before (recognition). Schonfield (1965, 1967) and Schonfield and Robertson (1966) demonstrated that aged human adults performed less well than young adults on free recall of a word list, but performed at the same level on recognition tests. Laurence (1967) also found differences between young and old adults on free recall, but when they were given category cues of their word lists at the time of recall, the aged adults obtained scores which approximated young adult scores. Theoretically, the recognition scores provide a test of memory which did not require retrieval from storage, but only matching of a stored trace; whereas free recall scores would measure the functional properties of the retrieval system. Hence, the general conclusion made from these experiments was that the major memory deficits of the aged involved those of retrieval abilities. The implication here, is that elderly people store the information, but that it is not as accessible to them as to the younger subjects.

McNulty and Caird (1966, 1967) have criticized the foregoing conclusions which implicate retrieval as the primary age deficit. They contend that partial learning of items might occur and be sufficient information to allow a correct choice on a recognition test (McNulty, 1965, 1966). Hence the amount of material learned may actually decrease with age without the recognition measure detecting
this difference (if the recognition task is sufficiently easy).

Drachman and Leavit (1972) have done a series of experiments which address this controversy. They gave a battery of tests to active, healthy old and middle-aged people. The ordinary digit span that most normal adults possess is about seven items. These experimenters used tests of this digit span and tests of memory for more than seven items (or supra-span digit storage tests) in immediate memory tests (where the lists are repeated immediately after presentation). Additional tests were given where the individuals were expected to recall items without being given cues for the correct response (uncued recall), and also after being given cues for the material they were to remember (cued recall). The final test required the subjects to produce their own list in a defined period of time, after they were given a particular category word. Such a category word might be 'vegetable', and they would respond with as many vegetables from their own vocabulary as asked for in the particular time period. They were then tested for retrieval of these items which were presumably from an accessible storage system. The results produced evidence for impairment of some, but not all memory functions measured by this test battery. Immediate memory span was unimpaired which is in agreement with earlier work (Birren, 1959). However, the task of recall of supra-span lists (lists of more than seven items) showed a major difference between old and middle-aged adults, with the older individuals having much more difficulty with the lists. The old adults obtained normal scores
in the test of retrieval by category. Since the retrieval mechanism for 'old material', or material they produced themselves was intact, Drachman and Leavit conclude that an important retrieval mechanism is intact and therefore that storage might be the primary cause of impairment in their subject population. This is essentially in agreement with McNulty and Caird (1966), who contend that partially learned items, or incomplete storage, rather than retrieval could account for old and young adult performance being comparable on recognition tests or cued recall tests.

The concept of partially learned items is similar to a model proposed by Wickelgren (1970) involving a theoretical threshold level for recall of stored items. In this view, a well-learned item will be of sufficient signal strength to exceed a threshold level at the time of recall, whereas a partially learned item may not reach recall threshold because of signal weakness. This provides an adequate framework with which to view storage and retrieval mechanisms, with the strength of signal in the storage system being most obviously altered in the aged individual.

McNulty and Caird (1967) predicted that if storage systems were affected by age, it should be possible to produce an experimental situation in which the elderly's performance on recognition and recall would be equally deficient (the signal below threshold for both). Erber (1974) has recently found that if she increased the level of task difficulty, an age difference could be obtained on a recognition memory task as well as on recall tasks for the older (non-institution-
alized) adults. This would be in keeping with the idea that partial learning must exceed a certain threshold in order for recognition to occur. Gordon and Clark (1974) have also found evidence for the existence of storage deficits of the elderly. They found that both immediate and long-term (one week) recall and recognition of prose was influenced by the age of the person, with the one week interval being most severely affected. Since the prose recognition test was quite difficult the signal was presumably quite weak, and hence not available for use even in the recognition tests.

Since many tests of immediate recall (if not too difficult) show no age effect, whereas tests of short-term recall with some interpolated task do show an age effect, this might be construed to be due to interference effects rather than signal weakness. Boyarski and Eisdorfer (1972) designed an experiment under the assumption that the registration process is normal in old individuals, and that interference effects were critical to memory declines. They utilized bright, active 60-83 year old individuals to investigate the pattern of forgetting due to interfering associations. The task required the learning of lists of paired associate nonsense syllable-adjective items. The kinds of errors the older subjects made led the experimenters to conclude that forgetting in brighter older persons may be due to interference from distracting associations brought to the learning situation from their pasts. Although this may be correct, as a younger group of bright individuals was not included, no comparison of interference effects across age groups
could be made. Thus this interference effect may not be a specific
age effect.

In fact, one might predict that there would not be an age-
related interference effect since Canestrari (1966) could find no
difference in relative performance of old individuals compared with
young individuals when subjected to influences of interference (using
paired associate lists of either high or low associative strength).
In this study, the linguistic habits of the subjects were equated,
and paired associate material was presented to the appropriate groups
and learned to criterion. There were age related differences in
performance by the elderly on the more difficult lists, however it
was determined that young and elderly individuals experienced the
same relative degree of interference effects in learning lists which
had different amounts of interference.

Memory-learning decrements with age are perhaps not as
severe as the foregoing might have implied. Improvement of memory
abilities occur under the proper conditions and makes old adult per-
formance comparable to young adult performance. Such conditions
include repeated testing sessions with batteries of tests (Peak, 1970),
with practice on memory drum tests (Taub, 1972) or with digit recall
(Taub & Long, 1972; Taub, 1973). These improvements in old adult
performance can be maintained for considerable time intervals (at
least six months). Since practice effects increase the old individuals
performance relative to that of young individuals, Taub and coworkers
suggest that the strategy of old adults may improve with practice, and,
perhaps related to the strategy changes, signal strengthening may occur with practice.

Since evidence can be cited to substantiate or to refute deficits in registration, retrieval, retention, long-term and short-term memory in old humans, developing a theoretical position favoring any one of these areas appears meaningless. This avoids taking a position on such issues as whether short-term memory or long-term memory is more disrupted, or what the distinction is, if one exists (Weiskrantz, 1970), between these processes. For the purposes of this work, such a theoretical entanglement is not necessary. It is clear that human learning-memory operations change with advanced age.

Animal experiments. There have been instances where investigators, using human subjects, have not found short-term memory problems (or deficits after short-time intervals), but have observed age related differences after 24 hours (or long-term memory differences (Wimer & Wigdor, 1958; Wimer, 1960). Generally, it has been thought that these results were due to failure to equate original learning levels (Talland, 1968; Hulika & Weiss, 1965). Medin, O'Neil, Smeltz and Davis (1973) did find such a deficit in an experiment involving concurrent discrimination problems with old and middle-aged rhesus monkeys. This was a rather unique subject pool since it included monkeys of two age groups which had received very similar treatment throughout their respective life spans. The concurrent (or serial) discrimination task requires subjects to learn a number of discriminations at one time, the first trial of each separate
discrimination problem presented before the second trial of any problem. The results on the trials for day one were essentially equivalent for old and middle-aged monkeys, suggesting that original learning was equivalent. However, on trial one and subsequent trials of day two, middle-aged monkeys were superior to the old monkeys on the discrimination tasks. In other words, the old animals tended to show greater forgetting over the 24-hour retention interval. It was thus concluded that a 'long-term' deficit occurs (although problems with consolidation into long-term stores is an equally tenable conclusion).

Considerable evidence has been accumulated concerning the requirement of a consolidation period for learned responses to become stable in memory. Many of the studies can be criticized because of lack of control for state dependent effects; however, Doty and Doty (1964, 1966) have performed two studies pertaining to consolidation and its age dependent effects in rats, which cannot be criticized on those grounds. Their hypothesis in the 1964 paper was that the interval required for memory to consolidate is significantly longer among immature and aged organisms than in young adults. Chlorpromazine (a major tranquilizer) was administered at varying intervals after training to groups of rats of different ages, the drug being expected to interact with the consolidation process by slowing or disrupting it. Using a simple avoidance task, the performance of 30-day and 600-day old rats was disrupted when treated with chlorpromazine one and two hours after their daily trials; whereas
the performance was unimpaired for 120-day old rats at the same time intervals. Chlorpromazine administered 10 seconds following the trials significantly impaired all age groups' subsequent performance, while failing to produce a significant difference between groups. This tends to rule out differential drug effects interacting with age as the major cause for group differences at the one- and two-hour time point. Therefore, they conclude that consolidation is a more extended process among immature and aged rats than in young adults.

In their 1966 study, they administered amphetamine to 120-, 500- and 730-day old rats. The drug was given at intervals of 10 seconds, one hour and four hours following 10 learning trials on four consecutive days. The variables of age and task difficulty at these time intervals were of interest here. The hypothesis tested was that amphetamine (a stimulant) would speed consolidation (e.g., improve performance on a variety of learning tasks), with the older rats showing greater relative learning increments. The tasks included: a) a simple conditioned avoidance problem, where movement out of the start compartment into either of two opposite compartments when the lights in both sections were turned on, constituted the correct avoidance response; b) a simple discriminated avoidance problem, where the rats were required to make a brightness discrimination before entering one of the compartments opposite the start compartment; and c) a discriminated avoidance reversal problem, where the rats learned the simple brightness discrimination avoidance task, but had to reverse the discrimination on four subsequent days with 10
trials on each day. They found that the drug improved performance on simple avoidance and discriminated avoidance responding in 500- and 730-day old rats at one and four hours after learning, while the performance of the younger adults was not improved. Since there was no effect on the performance of the younger groups at these time intervals, the younger rats apparently did not benefit from the treatment, or the consolidation of the material took place before drug administration. On the avoidance reversal task, (presumably a more difficult task) amphetamine tended to improve performance of all age groups only at the four-hour injection interval. Doty and Doty interpret this as an indication that the consolidation interval necessary for a difficult task is much greater than for a simple task. Again, as in the chlorpromazine study, the results on simple avoidance and discriminated avoidance indicate that the length of consolidation interval may increase with age.

Until recently, most research with rats had failed to obtain age differences in learning for young and aged groups. Although there is some evidence that the retention of simple avoidance tasks may differ between young and old rats (Gold & McCaugh, 1975), the studies that did not find age differences typically used tests of low complexity (Birren, 1962; Botwinick, Brinley, & Robbin, 1962; Kay & Sime, 1962) and parallel the human literature showing no deficits for simple tasks (Birren, 1959; Comfort, 1964; Palmore, 1970; Strehler, 1960). For more complex tasks, however, senescent humans (Timiris, 1972) and rats (Goodrick, 1968, 1969, 1972) do not perform as well as their younger counterparts. Goodrick has compared levels of motivation
and test complexity in young and senescent rats. He stresses the importance of differential deprivation for maze problems and has found learning deficits among highly motivated senescent Wistar albino rats. Such deprivation involves removing a greater proportion of, for instance, water or food, from old animals than from young animals. This is presumed to be sufficient control for the possibility of unequal motivation.

Goodrick's 1972 experiment compares performance on four mazes which varied in complexity. No age differences were detected for a straight runway, a 1-choice or a 4-choice T-maze. However, the young rats made significantly fewer errors on a 14-choice maze than the old rats. This difference was due at least partially to perseverative errors at specific choice points which were made more frequently by the older age groups. This perseverative tendency was also found in extinction trials, where senescent rats made fewer errors and ran faster to the goal box than young rats. Several other experiments, using avoidance tasks (Denenberg & Kline, 1958), bar-press tasks (Corke, 1964) and reversal tasks (Stone, 1929a, b) have also interpreted their results in terms of older rats showing greater perseverative tendencies. The older rats in Goodrick's study were also observed to show less exploratory behavior than young rats in open field test.

Conclusions. The foregoing evidence gathered from human and animal literature supports the contention that changes in learning-memory processes occur with advanced age. As long as the tasks are simple, no detectable differences emerge between age groups in
either animals or humans. However, with greater task difficulty come measurable differences between age groups in a number of verbal learning situations, concurrent discrimination problems and complex maze situations. It should be mentioned that these differences are not due to physical disabilities or a function of timed tests, both of which are variables known to bias results against old organisms (Elias & Elias, 1976).

The general theme which pervades the preceding literature is a decline in the reliability of memory-learning processing. The conditions under which one finds these deficits appear situation specific. Nevertheless, the factor of decreased accuracy of performance with complex task requirements transcends experimental design constraints.

The Possible Role of the Hippocampus in Memory and the Aging Process

The observation that normal aging is accompanied by a decline in the ability to acquire new information (Craik, 1968; Kral, 1966; McGhee, Chapman, & Lawson, 1965) and that lesions of the hippocampal area in humans result in deficits of memory storage (Milner, 1958) has led to speculation about the relationship between hippocampal dysfunction and senescence. First, a description of such brain damaged humans is in order.

Milner and coworkers (Milner, 1958, 1965, 1970; Scoville & Milner, 1957) have extensively investigated patients with hippocampal lesions. The size of such lesions varied from complete bilateral destruction, to more circumscribed damage. Generally, the memory loss Milner reports is characterized by the inability to per-
manently retain new information in the period after the brain damage. However the patient’s memory for very short periods after the presentation of a stimulus, or for events before the damage, is intact. Hence the defect may reside in the inability to transfer new information into more permanent stores (Errin & Anders, 1970), or may reflect a rapid decay of the stored trace.

Maze learning performance of patients with hippocampal lesions has been compared with normal subjects and patients who had undergone cortical excisions in other parts of the brain. Bilateral hippocampal patients showed the most severe deficits on memory scores with no apparent improvement on stylus maze problems (Milner, 1965). She notes the similarity of her results with studies done on rats (Kaada, Rasmussen, & Kviem, 1961; Kveim, Setekliev & Kaada, 1964) with hippocampal lesions, where impairment of multiple choice maze learning is observed, and with monkeys (Kimble & Pribram, 1963) which showed a decrease in the ability of acquire appropriate behavior sequences. Milner also found that unilateral hippocampal lesions produced effects dependent upon which hemisphere was damaged. With either visual (Milner, 1965) or tactile (Corkin, 1965) stylus maze tasks, they found a greater deficit for right hemisphere hippocampal lesions than for left hemisphere hippocampal lesions. These findings suggest that the ability to perform spatial tasks is not organized along modality specific lines, and requires the integrity of the right hippocampus.

Clinical evidence then consistently indicates that lesions
to hippocampal areas bring about deficits in memory function. Experimental evidence indicates that damage to similar structures in animals is, with the exception of the experiments cited above, at best contradictory. Attempts to obtain memory deficits comparable to those observed in humans, in animals other than human, have been unsuccessful. Part of this may be due to the more discretely localized lesions in the animal studies than in many of the human case studies, but surely cannot account for the consistent large discrepancies.

The results obtained from animal experiments have generally included the following: either no change under the conditions measured (McLardy, 1970; Migler, 1961; Woody & Erwin, 1966); or changes such as perseverative behavior (Entingh, 1971; Kimble, Kirkby, & Stein, 1966; Leaton, 1965), or no change in perseverative behavior (DeCastro, 1974); inability to perform certain reversal tasks (Kimble & Kimble, 1965; Silveira & Kimble, 1968; Thompson & Langer, 1963), schedules which require altering response rates (Clark & Isaacson, 1965; Ellen & Wilson, 1963; Schmaltz & Isaacson, 1966), or altering a preferred approach tendency (Fried & Goddard, 1967); deficits in complex avoidance (Musty, 1969), or maze tasks (Jarrard & Lewis, 1967; Madsen & Kimble, 1965; Thomas, 1971); inappropriate initiation of responses (Kim, Choi, Kim Chang, Park, & Kang, 1970); low levels of spontaneous alternation (Douglas & Isaacson, 1964; Kirby, Stein, Kimble, & Kimble, 1967; Roberts, Dember, & Brodwick, 1962), and poor performance in learned alternation (Mahut & Cordeau, 1963; Racine &

The preceding section is by no means comprehensive, however certain contradictions become apparent when attempting to relate these studies to a simple response inhibition deficit which has been elaborated in a number of similar ways in the recent literature (see Kimble, 1968; Douglas, 1972; Altman, Bruner, & Bayer, 1973; Jarrard, 1973). Nadel, O'Keefe, and Black (1975) have described the difficulties which arise for the response inhibition interpretations of hippocampal function after a very complete consideration of the hippocampal literature. Among the most notable problems include the contradictory extinction results, the critical nature of intertrial interval in the behavior measured, the seemingly measurement specific deficits in passive avoidance (in some situations hippocampal animals appearing normal and in others abnormal); the nature of exploration and maze learning deficits, and the apparent spatial deficit in reversal tasks. O'Keefe and Nadel (1974) consider hippocampal function to involve spatial abilities, and contend that this spatial deficit underlies all reported changes of behavior in animals and humans with
hippocampal damage. They may indeed be correct in their interpretation of hippocampal function, and there may be no human-animal paradox in the workings of the hippocampus. However, quite interesting line of research has developed out of the postulate that the difference between the results from human and animal literature lies in the fact that the reported human cases have had electrographic disturbances as well as hippocampal lesions or ablations. It is possible that the epileptic activity, and lesion, or an interaction of the two is responsible for the memory deficit found in the clinical literature. Following from this hypothesis, the electrographic normality and/or intact nature of the hippocampus would be necessary for normal recent memory functioning.

The initial support for this hypothesis comes from work using various protein synthesis inhibitors such as puromycin (Cohen & Barondes, 1967) and actinomycin D (Nakajima, 1969). Injecting these drugs into the hippocampal area produces electrographic seizure activity and interferes with learning. Isaacson and his students have been exploring such an hypothesis (Isaacson, 1972; Olton, 1968; Schmaltz, 1971; Schmaltz, Wolf & Trejo, 1973) by examining the behavioral changes in rats which follow the establishment of a penicillin induced epileptogenic foci in the hippocampus. Such a focus is essentially a group of hyperexcitable cells capable of autonomously generating abnormal electrical activity. The effect of penicillin application to the hippocampus or entorhinal area is to produce impaired performance in avoidance tasks (Schmaltz, 1971). Such an effect can be obtained with
penicillin when applied unilaterally or bilaterally, with or without associated surgical destruction.

Since animals with hippocampal lesions will perform as well as or better than controls in a simple active avoidance situation, the behavior of the animals with such penicillin foci can be dissociated from the behavior of animals with lesions. Data for operant discrimination tasks are not as clear. Although animals with penicillin foci in the hippocampus did tend to make fewer correct responses than control animals (Woodruff & Isaacson, 1972) they did not make more errors than controls (Schmaltz et al., 1973). There is work currently in progress (Wann, personal communication) comparing different appetitive and aversive task performance in rats with penicillin foci in the hippocampus. The results from this work will allow critical comparisons of animals with foci and lesions on these types of tasks.

The implications of such an electrographic explanation for memory deficits following hippocampal damage in humans is interesting in relation to the aging literature. Healthy aged individuals would perhaps now be expected to have notable lesions of the hippocampal formation; however, it is known that temporal area EEG abnormalities occur as an individual ages. Although the temporal area EEG changes which accompany age may be very different than those produced by epileptic foci in the hippocampus, these changes may be symptomatic of the known age-related alterations of memory function. The following will include the kinds of electrographic changes which occur in
older human subjects.

Electroencephalographic investigations of healthy older individuals have generally found that there are shifts in the distribution of wave forms towards the slower frequencies (less alpha components and an increase in delta and theta) (Busse, Barnes, & Silverman, 1954; Hughes, 1962; Kooi, Guvener, & Tupper, 1964; Obri, 1954, 1969; Thompson, 1975). Furthermore, visually evoked responses recorded from the scalp of humans change as a function of age (Thatcher, 1975). Subjects aged one month to 81 years were studied in this regard, and it was found that there were no significant differences in overall amplitude of the evoked response from age 15 to 81; however, between age 62 to 70 a reciprocal alteration in the form of the potential occurred, e.g., an amplitude increase was seen in the early component, and a decrease noted in the later component of the response. The source of this change remains obscure; however, some investigators believe that the late components of the evoked response are an indication of the efficiency of information processing (Thatcher, 1975). Hence the decrease of the amplitude of the late component in the older subjects may be interpreted in terms of decreased efficiency of certain information processing systems. At the present, this remains speculative.

Drachman and Hughes (1971) have experimentally compared the impairment of memory in old subjects with that of patients with lesions of the hippocampal complexes. The results of their study support the view that learning-memory functions decline in normal
aged individuals and further support the contention that temporal area EEG abnormalities are common among aged. However, the patients examined with a variety of hippocampal complex lesions could not be related in any simple or direct way to aged individuals on memory or non-memory types of tests. They therefore conclude that in aged people, a different "more widespread pathology must exist".

The foregoing conclusion is actually only one of a number of possible interpretations of the failure to correlate memory performance in normal aged and hippocampally damaged individuals. First of all, the hippocampal patients were young (18-44), had a variety of reasons for this pathological condition, and had variable-sized lesions which presumably did not influence the hippocampus alone. Secondly, it is not typically assumed that old people have totally ablated or lesioned hippocampal complexes, as previously mentioned. Further, the younger individuals with such damage may not demonstrate certain deficits because of compensatory functional take over by other undamaged brain structures, and this capacity may diminish as a function of age. Therefore one is compelled to conclude that this study does not rule out the possibility that the hippocampus is maximally involved in producing differences between young and old organisms in memory function. Nevertheless, it may not be assumed that changes in the aged brain occur solely in this structure.

The hippocampus is the site of the main pathological changes due to the Korsakoff syndrome, the etiology of which is most frequently related to chronic alcoholism. The clinical symptoms include
severe anterograde amnesia for events occurring after the onset of the disease. Early experimenters (Talland, 1968) reported a deficit in such patient's ability to retain and recall newly presented materials, as well as to incorporate them over longer time periods. However, more recent studies have challenged those results and have shown that patients can exhibit savings when retested on recently learned material (Weiskrantz & Warrington, 1970a, b) and they can retain non-verbal stimulus materials for a short period of time (Butters, Lewis, Cermack & Goodglass, 1973). Further, Gardner, Boller, Moreines, and Butters (1973) have data to support the notion that at least some elements of the original input is stored by Korsakoff patients since the correct item can be produced upon cueing. These workers note that Korsakoff patients perform similarly to aged individuals in the sense that their recall can be aided by cueing. However, the aged and Korsakoff individuals can be distinctly contrasted in tests such as retrieval by category, where old individuals perform well and Korsakoff patients perform poorly. Such findings again warn against being hasty to liken normal old-age deficiencies to pathological syndromes.

Of interest, however, in this regard, is one type of senile brain disease, known as Pick's disease. In Pick's disease, where frontal and parietal lobes atrophy, but the hippocampus is spared, there is relative preservation of memories for events since the onset of the illness (Gaitz & Scott, 1978). This observation is particularly interesting since the etiology of other forms of senile and pre-senile
dementias, which destroy the hippocampus, always include a severe recent memory impairment (Hirano & Zimmerman, 1962).

**General Characteristics of Senescent Nervous Systems**

Since neurons do not reproduce after early post-natal life, most of them have a life span paralleling that span of the organism, and thus have often been considered a particularly critical cell type to study if mechanisms controlling aging are to be elucidated (Timiras & Vernadakis, 1971). In general, it has been found that there is a decline in the number of neurones as age advances (Brody, 1955; Inukai, 1928). However, methods have been so tedious that proper comparisons between anatomical areas and between species has been lacking. Using more advanced instrumentation, Brody (1975) has recently been able to compare cell counts in different localized areas of human brain. His data indicate that neurons do not decrease at approximately similar rates in all brain areas. In layers two and four of many cortical areas there is a striking decrease of granule cell density whereas there is no parallel decrease in brain stem cell group populations. Since the neuron is an information carrying element in the nervous system, losing these elements must have an effect on information capacity. Whether the reported losses are sufficient to cause functional change remains speculative.

The ratio of glia cells to neurons is another cellular change noted upon examination of senescent human, monkey, and rat brains. In mature young brains the glia/neuron ratio is approximately .98; whereas for senescent brains, the ratio reaches 1.75 (Brizzee,
1975). The areas contributing most to this change are considered to be cortical rather than sub-cortical. Since the greatest neuron loss is in cortical areas, a slower rate, or no decline in the number of glia in these areas, could account for the change with age in the glia/neuron ratio. However, glial proliferation is also a possibility.

The increasing intraneuronal accumulation of lipofuscin granules is considered to be one of the most consistent cytological changes which accompanies aging (Hasan & Glees, 1972; Sekhon & Maxwell, 1974; Sohal & McCarthy, 1973; Spoerri & Clees, 1973). These deposits are thought to occur in lysosomes as a result of the accumulation of indigestible cellular 'waste materials'. Although the accumulation of lipofuscin has often been considered an important factor leading to cell death, workers in the area today are beginning to question this position. One such laboratory (Brody, 1975) has found that the inferior olivary nucleus tends to accumulate large amounts of lipofuscin; but, as other nuclei in the brain stem, does not show a significant amount of cell death with age. Hence, it is now thought that lipofuscin does not kill cells. It has been suggested that lipofuscin may actually be beneficial, since it could act as a sink for free radicals and immobilize toxic compounds (Brizzee, 1975).

After the age of 65, human neurons often show evidence of neurofilament degeneration, often termed neurofibrillary tangles. Such tangles do not occur spontaneously in laboratory rats; however, with application of aluminum, such twisted filaments occur (Crapper,
1975). The relationship between human exposure to aluminum compounds and the development of these neurofibrillary tangles has yet to be determined.

It has recently been demonstrated that on the average, there is less ribosomal ribonucleic acid (rRNA) in individual cells in senescent brains (Strehler, 1975). Since the amount of rRNA which would hybridize to DNA decreased with age, this species of RNA may either be transcribed more slowly, or broken down faster; in either case, protein synthesis in general may be decreased.

The activity of a number of enzymes controlling transmitter metabolism in the central nervous system also change as a function of age. Recent findings indicate that the changes which occur can be quite area specific, so that measurements of whole brain activity are likely to be misleading. The following is a general outline of changes occurring in human brain (from McGeer, 1975): tyrosine hydroxylase (the rate limiting enzyme for catecholamine synthesis from tyrosine) decreases predominately in subcortical areas and monoamine oxidase (plays a role in the degradation of catecholamines) increases, the net result of both being to decrease catecholamine activity; choline acetylase (acetylcholine synthesis) decreases most notably in cortical areas; levels of serotonin progressively decrease with age and glutamic acid decarboxylase (responsible for the conversion of glutamic acid to GABA) also decreases with age. Such changes in transmitter metabolism must be evaluated carefully since there may be changes in receptor sensitivity. Even
though the activity of certain transmitters may decrease, the receptor may either compensate or hypercompensate, producing entirely different results than what would be anticipated from the enzyme literature alone.

The extracellular space in whole brain of three-month and 26-month old rats has recently been measured by electron microscopy, after preparation with freeze substitution techniques (which prevent the usual changes in cell volume accompanying fixation). It was found that 21% of brain in the three-month old rats was extracellular space; whereas in senescent (26-month old) brain, 9.6% is extracellular space (Boudareff, 1975). The decrease in this space could have profound effects on a variety of physiological parameters: some of these would include the mobility of transmitter substances, conformational changes possible in cell membranes, or influences on the ionic concentration balance across the membrane. This, along with the fact that dendritic spines (and thus spine synapses) decrease in senescent organisms, could profoundly change the transmission properties of the brain areas involved. Decreases in spines have been found to occur in pyramidal cells in visual cortex of 36-month old rats as compared with three-month old rats (Feldman, 1975). This loss of spine synapses is not paralleled by a decrease of synapses on the dendritic shaft.

The foregoing is of particular interest because of current speculation as to the role that spines may play in synaptic efficacy. It has been suggested that swelling of spines or decreasing the length
of the spine could increase synaptic efficacy by decreasing the attenuation of synaptic potentials conducted passively to the parent dendrite (Rall, 1970). This hypothesis has become particularly interesting in light of the recent morphological evidence for an increase of dendritic spine area in granule cells of the fascia dentata after 30 seconds of 50 Hz stimulation of perforant path fibers (Van Harrevelt & Fikova, 1975).

**Hippocampal Changes With Age**

A number of investigators have attempted to show a relation between brain ribonucleic acid (RNA) and learning-memory phenomena in the hippocampus and other brain areas. Such experiments have typically involved training animals on avoidance tasks (Zemp, Wilson & Glassman, 1967; Zemp, Wilson, Schlesinger, Boggan & Glassman, 1966) or spatial reversal tasks (Bowman & Strobel, 1969) and measuring the incorporation of radioactive uridine into nuclear and ribosomal-polysomal RNA fractions. The general results indicate that the radioactive label increases significantly in brain samples which include the hippocampus. These results support the hypothesis that the proper functioning of protein synthesizing mechanisms in the hippocampus and/or temporal cortical areas may be required for proper functioning of learning-memory processes (Flexner, Flexner, De La Haba & Roberts, 1964; Flexner, Flexner & Roberts, 1967; Ott & Matthies, 1973). A difference in the amount of such RNA synthesis in the hippocampus has been noted between old and young rats. Altshuler, Kleban, Gold, Lawton and Miller (1971) have compared the
amount of RNA synthesis in the hippocampus between young and aged rats after avoidance training. While their young rats showed large increases in RNA synthesis, at the time point measured, their aged animals did not evidence quantitative increases with training. This difference might be explained in terms of an overall slowdown in transcription of RNA species. Perhaps if measurements were taken at later time points, the aged animals might also have shown measurable increases.

Although levels of catecholamines are depressed in old organisms, autonomic nervous activity (ANS) may actually be heightened. It is well established that as sympathetic nervous system activity increases, performance of many tasks improve, until a maximum is reached, and with further increase the behavior deteriorates. Eisdorfer (1970, 1975) has tested the hypothesis that older humans are actually on the far side of the maximum (or sympathetically overaroused). He predicted that administering propanalol (a β-adrenergic blocker) to his old subjects should improve their performance by decreasing sympathetic activity. In fact, in verbal learning tasks, the performance of the older adults did improve, indicating that they were on the over-reactive end of the performance curve. He concludes that this autonomic hyperreactivity is due to end organ sensitivity. This may in fact be true; however, other factors may interact with receptor sensitivity to produce these results, such as the influence of certain brain structures on hypothalamic functioning. Although hippocampal stimulation has been
found to both increase (Gergen, 1967; Poletti, Kinnard & MacLean, 1970) and decrease (Dafney & Feldman, 1969; Gergen, 1967; Stuart, Porter & Adey, 1964) hypothalamic unit firing, it is generally thought that the hippocampus can exert an inhibitory action upon hypothalamic-hypophyseal output (Isaacson, 1972; Strand, 1975), or at least plays a role in modulating it (Olds, 1970). If the interaction between the hippocampus and hypothalamus changes with age, this may contribute to the observed hyperreactivity.

The sub-microscopic structure of the hippocampus is particularly vulnerable to age-related changes (Blessed, Tomlinson, & Roth, 1968; Hirano & Zimmerman, 1962; MacLean, 1963; Roth, Tomlinson, & Blessed, 1967). For instance, lipofuscin accumulation begins at an earlier age and more pigment per section volume is found in the hippocampus compared with the cerebral cortex or cerebellum (Reichel, Hollander, Clark & Strehler, 1968). The neurofibrillary tangles found in human brains are most pronounced in the hippocampus (Wisniewski, 1975). Further, elevated aluminum content occurs only in the hippocampus and frontal lobes in a variety of senile dementias. This is interesting in the light of animal literature where neurofilament tangles can be induced by aluminum application (Crapper, 1975).

The zinc and lead content of the hippocampus changes in an age-related fashion. The region where mossy fibers terminate is a region of high electron density in the hippocampus, as high concentrations of zinc (Fjerdingstad, Danscher & Fjerdingstad, 1974) and lead (Fjerdingstad, Fjerdingstad & Danscher, 1974) confer greater contrast
of the hippocampal terminal endings for electron microscopy. Hasan and Glees (1973) found an increase in electron density of these terminals with advanced age. This may be due to an increase of hippocampal heavy metal content with age; the functional significance of which cannot as yet be determined.

Recent evidence suggests that the number of synapses on old rat granule cell dendrites in the hippocampus decreases by 30% in the region of termination of the perforant path (Bondareff & Geinisman, 1976). The change in the number of counted synapses was not due to a change in tissue volume (there was no statistically significant difference in the depth of the molecular layer between age groups) or to a change in the number of granule cells. This will become a particularly important piece of information for the interpretation of the data in this thesis.

The evidence from clinical memory literature, lesion work, pathological dementias, electrophysiological, biochemical and ultrastructural studies, indicates that some of the critical changes which accompany aging are particularly pronounced in the hippocampus. The extent to which hippocampal change is related to the decrease in reliability of learning-memory processes in senescent organisms is of empirical interest here. More specifically, the modifiability of nervous tissue may include changes in the efficacy of individual synapses. This process has an experimental model in the potentiation of synaptic transmission in hippocampal granule cells produced by high-frequency stimulation of their afferents. The relevance of
long-lasting potentiation in the hippocampus as a model of learning-memory process is considered below.

Long-Lasting Changes in Synaptic Efficacy as a Model of Learning-Memory Processes in the Nervous System

The assumption that the locus of the transformation of experience into permanent memory is the region of functional contact between neurons (i.e., the synapse) has been central to both historical and modern approaches to theories of learning (Brindley, 1969; Hebb, 1949; Little & Shaw, 1975; Marr, 1971; Rosenzweig, Mollgaard, Diamond & Bennett, 1972; Sherrington, 1947; Uttley, 1976a,b,c). The verification of this assumption has proved extremely difficult. It may never be possible to tease out each neuron, synapse, and/or metabolic change contributing to the storage of the information necessary to perform a particular task. This situation is not unlike problems in other disciplines, where the physical constructs of theories cannot be directly gathered for measurement. However, it is possible to collect data which may contribute to correlations between specific brain functions and representation of experience. In this manner, a theory of memory can be built from better and better approximations to actual physiology, and extended to what is known of memory in intact organisms.

While it may not be possible to specify sufficient conditions for a proof of the hypothesis that memory is based on synaptic modification, certain necessary conditions can be outlined. First, synapses must be capable of permanent modification. Second, when
learning occurs in a particular neural system, it should be accompanied by a measurable synaptic change. Further, if the synapse is prevented from changing, no learning should take place. Lastly, if a synaptic change correlated with storage of information is reversed, the memory should be lost. The theory can be disproven in principle, by finding that learning occurs even though there is no synaptic change in the system; (however, in the mammalian brain, a demonstration of this is technically impossible at present). There are numerous experimental paradigms which can contribute answers to these basic questions, some of which will be described below. The purpose of this section is to develop the rational for choosing potentiation of hippocampal synapses as a major experimental approach for this thesis.

Quite obviously missing in the following are the studies of synaptic plasticity in invertebrates which have appeared recently in the literature (Kandel, Castellucci, Pinsker, & Kupfermann, 1970; Kandel & Spencer, 1968). Since the primary concern of this thesis is the aging of the mammalian nervous system, and since it is unclear as to what extent invertebrate nervous systems are comparable, these will not be considered. Vertebrate spinal and neuromuscular monosynaptic reflex pathways have also been studied in detail (Eccles, 1964; Esplin & Zablocka-Esplin, 1971; Magelby, 1973; Magelby & Zengel, 1975a, 1975b; Zablocka-Esplin & Esplin, 1971). Although comparisons between these lower order synaptic systems and cortical synapses may encourage interesting discoveries, the direct relationship between them remains speculative; and will not be examined here.
One approach to the questions outlined above includes studies of neuronal unit activity during behavioral conditioning in mammals. This work is illustrated by the study of Olds and Hirano (1969), where hippocampal and midbrain unit activity in freely moving rats increased after appetitive Pavlovian conditioning to acoustic stimuli. This was later confirmed and extended by finding decreased unit firing in habituation situations, increased firing under discrimination learning contingencies, and decreased firing during extinction (Hirano, Best & Olds, 1970). More combinations of this basic design have been accomplished by simultaneously monitoring unit activity in many related brain areas (Segal, 1972; Segal, Disterhoft & Olds, 1972; Segal & Olds, 1972, 1973). These kinds of correlations are certainly relevant since cell firing rates should be related to these behavioral conditions if they are involved in producing these states. Unfortunately, they cannot be directly related to synaptic plasticity, since changes in neuronal firing rates could result from a wide variety of alternate mechanisms such as changes in the cell's ionic or hormonal balance.

Studies which interfere with memory processes by removing or electrically stimulating brain areas also contribute to the understanding of regional differences in brain function. The data from these kinds of experiments are consistent with the interpretation that the excision, lesion, or electrical stimulation disrupts memory-learning processes in the structure manipulated. It is, however, difficult to specify or to localize these effects. Similarly,
chemical stimulation techniques may act through mechanisms other than alteration of synaptic efficacy, such as circulating levels of hormones (Nakajima, 1973).

Thus, although numerous studies are able to correlate specific experimental manipulations with memory processing, none of these approaches can resolve the question of whether synaptic modification is the underlying mechanism of memory. The first necessary condition outlined above is that synapses must be permanently modifiable. One approach to this question is exemplified by the kindling method (Goddard, 1967; Goddard, McIntyre, & Leech, 1969) which has strongly suggested that synapses in chronic preparations can be progressively modified and can remain modified over long periods of time. This approach involves creating what Goddard has called an 'artificial engram'; or a long-lasting alteration in brain response under conditions which can then be examined and compared with certain theoretical properties considered important in information encoding and storage (Goddard & Douglas, 1975). The kindling technique involves low-intensity (non-epileptogenic) brain stimulation of rats with chronically implanted stimulating and recording electrodes. The stimulation is optimally given once a day over several weeks. At first, this stimulation produces no behavioral response or electrographic after-discharge. However, with subsequent trials, behavioral convulsions are observed and epileptiform after-discharge can be recorded. Once this change has taken place, it appears to remain stable. Kindling has met many necessary conditions for being an
appropriate model of normal memory, which is best summarized as follows:

It involves a non-degenerative change in neural processing based, at least in part, on relatively permanent alterations of excitatory synapses. It can be induced by specific forms of neural activation at specifiable locations in the adult mammalian brain, and can affect behavior in a lasting way. And, the effect shows other similarities to learning such as transfer, interference and spontaneous recovery. (Goddard & Douglas, 1975, p. 392)

As they further mention, kindling has not been shown to be influenced by environmental sensory input. While changes in the efficacy of synaptic transmission is the most likely explanation of the kindling effect, there is no clear demonstration that this is the case. It would be preferable to work with a model system where the changing synapses were quite precisely defined so that examination of the mechanism of change would be relatively simplified. Such a model has recently been developed and will be discussed below.

High-frequency (HF) stimulation of neurons from a variety of nervous systems can produce an increased probability of such cells firing to a subsequent test pulse; this effect is termed post-tetanic potentiation. Early evidence that potentiation could occur has its roots in modern spinal reflex physiology (Lloyd, 1949; Eccles & Rall, 1951) where the effects of post-tetanic potentiation of spinal motor neurons lasted several minutes after discontinuation of the HF stimulation. This post-tetanic potentiation is characterized by an increase in the size of the excitatory post-synaptic potential (EPSP), and thus in an increase in the probability of
discharge of the post-synaptic neuron. Using intracellular recording techniques, this was found to result from the number of quanta of transmitter released per impulse (Hughes, 1958; Eccles & Krnjevic, 1959).

It was not until quite recently however, that similar potentiation was demonstrated to last for long periods of time in some central nervous system synapses (Bliss & Lomo, 1970, 1973; Bliss & Gardner-Medwin, 1971, 1973). This is a necessary correlate of a more permanent kind of memory mechanism. Bliss, et al., potentiated the synaptic response of granule cells of the hippocampus by tetanizing its major input from the entorhinal cortex. (This procedure and hippocampal anatomy will be described more thoroughly in sections to follow.) Both anesthetized and unanesthetized animals were used in these studies without any apparent differences in the parameters necessary for potentiation. Further, they found that when a potentiated response was obtained it would sometimes remain elevated over a considerable time interval. In this mono-synaptic pathway an increase in synaptic efficacy is the only conceivable interpretation of the mechanism that causes test stimulation to become more effective (if more fibers are not firing). This technique was therefore chosen as the primary focus of this thesis.

Douglas and Goddard (1975), using a slightly revised procedure, obtained results which were even more striking. That is, with their procedure, potentiation could be obtained in 100% of the preparations. This increased evoked response was found to be a
consistent and relatively permanent alteration, with certain com-
ponents remaining potentiated for at least two months after discon-
tinuation of the potentiation procedure. This technique is particu-
larly useful since physiologically relevant stimulation parameters 
can be used (Racine, Newberry, & Burnham, 1975; Douglas, 1977), 
and the evoked responses recorded after potentiation often fall within 
the range of normal responses which can be elicited by raising the 
amplitude of stimulation. Furthermore, HF stimulation of the perfor-
ant path results in persistent increases in unit activity of the 
fascia dentata (Deadwyler, Gribkoff, Cotman & Lynch, 1976) again 
suggesting that the changes produced after HF stimulation may be 
physiologically relevant.

Racine and coworkers have found an interesting interaction 
between the effects of kindling and post-tetanic potentiation when 
stimulating the amygdala. The late components of the evoked poten-
tials recorded from a number of brain areas were enhanced following 
kindling of the amygdala, as compared with control evoked responses 
before kindling (Racine, Gardner, & Burnham, 1972). Furthermore, 
prior post-tetanic potentiation of the amygdala and septum was 
shown to facilitate kindling of the amygdala (Racine, et al., 1975). 
Hence the changes which occur following application of these techni-
ques may have certain mechanisms in common (although other inter-
pretations are possible). Racine, et al. (1975) claim that both these 
methods produce increases in essentially similar components of the 
evoked response measured, although it is difficult to determine this 
from the figures in their paper. The long-term effects of tetanic 
stimulation and kindling were dissociable from recruiting response
triggered in the amygdala (Racine, et al., 1975). The "recruiting" paradigm involves stimulating the amygdala with 10 Hz and recording evoked potentials from the pre-optic area and hippocampus. These potentials begin to grow in amplitude after the first few stimuli. However, such recruiting responses do not permanently augment the evoked response in these structures. Since the neural activity caused by the recruiting response was not sufficient to cause a long-lasting change, it follows that the neural activation must be rather specific to produce a permanent electrophysiological change.

A more rigorous demonstration that the effects of long-lasting potentiation are specific has recently been accomplished by stimulating separate inputs to the granule cells of the hippocampus. After establishing convergence of two pathways onto the same granule cells, high-frequency stimulation of one pathway which produced long-lasting potentiation of the response evoked from that pathway, did not lead to an increased response from the other pathway (McNaughton & Barnes, 1977). Since this result could be obtained from either of the two pathways, this provides conclusive double dissociative evidence that the long-lasting potentiation phenomenon is selective and does not depend on some general change in the post-synaptic cell.

Another approach to the study of the relationship of changes in the brain to the possible mode of information storage comes from studies which can correlate changes in well defined behavior sequences to precise manipulations of brain function. This kind of work is most elegantly exemplified by Adamec (1975) in his studies on predatory attack behavior in cats. It was known from the
work of Flynn and his co-workers (Egger & Flynn, 1963; Flynn, Vanegas, Foote, & Edwards, 1970; Siegle & Flynn, 1968) that hypothalamic control over predation is facilitated by stimulation of the ventral hippocampus and inhibited by stimulation of the basolateral amygdala. Adamec took these findings a step further by relating the excitability (or after-discharge threshold) in these structures to the naturally occurring behavior of cats which were rat killers and those which would not spontaneously kill rats. He was able to correlate differences in the epileptic excitability of structures thought to be responsible for the different aspects of predatory response with actual rat-killing behavior in the cats. Further, by increasing this excitability in 'killers' to the level of 'non-killers', by partial kindling of the basolateral amygdala (which lowered the after-discharge threshold), he was able to abolish their spontaneous killing behavior. This finding further correlated with the fact that the basolateral amygdala seemed to have an excitatory influence on defensive behavior in cats (Stokman & Glusman, 1970). This is particularly good evidence in support of the idea that changes taking place during such repetitive stimulation may be similar to changes which can naturally change the disposition to attack in these animals.

Another approach which can be used to relate synaptic function to memory is to compare synaptic responses from brains of animals which are known to have memory deficits with those which do not have such deficits. If in the one case encoding-storage processes
were not functioning normally, and if synaptic changes are involved in the reliability of such information processing, measurable differences between the workings of these synapses would necessarily be required. If the predicted result was found to be correct, then it would serve as another supportive correlation for the theory that synaptic modification is critically important in memory formation. It would also give information about the nature of the deficit being studied. The foregoing is the basic rational behind comparing senescent and middle-aged synapses of the fasic dentata. Before a more detailed discussion of the proposed methods, some basic hippocampal anatomy and physiology will be reviewed.

**Hippocampal Connectivity**

Although the general anatomy of the hippocampus and dentate area is unmistakably clear, the internal and external connections continue to be a matter of some dispute. The following will include a currently viable description of the entorhinal cortex as it connects with the hippocampus (in the rat). Since the plane of section in most anatomical descriptions is horizontal, with extreme dorsal or ventral views excluded, caution must be taken when extrapolating to these areas.

The hippocampus is composed of three parts: the subiculum, Ammon's horn, and the dentate gyrus. The perforant path projection from the entorhinal cortex to the fascia dentate is of particular interest in relation to the experiments in this thesis, and will be specifically emphasized in the following. The entorhinal cortex
is classically sub-divided into two regions, containing large cells in its medial extent (area 28a), and smaller cells throughout its lateral extent (28b). Medially adjacent to the entorhinal area is the parasubiculum (area 49), the presubiculum (area 27) and finally subiculum, which represent a transition zone between entorhinal cortex and the remainder of the hippocampus. Lorente de No (1934) divided Ammon's horn into four zones: CA1, CA2 (corresponding to Cajal's (1911) regio superior), and CA3 and CA4 (corresponding to Cajal's (1911) regio inferior). The scattered cells of the subiculum are replaced by the orderly, densely packed band of pyramidal cells of Ammon's horn which demarcate the CA1 field. The pyramidal cells of CA3 are larger and more loosely packed than those of CA1, with CA2 being a transition zone between these two cell types. The region between the fascia dentata and CA3 is called either CA4 or the dentate hilus, and is made up of modified pyramidal cells and polymorph cells.

The dentate area is composed of a densely packed layer of cells, the stratum granulosum. Beneath it, the stratum polymorph contains the proximal portions of the unmyelinated mossy fibers, basket cells, and some polymorph cells. Above the stratum granulosum lies the stratum moleculare which contains primarily the dendrites of the granule cells, a small number of scattered neurons which have been little studied, and is bounded by the hippocampal fissure.

There are two major efferent systems from the entorhinal cortex to the hippocampal formation. One projects bilaterally to
regio superior and subiculum (Blackstad, 1956; Raisman, Cowan & Powell, 1965) and one constitutes a major ipsilateral input to the fascia dentata and regio inferior (Hjorth-Simonsen, 1972; Hjorth-Simonsen and Juene, 1972). These two systems appear to have different cells of origin in the entorhinal cortex: the projection to regio superior arising from layer 3 pyramidal cells and the projection to the dentate arising from layer 2 stellate cells (Steward & Scoville, 1976; Swanson & Cowan, 1977). The entorhinal projections to regio inferior have not been clarified.

The lateral entorhinal area gives rise to the lateral perforant path and terminates in a continuous band occupying the other half of the strata moleculare of CA3 and the outer one-third of the dentate molecular layer (Hjorth-Simonsen, 1972). The medial perforant path originates in the medial part of the entorhinal area and projects to the inner half of the stratum moleculare of CA3 and to the middle-third of the dentate molecular layer (Hjorth-Simonsen & Jeune, 1972). The inner zone of the molecular layer receives septal input, a commissural projection from the contralateral hippocampus, and association fibers from CA3 and CA4 (Zimmer, 1971). The outer two-thirds also receive sparse projections from the medial septum and diagonal band nuclei. The locus ceruleus also projects to the hilus of the fascia dentata, but does not appear to terminate on the granule cells (Swanson & Hartman, 1975). Figure 1 is a schematic diagram of the locus of termination of various projections onto the granule cells of the dentate gyrus.
The entorhinal cortical projection to the dentate gyrus is mostly ipsilateral in the rat. However, substantial contralateral entorhinal-dentate projections occur in the rabbit (Hjorth-Simonsen & Zimmer, 1975); and sparse projections have been reported in rat (Goldwitz, White, Steward, Cotman & Lynch, 1975; Zimmer & Hjorth-Simonsen, 1975). The afferents to the dentate from the septum are presumed to be cholinergic (Storm-Mathisen, 1970) while the transmitter(s) for the other afferent fibers to the molecular layer of the dentate remain unknown. Although little is known about the nature of entorhinal afferents, it has recently been found that the presubiculum gives rise to a topographical projection to both the ipsilateral and contralateral entorhinal cortex in guinea pig (Shipley, 1975). Also, the hippocampus is thought to project back onto the entorhinal area (Hjorth-Simonsen, 1971). The efferent fibers of the granule cells of the dentate gyrus (often termed mossy fibers) travel through the hilus of the fascia dentata towards the pyramidal cells of CA3 and CA4 where they form synapses with the apical dendrites (Andersen, Blackstad, & Lomo, 1966; Blackstad, Brink, Hern & Jeune, 1970; Blackstad & Kjaerheim, 1961; Swanson & Cowan, 1977). The mossy fiber axon also gives off a recurrent collateral which synapses with basket cells in the subgranular region, which in turn provide a feedback inhibition onto the granule cells (Anderson, et al., 1966).

The Perforant Path - Granule Cell Evoked Response

The evoked potential recorded from the dentate molecular
layer after stimulation of the perforant path is comprised of three components, each giving different information about the functioning of the perforant path - dentate connection. The first component (see 1 in Figure 1) corresponds to a pre-synaptic fiber potential, and is due to the arrival of action potentials along the pre-synaptic fibers of the perforant path. The detection of this component is possible with proper electrode positioning but is not seen in all preparations. The second component (see 2 in Figure 1), is due to monosynaptic EPSP's from the dendrites in the dentate molecular layer. Finally, the third component (see 3 in Figure 1), referred to as a population spike, represents the synchronous action potentials of a number of granule cells (Andersen, Bliss, & Skrede, 1971; Lomo, 1970). After stimulation of the perforant path, the second and third components increase in amplitude, and the third decreases in latency. No change is observed in component 1. This indicates that the potentiated response is not simply due to more perforant path fibers firing but that the change takes place at the synapse.

The perforant path - granule cell response reverses polarity when the recording electrode is lowered from the molecular layer into the hilus (see Figure 1). The polarity reversal is due to the pattern of current flow in the extracellular space. When the electrode is in the synaptic zone in the molecular layer, it is located in a current sink whose source is located in the granular and sub-granular regions. Therefore, a negative going response is recorded in the molecular layer and a positive going response is recorded in the region of the cell-body (Lomo, 1970, 1971a).
Figure 1. In the upper portion of this figure is a diagram of the distribution of synaptic connections in the fascia dentata (B-basket cell terminals; C-commissural afferent terminals; MPP-medial perforant path; LPP-lateral perforant path; S-septal afferent terminals; A-ipsilateral association fiber terminals; MOL-molecular layer; CR-granule cell body layer).

The lower portion of this figure shows granule cell responses to perforant path stimulation at three different depths (A, B and C) which correspond to the position of A, B and C on the granule cell of the upper diagram. The three components of the evoked response are shown as: 1 (the pre-synaptic fiber response), 2 (the field excitatory post-synaptic potential) and 3 (the compound action potential or "population spike").
This electrophysiological technique has been successfully employed in a variety of species and locations in the hippocampus. Both anesthetized intact organisms (Adnérsen, Teyler, & Wester, 1973; Bliss & Lomo, 1973; Gloor, Vera & Sperti, 1964; Steward, White, Cotman & Lynch, 1976; Teyler, Alger, Bergman & Livingston, 1977; White, Goldowitz, Lynch, & Cotman, 1978) and slices of excised tissue (Alger & Teyler, 1976; Andersen, Sundberg, & Sveen, 1977; Bliss & Richards, 1971; Dudek, Deadwyler, Cotman, & Lynch, 1976; Lynch, Dunwiddie, & Grubkoff, 1977; Schwartzkroin, 1975; Schwartzkroin & Wester, 1975; Skrede & Westgaard, 1971; Teyler et al., 1977; Yamamoto, 1972) have generally confirmed and extended the findings obtained in awake preparations. Since this thesis deals with awake implanted rats, the chronic preparation is emphasized.

Using awake, freely moving rats, Douglas and Goddard (1975) found that the largest change in the evoked response occurs after the first potentiation stimulation train, with the response reaching 30% of its maximum value at that time. The population spike remained potentiated at least twelve days after the final potentiation treatment, but returned to baseline levels at a measurement taken two months later. The second component, however, remained potentiated even at the two-month time point. The foregoing description is characteristic of data gathered for mature rats, and certainly suggests a number of measures which might be relevant for examination of the age variable in these animals (which will be discussed later).

The rational behind the predictions that will be made in the
experimental sections are as follows. If certain forms of memory
disfunction are related to changes in synaptic function of the dentate
granule cells, it can generally be predicted that there will be
differences between age groups in at least one of the aforementioned
components of the evoked response. One way of viewing this general
scheme is in terms of the systems approach to archicortex described
by Marr (1971). Marr defined an event as being stored in a neural
system if input of a subset of that event resulted in output of the
whole event. He showed that the reliability of the system, or the
probability of one stored event being distinguished from another,
decreases as the number of stored events (and hence number of po-
tentiated synapses) increases. It was assumed (although this is not
required by the model) that the number of synapses in the system was
constant over time. An interesting corollary of this is that as
the number of stored events increases, the size of the subset
required for reliable retrieval of that event approaches the size
of the whole event. This model therefore predicts the following
differences in the measured evoked response between mature
and senescent organisms. The amplitude of the evoked response
of the old animals should be higher (since the synapses would
be expected to be more modified and thus more powerful). There-
fore the initial percent potentiation may be less (the old rats
nearer to the maximum possible amount of potentiation) and the maxi-
imum potentiation would be reached sooner in these rats. The
reliability of retrieving this information would be reduced in the
old animals (there would be more stored events and therefore less
reliable retrieval). The durability of the elevated response could also be altered. The extent to which these predictions are met will thus be examined.

**Experimental Variables**

The variables measured in the studies designed for this thesis involve behavioral and neurophysiological tests of middle-aged and senescent rats. As has been developed in the foregoing discussion, the physiology will include monitoring the response of the hippocampal granule cells and the behavior will include tasks which are thought to be relevant indicators of hippocampal function (a spatial discrimination task, and a spontaneous alternation task).

The time of day at which the physiological and behavioral measures are recorded will be kept constant for any particular animal and balanced across groups. The time of day when the perforant path - granule cell synapse is measured has recently been shown to be an important variable. The synaptic response is more powerful in rats when tested at a time of day which corresponds to the colony room period and is least powerful during the time corresponding to the colony room light period (Barnes, McNaughton, Douglas, Goddard, & Adamec, 1977). Furthermore, there is scattered evidence suggesting that circadian rhythms in old organisms may be altered with age. The period of circadian activity rhythms of golden hamsters and two species of deer mouse became shorter as the animals aged after being released into constant darkness (Pittendrigh & Daan, 1974). This does not necessarily
indicate that this would occur in normal light-dark situations. In the human literature, however, there is evidence of altered urinary steroid rhythms in older individuals (Montalbetti, Ghiringhelli, Gonini, & Bonanomi, 1967), and that a number of physiological rhythms may shift in their phase relation with one another as individuals age (Cahn, Fol, & Huston, 1968). It is possible that such internal discordance contributes significantly to the aging process. Certainly sleep patterns change dramatically as an individual ages (Fischer-Williams, 1976; Thompson, 1975). All these factors suggest that the time of day variable is important to include in an experiment comparing young and old organisms.
GENERAL METHODS

Subjects

Male Long Evans rats were obtained from the retired breeder stock at Quebec Breeding Farms at eight months (± one month) of age. All rats were housed in single cages in a room which was maintained at approximately 21°C and set on a 16-8 hour light-dark schedule (07:30-23:30 light). The old rats were maintained in this room for 20 months and the young rats for two months before the start of this experiment. Under optimal conditions the life expectancy of the rat is about three years (Stone, 1929a, b). Rats are considered senescent at approximately two years of age, which is thought to be comparable with 60 years in man (Munn, 1950; Stone, 1929a, b; Tryon, 1931). The two age groups in this study will hereafter be referred to as senescent normal (SN) and mature adult (MA). The experiment was run over a six-month period so that the SN rats were tested between the ages of 28-34 months, and the MA rats were tested between the ages of 10-16 months. The SN rats were therefore quite near their maximum expected life span of 36 months, which approximates a human age of between 70-85 years. The MA rats had lived approximately one-half their expected life span corresponding to a human age of between 30-45 years.

Random assignment to groups. Out of the original population of 50, 34 SN rats were alive and healthy at the time of random assignment to groups. There were a variety of causes of deaths over.
the 20-month period, such as respiratory, bladder, and intestinal disease. All of the 40 MA rats were included at this time. One month after the arrival of the MA rats, all animals were assigned numbers and were etherized so that their ears could be marked with the appropriate number code. After ear marking, 32 SN and 32 MA rats were assigned to groups with a random number table. This was accomplished by consecutively filling each of eight groups with the numbers of four MA and four SN rats, leaving a total of two extra SN and eight extra MA rats. These extra rats were kept to replace losses in group size due to deaths occurring during the experiment. This loss was anticipated to occur in the SN groups because of their advanced age; deaths due to surgical anesthesia and illness was also possible in the MA groups. Appendix A lists the time of the occurrence and the causes for diminished group size during the experiment.

Each group was then assigned a cage position on one of two double colony racks. This order was chosen to minimize possible confounding variables due to housing differences between age groups (such as light and temperature gradients). The rats remained in this position in the colony room throughout the experiment.

Handling. The method used for handling all rats consisted of picking them up by grasping them around the body and placing them onto the top of a cart in the colony room. After six rats were placed on the cart, they were generally left there for a period of 5-15 minutes, during which time the health status of each animal
was noted. By the time that the experiment started, (although the SN rats had received more total handling) none of the rats exhibited exaggerated emotional responses to being picked up or held. The one other form of handling experience these rats received before the beginning of the study is described in Appendix D (the odor adaptation trial).

The weights of the rats and incidence of tumors are discussed in Appendices B and C respectively.

**Overall Design**

Thirty-two MA and 32 SN rats were randomly assigned to one of eight groups (as discussed above). Thus each group consisted of four SN and four MA rats. For all treatments (with certain exceptions to be described later) each group was tested within the same two-hour time period shown in Table 1. Therefore, most treatments can be viewed in terms of age and/or time-of-day effects.

**Table 1**

<table>
<thead>
<tr>
<th>Group #</th>
<th>Time of Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12:00-14:00</td>
</tr>
<tr>
<td>2</td>
<td>14:00-16:00</td>
</tr>
<tr>
<td>3</td>
<td>16:00-18:00</td>
</tr>
<tr>
<td>4</td>
<td>18:00-20:00</td>
</tr>
<tr>
<td>5</td>
<td>20:00-22:00</td>
</tr>
<tr>
<td>6</td>
<td>22:00-02:00</td>
</tr>
<tr>
<td>7</td>
<td>02:00-04:00</td>
</tr>
</tbody>
</table>

The sequence of experimental events as well as the actual sequence of the experiment in time (the date) can be seen in Table 2 (over three pages). Although each group received the same treatment
in exactly the same time sequence, these treatments were staggered by one day for each successive group. The reason for staggering the groups was purely practical since it distributed the demand on computer and experimenter time. The rational for each treatment will be discussed briefly at the beginning of each experimental section to follow. As mentioned above, not all treatments were measured at the individual group's designated time of day. These exceptions came at the end of the study and are shown on the third page of Table 2.

The choice of chronological order of the experiments in the thesis is as follows (see Table 2 for the location of the task in time, using the symbol codes in parentheses below). The spatial discrimination test (M) was given at the beginning of the experiment both because the apparatus affixed to the head for electrophysiological recording would have proved cumbersome on this maze, and because the testing studio was only available during this period of time. Surgery (S) for electrode implantation thus followed the spatial maze task. Spontaneous alternation maze tests (A) were interspersed throughout the experimental period to monitor recovery from surgery and to determine whether certain electrophysiological manipulations influenced performance on this task. The electrophysiological measures taken before HF stimulation (T) were those which were thought not to interfere with the results from this major experimental section, such as EEG recording (E), and low-frequency stimulation (P, I, and B). After HF stimulation, other physiological
measurements were taken (double pulse inhibition and facilitation, DP), the effects of Nembutal anesthesia on the evoked response was examined (ADP), and blood pressure was measured (BP). Brain fixation and histology concluded the experimental section (F1X).

Although the design of this experiment was carefully considered (split-plot factorial, pr.q (Kirk, 1968)), and the orthogonal comparisons (e.g., between age groups, and between times of day) were pre-planned, the study was complicated by deaths and a variety of equipment and program failures which produced cells with unequal n, and at times cells which were empty. The difficulties which arise from a situation like this are numerous and the standard methods of analysis become unreliable. The t ratio, therefore, was used most often to make comparisons of age or time of day, and α was set at the .05 level. Thus the error rate in this study is per comparison, and the probability that at least one of these independent comparisons will be significant by chance is approximately equal to the number of comparisons times α (.05).
<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Date</td>
<td>Dec</td>
<td>26</td>
<td>27</td>
<td>28</td>
<td>29</td>
<td>30</td>
<td>31</td>
<td>Jan</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Date</td>
<td>Jan</td>
<td>19</td>
<td>20</td>
<td>21</td>
<td>25</td>
<td>26</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29</td>
<td>30</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Feb</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 2 (continued)

#### Experimental Design (page 2)

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>E1</th>
<th>E2</th>
<th>I1</th>
<th>P1</th>
<th>P2</th>
<th>B1</th>
<th>B2</th>
<th>T1</th>
<th>B3</th>
<th>B4</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>E1</td>
<td>E2</td>
<td>I1</td>
<td>P1</td>
<td>P2</td>
<td></td>
<td>B1</td>
<td>B2</td>
<td>T1</td>
<td>B3</td>
<td>B4</td>
</tr>
<tr>
<td>3</td>
<td>E1</td>
<td>E2</td>
<td>I1</td>
<td>P1</td>
<td>P2</td>
<td></td>
<td>B1</td>
<td>B2</td>
<td>T1</td>
<td>B3</td>
<td>B4</td>
</tr>
<tr>
<td>4</td>
<td>E1</td>
<td>E2</td>
<td>I1</td>
<td>P1</td>
<td>P2</td>
<td></td>
<td>B1</td>
<td>B2</td>
<td>T1</td>
<td>B3</td>
<td>B4</td>
</tr>
<tr>
<td>5</td>
<td>E1</td>
<td>E2</td>
<td>I1</td>
<td>P1</td>
<td>P2</td>
<td></td>
<td>B1</td>
<td>B2</td>
<td>T1</td>
<td>B3</td>
<td>B4</td>
</tr>
<tr>
<td>6</td>
<td>E1</td>
<td>E2</td>
<td>I1</td>
<td>P1</td>
<td>P2</td>
<td></td>
<td>B1</td>
<td>B2</td>
<td>T1</td>
<td>B3</td>
<td>B4</td>
</tr>
<tr>
<td>7</td>
<td>E1</td>
<td>E2</td>
<td>I1</td>
<td>P1</td>
<td>P2</td>
<td></td>
<td>B1</td>
<td>B2</td>
<td>T1</td>
<td>B3</td>
<td>B4</td>
</tr>
<tr>
<td>8</td>
<td>E1</td>
<td>E2</td>
<td>I1</td>
<td>P1</td>
<td>P2</td>
<td></td>
<td>B1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Date  | Feb | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 1  | 2  | 3  | 4  |

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>B5</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>I2</th>
<th>A</th>
<th>B7</th>
<th>A</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>B8</th>
<th>B8</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>B5</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
<td>I2</td>
<td>A</td>
<td></td>
<td>B7</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>B5</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
<td>I2</td>
<td>A</td>
<td></td>
<td>B7</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>B5</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
<td>I2</td>
<td>A</td>
<td></td>
<td>B7</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>B5</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
<td>I2</td>
<td>A</td>
<td></td>
<td>B7</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>B5</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
<td>I2</td>
<td>A</td>
<td></td>
<td>B7</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>B5</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
<td>I2</td>
<td>A</td>
<td></td>
<td>B7</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>B5</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
<td>I2</td>
<td>A</td>
<td></td>
<td>B7</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<p>| Date  | Mar | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>B9T5 B10 A</th>
<th>B11 A DP</th>
<th>ADP</th>
<th>BP</th>
<th>FIX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B9T5 B10 A</td>
<td>B11 A DP</td>
<td>ADP</td>
<td>BP</td>
<td>FIX</td>
</tr>
<tr>
<td>2</td>
<td>B9T5 B10 A</td>
<td>B11 A DP</td>
<td>ADP</td>
<td>BP</td>
<td>FIX</td>
</tr>
<tr>
<td>3</td>
<td>B9T5 B10 A</td>
<td>B11 A DP</td>
<td>ADP</td>
<td>BP</td>
<td>FIX</td>
</tr>
<tr>
<td>4</td>
<td>B9T5 B10 A</td>
<td>B11 A DP</td>
<td>ADP</td>
<td>BP</td>
<td>FIX</td>
</tr>
<tr>
<td>5</td>
<td>B9T5 B10 A</td>
<td>B11 A DP</td>
<td>ADP</td>
<td>BP</td>
<td>FIX</td>
</tr>
<tr>
<td>6</td>
<td>B9T5 B10 A</td>
<td>B11 A DP</td>
<td>ADP</td>
<td>BP</td>
<td>FIX</td>
</tr>
<tr>
<td>7</td>
<td>B9T5 B10 A</td>
<td>B11 A DP</td>
<td>ADP</td>
<td>BP</td>
<td>FIX</td>
</tr>
<tr>
<td>8</td>
<td>B9T5 B10 A</td>
<td>B11 A DP</td>
<td>ADP</td>
<td>BP</td>
<td>FIX</td>
</tr>
</tbody>
</table>

Date: Mar 25 26 27 28 29 30 3 4 5 6 7 8 15 20

April May June

a. "M" represents days tested on circular maze.
b. "A" represents days tested on spontaneous alternation.
c. "S" represents the day of surgery for an entire group.
d. "E" represents days of EEG data collection.
e. "I" represents days where stimulus-response relationships were determined.
f. "P" represents low frequency stimulation data collection.
g. "B" represents baseline potential data collection.
h. "T" represents high-frequency stimulation runs.
i. "D" represents double pulse stimulation runs.
j. "ADP" represents anesthetic double pulse stimulation runs.
k. "BP" represents blood pressure measurements.
l. "FIX" represents brain fixations.
SECTION 1: CIRCULAR MAZE (Procedure, Results and Discussion)

The rats in the present study were tested on a behavioral task (shown as M in Table 2) thought to be sensitive to alterations in general hippocampal function (i.e., spatial memory function). The requirements of the task were such that proper performance demanded use of the general location of the goal in space rather than cues such as "turn left", or follow an old odor trail. The latter cues, if utilized, would actually be a distraction since the rat's initial position in the maze was randomized, and the odor trails of adjacent trials were randomly rotated. The maze will be described in more detail below; however, if there were differences between age groups on the performance of this task, it would suggest possible differences in the working of the hippocampus. These behavioral data will thus be correlated with electrophysiological measures taken from the hippocampus of these rats in a later experimental section.

The Equipment and Maze

The experiment was run in a large television studio. The maze was placed at one end of the studio and was surrounded by studio drapery and partition screens. This shielded the maze area from the view of the equipment (which was set up at the opposite end of the room from the maze) and provided a rather uniform surround. Two flood lights were aimed at the maze in such a way as to minimize shadows. A television camera was secured approximately six meters above and was focused on the maze top. The output from this camera
went into the first channel of a video mixer. A second television camera was focused on the beam of an oscilloscope which was in xy input mode. The output of this camera went to the second channel of the video mixer. The third channel of the mixer displayed an image of the oscilloscope spot superimposed on the image of the maze surface. Finally, the mixed image was monitored on a 24-inch television screen.

The position of the oscilloscope spot was controlled by a dual potentiometer-coupled joy stick (see below). The experimenter could thus keep the spot centered on the rats head during his exploration on the maze surface. The x and y potentiometer voltages were transmitted to the x and y inputs of the oscilloscope that had the TV camera focused on it. These x and y values were calibrated so that the spot from the oscilloscope reached just past the outermost edge of the maze displayed on the television screen at the full extent of the joy stick positions. The x and y outputs from the potentiometers also went to a second oscilloscope, where the signals were amplified. The amplified signals were recorded on an Ampex FM tape recorder. In this manner, the x and y values of the position of a rat on the maze could be recorded and then transferred from tape to the magnetic disk of a PDP-11/10 computer for analysis.

The maze used for this spatial memory discrimination task was circular (1.22 m in diameter), with 18 (9.5 cm) circular holes lining the circumference. The rotatable surface of the maze stood .91 meters off of the floor on a metal stand. Underneath this sur-
face was a tunnel which was constructed like a drawer so that a rat would be removed easily.

The start box was a cylinder made from a bottomless one gallon paint tin with metal weights mounted on the bottom for stability. It was hung from a beam approximately six meters above the maze. The start box was coupled to a system of weights and pulleys, such that by releasing a cord the experimenter could cause the start box to move quickly and smoothly from the maze surface to a resting position six meters above the maze. This allowed the experimenter to be seated at the video screen, raise the start box, and immediately begin timing and following the rat with the joy stick. See Appendix F for the details of the maze structure.

Summary Procedure

Each group received two trials per day for six consecutive days. The eight groups were staggered by time of day as well as by day as seen in Table 2. The groups took between 90 and 120 minutes each to test. On Day 1, each rat was immediately placed in the tunnel for four minutes and was then removed and returned to its home cage for one minute. This was considered an adaptation trial. Trial 1 began after the rat had been in its home cage for one minute, and was conducted according to the procedure to be described below.

A "trial" consisted of the following:

1) The tunnel was kept in a fixed position while the maze top was rotated randomly above the tunnel for each trial.

2) The rat was placed into the start box with its nose facing in a
pre-selected random direction towards one of the eighteen holes (Figure 2.1).

3) Thirty seconds after the start, box was raised, and the trial was started (Figure 2.2).

4) The latency to enter the tunnel, errors (Figure 2.3), and the position of the rat on the maze, were recorded.

5) If the rat found the tunnel before four minutes elapsed, it was left in the tunnel for one minute, and the trial was ended (Figure 2.4); if the rat did not find the tunnel in four minutes (or jumped from the maze) it was picked up and placed into the tunnel for one minute.

6) On all days except Day 1 where there was one adaptation trial and one regular trial, the second trial was begun after the rat had been placed in its home cage for one minute. The procedure for this second trial was identical to the first trial.

7) The tunnel position was kept constant from Trial 1 to 7. On Trial 8 it was changed to a new position and kept constant from Trial 8 to 11.

The new position of the tunnel was moved 135° from the old position, and therefore was not exactly reversed in space. Before the experiment was begun, it was reasoned that a strict reversal may be easier to learn than a change that was somewhat less than 180°. The 135° rotation was thus employed to keep the task as difficult as possible, so that there would be a good chance that age effects could be detected. The trials where the position was changed from the initial direction will be loosely referred to as the reversal
Figure 2: Examples of various stages in the performance of the circular maze task. 1) The rat was confined in the start box for 30 seconds before the beginning of a trial. 2) The start box was raised after this period of time and the rat was allowed to move freely on the maze. 3) A nose-head-neck deflection into a hole that was not above the tunnel was considered an error. 4) The trial was terminated when the rat's four feet were no longer on the maze surface.
trials.

A summary outline of the days on which the trials were run is shown in Table 3. Appendix E gives a more detailed description of the testing procedure. The pilot work done on this maze which led to the development of the procedure used in this study is discussed in Appendix D.

Table 3
Summary Outline of the Trials Given on the Circular Maze

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adaptation</td>
<td>Trial 2</td>
<td>Trial 4</td>
<td>Trial 6</td>
<td>Trial 8</td>
<td>Trial 10</td>
</tr>
<tr>
<td>Trial 1</td>
<td>Trial 3</td>
<td>Trial 5</td>
<td>Trial 7</td>
<td>Trial 9</td>
<td>Trial 11</td>
</tr>
</tbody>
</table>

Data analysis. The data were transformed into a variety of measurements, discussed below, with the use of Fortran programs written for the PDP-11/10 computer. Analysis of the data were complicated by two major factors: Equipment failures and the willingness of the rats to jump from the maze top to the floor.

When data were read improperly onto the tapes, they were irremediably lost. Because of the time of day variable, each group was tightly scheduled so that continuous double checking of the system was impossible. One entire day's data were lost in this manner (Trial 10 and 11 for Group 1, Trial 8 and 9 for Group 2, Trial 6 and 7 for Group 3, Trial 4 and 5 for Group 4, Trial 2 and 3 for Group 5, and Trial 1 for Group 6). This makes the appropriateness of statistical tests such as split plot ANOVA or MANOVA questionable, since very few animals have complete data (several animals cannot.
simply be discarded to make the cells equal). A much more complete
data collection system would involve filming all trials with a
Super-8 movie camera.

To avoid the second complication, the maze should have
been designed differently; including transparent sides around its
circumference and removable mesh coverings under each hole. This
would have prevented trial termination due to jumping or falling
from the maze.

General Description of the Results

Was this task measuring memory? The hippocampus is
thought to process information about the relation of objects in
space. If this function was altered in SN animals, these rats should
behave differently than MA rats on a task requiring memory of a
particular place in space. Requiring the rats in this study to learn
a spatial memory task rather than a task involving avoidance or fine
discrimination has other advantages. It is critically important in
aging research to separate performance factors from memory factors.
It is well established that certain avoidance tasks (Freund & Walker,
1971; Sprott & Staines, 1976) differentiate between old and young
animals along the performance dimension. Further, tasks which re-
quire refined sensory discriminations may tax the old rats physical
capabilities, thus producing behavioral differences which may have
little to do with actual memory mechanisms.

There are a number of features of this spatial memory task
which tend to support the notion that it is a measure of the working
of spatial memory. First, differences between the MA and SN rats arose in latency-independent measures. Therefore, motor ability cannot account for differences between the age groups. Second, the discrimination did not depend on fine visual or olfactory cues. That is, odor trials and detailed visual cues could not be used for the location of the tunnel position. Odor cues on the maze were actually a distraction, and the closest wall was without pattern. Since the studio was quite large and had air continuously circulating through it, it is unlikely that olfactory gradients could have been an important cue. The two spot lights aimed at the maze, plus general visual intensity gradients, however, were undoubtedly important. Since gross illumination detection is not likely to be disrupted in the old animals (Fields, 1953; Kay & Sine, 1962; Oldfield-Box, 1969; Stone, 1929a, b), the differences between age groups on this task most likely did not arise from sensory defects.

Motivation. Another factor, which is difficult to control in many kinds of research, is that of "level of motivation". Some people in aging research have attempted to equilibrate motivation by differentially depriving their old rats (i.e., depriving the old rats more severely) to ensure that the senescent rats would be at least as motivated as younger rats to perform a certain task. It is more desirable, however, not to weaken or stress the older animals since this too may be a confounding variable. Until the substrates of "memory" and "motivation" are unequivocally described, it remains a logical impossibility to completely dissect memory processes from
motivational processes.

However, there are a number of factors which suggest that the rats in this study were similarly compelled to find and enter the dark tunnel. First of all, there were no instances where a rat came back out onto the maze top after it had descended into the tunnel. Further, there were only two rats (one MA and one SN) that never found the correct hole, and therefore never actively entered the tunnel. Out of 704 possible times when a rat could have chosen not to go down the correct hole immediately upon finding it, this occurred only six times (four SN, two MA). And, in only one of these instances did the rat not go down the correct hole before the time limit had elapsed (one SN rat). All the above information suggests that both groups were well motivated to enter the dark tunnel.

Patterns of behavior on the maze surface. As the number of trials increased, there was a clear change in the behavior of the rats on the maze surface. There were four basic behavior patterns observable from the video-tracking plots (these plots are simply the $x$ and $y$ coordinate points connected sequentially). The following general sequences were seen in both age groups:

1) There were rats that remained in the middle or at one side of the maze during the entire trial, and hence failed to find the correct hole (representative examples from two rats are seen in the video tracking plots shown in Figure 3.1).

2) There was a pattern where many holes were investigated, not in a detectable order, with frequent returns to the center of the maze.
Examples of this can be seen in Figure 3.2.

3) There was a pattern of systematic excursion from hole to hole at the edge of the maze, with few or no returns to the center of the maze (examples are shown in Figure 3.3).

4) There were perfect or nearly perfect runs to the correct hole (with no returns to the center) which can be seen in Figure 3.4.

While there were animals in each age group that remained in the middle or at one side of the maze as described in pattern 1, this behavior was peculiar to very few animals (as discussed in the "motivation" section). The usual behavior sequence, however, involved a general progression from pattern 2 to pattern 3, and finally to pattern 4. On initial trials, the rats tended to explore many incorrect holes, often returning to the center after investigating the edge of the maze (Figure 3.2). As the rats became more experienced with the maze, the number of center crossings decreased, and after reaching the edge of the maze the animals began a more systematic search from hole to hole (Figure 3.3). In later trials many rats went directly to the correct position (Figure 3.4) (or one or two holes away from it before correctly locating the tunnel). These patterns indicate that the rats became both more accurate and more efficient in locating the correct position of the tunnel.

An interesting aspect of the first reversal trial was the tendency of the rats to return to a pattern 2 type behavior (Figure 3.2). Although the initial tendency on this trial was to go directly to the place where the tunnel has been in previous
Figure 3. Patterns of behavior observed on the maze surface. A representative example from each age group is shown for each pattern. 1) A pattern of limited movement (which was atypical). 2) A pattern which included examination of many holes with frequent center crossings. 3) A pattern of relatively systematic search from hole to hole. 4) A pattern which located the tunnel with a high degree of accuracy.
trials, they afterwards reverted to the non-sequential search strategy, as was often seen on Trial 1. On subsequent trials after the reversal, however, behavior patterns 3 and 4 were again seen. This progression was particularly noticeable in the MA rats.

**Cumulative plots.** One way to view the overall progression of the rats' movements on the maze from trial to trial, is to obtain a cumulative point plot for each group on each trial. This involves super-imposing each xy point collected for all the MA or SN rats on a particular trial. Since lines are not drawn between the points, the pattern of the positions that the rats explored on the maze, from trial to trial, can be viewed (see Figure 4 and Table 4).

On the first trial, it can be seen that each group spent a good deal of time in the center of the maze, and around the regions of the holes. By Trial 4, the pattern of movement on the maze (the number of dark fixation points and the density of the center) distinguished between age groups. Latency measures, of course, contribute to the density of the plots (the longer the latency, the more points for any given rat); however, the general impression from these plots is that the MA rats were more accurate in locating the tunnel position efficiently. On Trial 8 (the first reversal trial) the pattern of both groups changed to a generally more densely travelled maze surface. The holes near the old correct tunnel position are particularly "well investigated", and the center area is again more of a point of fixation. By Trial 11, it can be seen that both age groups continue to travel to the old tunnel position.
Figure 4. Cumulative plots of the pattern of exploration on the circular maze. These plots were constructed by super-imposing the data from all animals within each group for each trial. The arrow indicates the correct location of the tunnel. Notice that the position of the tunnel is changed on Trial 8.
however, it is obvious that the area around the new tunnel location is also a dense point of fixation. The four reversal trial plots also suggest that the SN rats do not reverse the task as well as the MA rats.

Table 4
Number of Animals Included in the Polar Histograms and Cumulative Plots

<table>
<thead>
<tr>
<th>Trial</th>
<th>SN Polar Plots</th>
<th>SN Cumulative Plots</th>
<th>MA Polar Plots</th>
<th>MA Cumulative Plots</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>28</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>27</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>25</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>25</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>25</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>25</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>28</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>28</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>10</td>
<td>26</td>
<td>27</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>11</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
</tr>
</tbody>
</table>

First hole investigated. Another descriptive measure of the rats' ability to locate the correct position of the tunnel in space is to note the first hole that the rat investigated after leaving the center of the maze. A good way to visualize the age data is by using polar histograms (see Figure 5 and Table 4). Around the circumference of each circle in this figure are 18 notches indicating hole positions, one of which is the hole over the tunnel (noted with an arrow). The length of the line from the center towards any of the notches represents the number of animals which first investigated that particular hole in that trial. It was expected that as the rats learned the task, they should eventually investigate holes in close
Figure 5. Polar frequency histograms of holes first investigated after leaving the center of the maze. The arrow indicates the correct position of the tunnel.
proximity to the tunnel.

There are four important observations to be made from these histograms:

1) In general, as the number of trials increase, the first hole investigated become clustered in the half of the maze which contained the tunnel (this is another indication that a spatial discrimination was learned).

2) The first hole investigations by the MA rats on Trial 1 were distributed away from the correct hole. The MA rats were therefore less likely to have found the correct hole than if their responses were more randomly distributed (see the mean angle measurement section).

3) The first hole investigations on Trial 8 were definitely in the direction of the old position of the tunnel.

4) In general, the MA rats tended to be more accurate, this is particularly noticeable on the reversal trials.

Percent correct responses. The maze had the flaw of the rat being able to terminate the trial by jumping (or falling) from the maze onto the floor (this occurred in both age groups). Because of this feature, the rats which "jumped" were not given the full four minutes on the maze surface. After such a jump, the rat was placed into the tunnel for the usual period. This was scored as a non-entry. Because these were not "true" non-entries, the percent correct response measure only includes the animals which remained on top and made a correct entry within four minutes. (see Table 5).
This measure suggests that initially the SN rats do not find the correct hole as often as the MA rats, although both groups subsequently maintain a rather high level of correct entries.

Table 5
Percent Correct Responses

<table>
<thead>
<tr>
<th>Trial</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA</td>
<td>86</td>
<td>70</td>
<td>91</td>
<td>91</td>
<td>94</td>
<td>94</td>
<td>91</td>
<td>91</td>
<td>91</td>
<td>93</td>
<td>94</td>
</tr>
<tr>
<td>SN</td>
<td>52</td>
<td>57</td>
<td>85</td>
<td>79</td>
<td>90</td>
<td>90</td>
<td>77</td>
<td>93</td>
<td>83</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>

Motor-Dependent Measures

Latency. Although latency independent measures are of greatest interest in this study (because they do not bias the results in terms of motor factors), the latencies of all rats on all trials were measured. The means can be seen in Figure 6. The MA rats' latencies were statistically different (lower) than the SN rats' latencies on all trials but Trial 7 and 9 (see Table 6). Since the MA rats had lower mean latency scores on all trials, the SN rats spent a consistently longer period of time on top of the maze than the MA rats.

Speed. The x and y value of the position of the rat on the maze was measured at a fixed interval of four times per second. From these data the average speed of the rat on the maze was determined by summing the distance between each point and dividing by the total time spent on the maze. The mean speed of each group is seen in Figure 6. The mean speed of the MA rats was always higher than that of the SN rats, and was statistically different on all trials but Trial 1, 2, 3, and 9 (see Table 6). It is interesting to note
Figure 6. Motor-dependent measures on circular maze. The mean latency, speed, and number of fixations are shown for both age groups across all trials.
that the mean speed on the first three trials is quite similar for both age groups; however, while the speed measures increase over trials for both groups, the SN rats do not show as large an increase as the MA rats. This general increase may be related to familiarity with the task.

The speed measure indicates that, in general, the SN rats make slower movements on the maze than the MA rats, which may partly account for the longer latency scores for the old animals. On initial trials, however, where speed scores are very similar between age groups, it is likely that other factors contribute to the longer latency scores.

Table 6

<table>
<thead>
<tr>
<th>Trial</th>
<th>t (Latency)</th>
<th>t (Speed)</th>
<th>t (Fixations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.59* (62)</td>
<td>.70 (52)</td>
<td>1.45 (52)</td>
</tr>
<tr>
<td>2</td>
<td>2.85* (62)</td>
<td>1.88 (52)</td>
<td>.87 (52)</td>
</tr>
<tr>
<td>3</td>
<td>2.41* (62)</td>
<td>.88 (52)</td>
<td>-.10* (53)</td>
</tr>
<tr>
<td>4</td>
<td>3.00* (62)</td>
<td>3.42* (53)</td>
<td>3.43* (53)</td>
</tr>
<tr>
<td>5</td>
<td>2.32* (62)</td>
<td>2.81* (51)</td>
<td>2.30* (53)</td>
</tr>
<tr>
<td>6</td>
<td>3.86* (62)</td>
<td>3.26* (46)</td>
<td>2.16* (47)</td>
</tr>
<tr>
<td>7</td>
<td>1.54 (62)</td>
<td>2.19* (47)</td>
<td>1.42 (47)</td>
</tr>
<tr>
<td>8</td>
<td>2.38* (62)</td>
<td>2.71* (53)</td>
<td>2.97* (53)</td>
</tr>
<tr>
<td>9</td>
<td>1.99 (62)</td>
<td>1.80 (53)</td>
<td>1.40* (53)</td>
</tr>
<tr>
<td>10</td>
<td>2.33* (62)</td>
<td>3.29* (52)</td>
<td>2.30* (52)</td>
</tr>
<tr>
<td>11</td>
<td>2.01* (62)</td>
<td>2.17* (52)</td>
<td>1.44* (53)</td>
</tr>
</tbody>
</table>

The numbers in parentheses indicate the degrees of freedom.

* \( p < .05 \)

**Fixations.** The number of points on the maze where the movement of the rat was confined to a small radius (about the size
of one of the holes on the maze) for at least two seconds was defined as a fixation point. The mean number of two-second fixations are seen in Figure 6. The pattern of improvement (fewer fixation points) over trials is similar to the pattern of improvement in errors (see Table 6). It was initially thought that this measure would be able to discriminate whether the rat tended to make repetitive (perseverative) errors or whether the errors were distributed more randomly on the maze. For instance, if a rat had fewer fixation points than errors, it might be reasoned that they repeated errors at a point of fixation. It soon became clear, however, that the fixation data were not independent of latency or speed, and therefore the possible interpretations were confounded. The decrease in the mean number of fixations does suggest that there was less "hesitation" in a particular position on the maze as the number of trials progressed.

Motor-Independent Measures

Total distance. As described in the mean speed section, the total distance the rat travelled on the maze could be calculated from the data collected with the video tracking system. This measure is motor independent. Given the differences between groups on the latency and speed measures, if there was no difference on the distance measure, it would tend to indicate that this maze distinguished between age groups on the basis of motor factors. This, however, was not the case, as shown in Figure 7. On Trials 4, 6, 7, and 9 through 11, the mean distance the MA rats travelled on the maze before entering the tunnel was statistically less than the distance the SN rats
travelled before finding the tunnel (see Table 7). Thus, even though the latency and velocity measures may differentiate between the age groups on the basis of motor factors, the total distance measurement also distinguishes between these groups. This suggests that something other than motor disability is operating to make the SN rats less accurate in locating the tunnel.

Table 7

The Values of t for the Motor-Independent Measures: Distance, Angle, and Errors (SN minus MA)

<table>
<thead>
<tr>
<th>Trial</th>
<th>t (Distance)</th>
<th>t (Angle)</th>
<th>t (Errors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.49 (52)</td>
<td>-2.26* (52)</td>
<td>0.11 (62)</td>
</tr>
<tr>
<td>2</td>
<td>0.20 (52)</td>
<td>-0.55 (50)</td>
<td>-1.07 (62)</td>
</tr>
<tr>
<td>3</td>
<td>0.81 (52)</td>
<td>1.26 (52)</td>
<td>-0.40 (62)</td>
</tr>
<tr>
<td>4</td>
<td>3.56* (53)</td>
<td>0.40 (52)</td>
<td>2.12* (62)</td>
</tr>
<tr>
<td>5</td>
<td>1.06 (51)</td>
<td>1.01 (51)</td>
<td>2.23* (62)</td>
</tr>
<tr>
<td>6</td>
<td>2.65* (46)</td>
<td>2.05* (46)</td>
<td>2.16* (62)</td>
</tr>
<tr>
<td>7</td>
<td>2.03* (47)</td>
<td>2.10* (47)</td>
<td>2.15* (62)</td>
</tr>
<tr>
<td>8</td>
<td>1.68 (53)</td>
<td>1.02 (54)</td>
<td>1.12 (62)</td>
</tr>
<tr>
<td>9</td>
<td>2.38* (53)</td>
<td>2.04* (53)</td>
<td>3.89* (62)</td>
</tr>
<tr>
<td>10</td>
<td>2.28* (52)</td>
<td>0.22 (52)</td>
<td>-0.07 (62)</td>
</tr>
<tr>
<td>11</td>
<td>2.34* (52)</td>
<td>2.65* (51)</td>
<td>2.48* (62)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate the degrees of freedom.

* p < .05

Errors. Another latency independent measure involves the number of errors made before entering the correct hole. An error was defined as a nose-head-neck deflection into an incorrect hole (or jumping from the maze entirely). Since this measure was collected by hand at the time of testing, it includes all 64 rats. The mean error scores are seen in Figure 7. On Trials 1, 2, and 3 and Trials 8 (the first reversal trial) and 10, the number of errors...
Figure 7. Motor-independent measures on circular maze. The mean distance, number of errors, and angular deviation from the tunnel are shown for both age groups across all trials.
were not different between groups. However, on all other trials the MA rats made statistically fewer errors than the SN rats (see Table 7). The errors for both groups reached a fairly constant level by Day 3; however, the number of errors on the best trials for the SN animals was approximately five, whereas the MA rats averaged only three errors on their best trials. Again this suggests that the SN rats were less accurate in remembering the "place" where the tunnel should be.

Other interesting aspects of this measure include the increase in errors in both groups on the second trial of Day 3 and 4. This may be a "massed trial" effect, or perhaps an odor cue distraction effect (from the odor trails present on the second trial of any day). Also, the fact that both groups of animals showed an increase in errors above their initial (Trial 1) level on the first reversal trial (8) is a good indication that they had learned the location of the previously correct hole.

Mean angle from the tunnel. The angle that each xy data point formed with the center (as vertex) and correct tunnel position was totaled and divided by the number of points, to give each rat an average angle score (the maximum being $180^\circ$). This angle was expected to decrease as the rats became more accurate in locating the tunnel. The results are shown in Figure 7. On Trial 1, the SN rats have about a $15^\circ$ advantage over the MA rats in the mean angular deviation from the correct hole (this was statistically significant, see Table 7). This indicates that all measurements on this maze
were actually somewhat biased against the MA rats because of their initial pre-disposition to explore the direction opposite the correct hole (as mentioned in the first hole investigated and cumulative point plot data). However, by Day 4 (Trials 6 and 7), the mean angular deviation of the MA rats was statistically lower than that of the SN rats (see Table 7). Further, on Trials 9 and 11, the MA rats were statistically more accurate than the SN rats in locating the tunnel. This suggests that the MA rats were better able to change the discrimination than the SN rats.

On the first reversal trial (8), there was considerably more angular error than on the first trial (1). The mean angular deviation measured from the previously correct location for Trial 8 is seen in Figure 7. In both groups the mean angle measurement is less when taken from the originally correct position, than when measured from the new correct position. This strongly suggests that the rats had learned to go to the correct place in space, rather than to go to the tunnel itself because of other uncontrolled cues.

Time of Day

No significant circadian effects were observed on any of the measures (motor dependent or independent) in this section. In addition, the between time-of-day variance was not extremely large, and it is therefore concluded that the behavior on this task does not exhibit consistent time-of-day effects. This stability over time of day stands in marked contrast to the circadian fluctuations in ongoing electrical activity (see Section III), excitability of the
perforant path synapses (Barnes, et al., 1977; see Section V), and spontaneous alternation behavior (see Section VII), which will be discussed later.

General Discussion of the Circular Maze Data

The data from the circular maze task indicate that the SN rats spent more time covering a greater distance, making more errors than the MA rats. In summary, the SN rats were less accurate than the MA rats in locating the place where the tunnel was positioned. In addition to this major conclusion, a number of other features should be noted.

Although there may have been ambulatory differences between age groups, e.g., the SN rats spent more time on the maze surface (longer latencies), and the SN rats did not increase the velocity of their movements as much as the MA rats over trials, motor factors most probably cannot account for the entire age difference. This is supported by a number of different motor independent measures:

1) the total distance data (the SN rats travelled further to find the correction position);
2) the number of errors (the SN rats made more errors before locating the tunnel);
3) the mean angular deviation from the correct tunnel position (the SN rats were not as accurate by Day 4, and on the reversal trials, in locating the tunnel);
4) the first hole investigated was, in general located qualitatively less accurately by the SN rats;
5) The cumulative point plots indicate that the MA rats located the tunnel more accurately than the SN rats.

All these measures converge to the conclusion that the differences in performance of the task was not merely a motor difference. The conclusion that the rats actually learned to locate the tunnel in space is substantiated by the following evidence:

1) The total distance, the number of errors and the mean angular deviation measures decreased over trials in both age groups, which would be predicted if the rats learned to narrow down the location of the tunnel in space;

2) The histograms derived from first hole investigations and the cumulative plot graphs indicate that both groups accumulated information as to the location of the correct hole.

3) Perhaps the most convincing evidence that the rats learned a spatial discrimination is the result from the first reversal trial, where the rats in both groups went to the former correct position rather than to the new tunnel position (seen most clearly in the mean angle, first hole investigated, and cumulative plot measures).
SECTION II: SURGERY

Surgical implantation of electrodes was completed over eight consecutive days (as in Table 2), beginning with Group 1 and ending with Group 8. The entire surgical procedure took between 12-16 hours per day for each group. The order that the rats were implanted within each group was the same as the cage assignments, beginning with the top left hand cage and ending with the bottom right hand cage in every group. The side of electrode implantation was alternated, in this order as well, beginning with the left side, so that two SN and two MA rats were implanted in the left hemisphere, and two SN and two MA rats were implanted in the right hemisphere in each group. The rats were anesthetized with 33 mg/kg Nembutal, which was supplemented as necessary to maintain surgical anesthesia.

Electrodes and Equipment

The recording electrodes consisted of a single strand of stainless steel wire, insulated with Teflon (114 μm O.D.) which was obtained from Medwire Corp. The bipolar stimulating electrodes were constructed from the same material, by twisting together two lengths of the wire and separating the tips 0.5 mm vertically. The ground and indifferent electrodes consisted of nicrome wire leads soldered to stainless steel screws. The ground electrode was screwed into the skull over the frontal sinus, while the indifferent electrode was screwed into the skull over the cerebellum. Six other screws were also distributed around the skull to insure dental acrylic
adhesion.

Surgery was performed using two Kopf stereotaxic frames. One frame was used for implanting electrodes in the left hemisphere, and the other for implanting the right side. Burr holes, approximately 2 mm in diameter, were drilled in the skull, with a dental drill, at 3.5 mm posterior, 1.8 mm lateral (relative to bregma) for recording, and 8.1 mm posterior and 4.4 mm lateral for the stimulating electrode. The dura was slit with a sharp needle, avoiding damage to the underlying neocortex as far as possible. The recording electrodes were vertically positioned in the upper blade of the fascia dentata by monitoring multiple unit injury discharges. The stimulating electrodes were then lowered while stimulating at a rate of 0.1 Hz until the largest evoked response was recorded. The depth of the recording electrode was then adjusted to give the maximally positive response. After the electrodes were in the configuration for maximal response, 20 test pulses were given at a rate of 0.1 Hz and an average of these potentials were stored for the "peak potential" measurement which will be discussed later.

Since it was believed that the electrodes tend to drift upwards during recovery from surgery, the recording electrode was moved down an arbitrary amount (0.2 mm) from this peak potential, before fixing it in place. The gold Amphenol connector pins attached to the electrodes were mounted into a plastic nine-hole connector (Molino & McIntyre, 1973). The whole array was held firmly to the skull with dental acrylic. An antibacterial agent (Furacin) was
applied to the open wounds after the dental acrylic was applied. Bicillin was then injected intramuscularly (.1 cc in each hind leg) to further combat infection. The rats were given one month to recover before any further electrophysiological measures were taken.

The electrical signals were amplified by means of a Grass P15B, AC preamplifier coupled to an oscilloscope amplifier. High and low filter cutoff frequencies (one half amplitude) were set at 3 KHz and 10 Hz respectively. The oscilloscope output was sampled by an online PDP-11/10 digital computer, at a rate of one point every 50 μsec. This computer was used here and throughout the other electrophysiological measures in this study, for signal averaging, computation and display purposes.

All stimuli here, and in the following sections, consisted of constant current, symmetrical, diphasic, square waves delivered via a photon-coupled stimulus isolator. The durations of each half cycle were kept constant throughout the experiment (unless otherwise specified) at 50 μsec. The current used to stimulate during surgery was 1 mA.

Measurements Collected During Surgery

A number of measures were collected during the process of stereotaxically implanting electrodes, each of which might be thought to compliment certain other sections of the thesis.

Skull dimensions. The following skull dimensions were measured in each animal to determine whether there were any differences between the two populations:
1) the distance between bregma and lambda;
2) the distance between bregma and the most medial edge of the parasagittal ridge;
3) the distance between lambda and the most medial edge of the parasagittal ridge.

The mean values for the bregma-lambda distance, and the bregma-parasagittal ridge distance were nearly identical between age groups (see Table 8). However, the lambda-parasagittal ridge measure was statistically smaller in the SN than in the MA rats (Table 9). This difference appeared to be due to a greater calcification of the posterior portion of the bone ridge, rather than to a narrowing of the skull in the posterior region.

<table>
<thead>
<tr>
<th></th>
<th>Bregma-Lambda (mm)</th>
<th>Bregma-bone Ridge (mm)</th>
<th>Lambda-bone Ridge (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>8.053</td>
<td>4.909</td>
<td>5.169</td>
</tr>
<tr>
<td>SE</td>
<td>.11</td>
<td>.08</td>
<td>.08</td>
</tr>
<tr>
<td>N</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>MA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>8.051</td>
<td>5.039</td>
<td>5.479</td>
</tr>
<tr>
<td>SE</td>
<td>.09</td>
<td>.08</td>
<td>.09</td>
</tr>
<tr>
<td>N</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>t</td>
<td>0.012</td>
<td>1.163</td>
<td>2.576*</td>
</tr>
</tbody>
</table>

Anesthetic rating. Each rat was injected with 33 mg/kg Nembutal in preparation for surgery. Ten minutes after this injection
each rat was rated, on a scale between 1 and 5 on their level of anesthesia (1 being completely awake, and 5 being anesthetized enough to be set up in the stereotaxic). The mean ratings show that the SN rats were somewhat more deeply anesthetized than the MA rats at this point in time (see Table 9) although this difference was not statistically reliable. Hence there was only a very small, if any, behavioral difference between the rats at this time point.

Table 9
Rating on a 1-5 Scale of the Depth of Anesthesia
at 10 Minutes after Injection

<table>
<thead>
<tr>
<th></th>
<th>SN</th>
<th>MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>\bar{x}</td>
<td>4.40</td>
<td>4.10</td>
</tr>
<tr>
<td>SE</td>
<td>0.17</td>
<td>0.20</td>
</tr>
<tr>
<td>n</td>
<td>30</td>
<td>33</td>
</tr>
</tbody>
</table>

\[ t = 1.00, P > .05 \]

Respiration. Respiration measurements were taken three times during surgery:
1) after the rat was put into the stereotaxic;
2) before the electrodes were lowered into the brain;
3) during the period when evoked responses were being recorded.
 Fifty cycles of inspiration-expiration were timed, and the number of cycles per second was calculated. Table 10 shows the mean rate of respiration for each of the three points in time described above. The respiration was not found to be statistically different between age groups at any of the time points measured.
Table 10

Respiration During Surgery

<table>
<thead>
<tr>
<th></th>
<th>Resp. 1 (Hz)</th>
<th>Resp. 2 (Hz)</th>
<th>Resp. 3 (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{x}$</td>
<td>1.151</td>
<td>0.964</td>
<td>0.955</td>
</tr>
<tr>
<td>SE</td>
<td>.04</td>
<td>.05</td>
<td>.03</td>
</tr>
<tr>
<td>n</td>
<td>32</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>MA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{x}$</td>
<td>1.138</td>
<td>1.027</td>
<td>1.000</td>
</tr>
<tr>
<td>SE</td>
<td>.04</td>
<td>.03</td>
<td>.04</td>
</tr>
<tr>
<td>n</td>
<td>33</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>$t$</td>
<td>0.226</td>
<td>1.133</td>
<td>0.845</td>
</tr>
</tbody>
</table>

Total amount of anesthetic. To maintain surgical anesthesia, a number of rats required supplemental doses of Nembutal (beyond the 33 mg/kg dose initially administered). The mean dose of anesthetic required (in mg/kg) is seen in Table 11. The MA rats required more anesthetic (statistically) than the SN rats to be maintained at a surgical level of anesthesia.

Table 11

Amount of Anesthetic Injected During Surgery

<table>
<thead>
<tr>
<th></th>
<th>SN (mg/kg)</th>
<th>MA (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{x}$</td>
<td>35.12</td>
<td>39.11</td>
</tr>
<tr>
<td>SE</td>
<td>.68</td>
<td>1.33</td>
</tr>
<tr>
<td>n</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>

$t = 2.67, p < .05$

Total time behaviorally anesthetized. The time of the initial injection of anesthetic, and the time when the rat began to show signs of being able to move and groom, were recorded. The
difference between these two times was calculated and represents an estimate of the duration of the influence of the anesthetic on the behavior of the rat. The mean "down" time for both age groups is seen in Table 12. The SN rats showed the behavioral effects of the anesthetic 40 minutes longer than the MA rats.

Table 12

<table>
<thead>
<tr>
<th></th>
<th>SN (min.)</th>
<th>MA (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \bar{x} )</td>
<td>242.50</td>
<td>203.50</td>
</tr>
<tr>
<td>SE</td>
<td>16.75</td>
<td>10.07</td>
</tr>
<tr>
<td>( n )</td>
<td>22</td>
<td>24</td>
</tr>
</tbody>
</table>

\[ t = 2.04, \ p < .05 \]

Measurement of peak evoked response. While the electrodes were being positioned for permanent implantation, a sample of 20 waveforms, collected at a rate of .1 Hz was recorded at the point where the maximum positive evoked response was measured. It was reasoned, that if the SN rats' synapses were more powerful than the MA rats' synapses as a result of more experience, that the averaged peak responses collected at surgery should tend to be larger in the SN rats. This was considered to be an appropriate time to make this comparison since the electrodes were optimally positioned (the electrodes tend to drift during surgical recovery).

The mean amplitude of the EPSP (from a point measured 2 msec after the onset of the stimulus), the mean point at which the spike began (the "rollover") and mean peak latencies of the population spike, however, indicate that the old rats actually tend to show a smaller response (although not statistically smaller; see Table 13).
If it is assumed that the electrode placements were randomly distributed over the two populations of rats (because of the low variability of the bregma-lambda distance), then it might be concluded that the amplitude of the responses obtained from these rats were not different. There appear, however, to be two factors which might interact to alter the interpretation of these results. First, if the anesthetic differentially influenced the amplitude of the SN rats' potentials, the recorded amplitude might give false information about the actual amplitude (when in a non-anesthetized state). Further, if there are actually fewer, (but more powerful) synapses on the old rats granule cells, the current flow measured by these extracellular records may not be larger than the current measured from the MA rats. This will be discussed more fully later.

Table 13
Amplitude of the Peak Positive Response Collected at Surgery

<table>
<thead>
<tr>
<th></th>
<th>EPSP (arb. units)</th>
<th>Spike Rollover (msec)</th>
<th>Spike Peak (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>38.58</td>
<td>3.93</td>
<td>6.96</td>
</tr>
<tr>
<td>SE</td>
<td>9.04</td>
<td>0.15</td>
<td>0.19</td>
</tr>
<tr>
<td>n</td>
<td>31</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>MA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>55.16</td>
<td>3.79</td>
<td>6.58</td>
</tr>
<tr>
<td>SE</td>
<td>10.12</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>n</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>t</td>
<td>1.219</td>
<td>0.794</td>
<td>1.616</td>
</tr>
</tbody>
</table>
Summary of Surgery Measurements

The skulls of the SN and MA rats were remarkably similar, with the exception of the thicker posterior bone ridge.

Although the rats were not behaviorally distinguishable at 10 minutes after injection (as indicated by the five point rating scale), the "total time anesthetized" score indicates that the SN animals took longer to recover. The SN rats remained influenced by the anesthetic longer in spite of the fact that the younger rats required more anesthetic (approximately 4 mg/kg more) to keep them surgically anesthetized. Further, the respiration of the two groups of rats did not differ statistically, even though the MA rats were given more anesthetic. The foregoing set of observations tend to support the idea that the SN rats are more influenced by the administration of pentobarbital (see Section IX for further evidence).

The peak positive evoked responses measured during surgery were not statistically different between age groups. Although a qualitative argument can be made about the reasons for these particular results, a quantitative statement of the influence of anesthetic and fewer synapses is not possible (this will be discussed more fully later).
SECTION III: EEG

Recent evidence has suggested that the generators of hippocampal theta activity reside in CA1 and in the dentate gyrus (Kramis, Vanderwolf, & Bland, 1975). Since this experiment involved chronic implantation of recording electrodes in the dentate gyrus, an opportunity to compare electrographic activity between age groups was provided. This itself might give important information about the age variable, and furthermore provide a description of the background against which the other physiological measures will be taken. A power spectral analysis program was available for detailed analysis of these data (Weiner, 1948, Dummermuth & Fluhler, 1967; Gramsbergen, 1976). This analysis included performing a fast fourier transform on the EEG, which breaks the EEG waveform into its sine and cosine components. The power spectral analysis uses this information to give a distribution of the variance in voltage found at each frequency (or "power" at each frequency). The Fourier method allowed comparison of the power between age groups in this study at different frequencies, as well as an examination of the power at individual frequencies over time.

EEG Procedure

Four weeks after surgery, EEG was measured from each animal in two 10-minute sessions on two consecutive days (E1 and E2 in Table 2). The first day of EEG measurement was the first time the rats were taken into the electrically shielded room and plugged
into the recording equipment. Each rat was taken into the recording room singly in its home cage on these two days (during the rat's designated time period) and EEG recording was begun immediately after the rat's head plug was connected. The recording and stimulating cables were attached in such a way that the rat was free to move around in its cage. Three hundred one-second samples of EEG were collected at a rate of one every two seconds, with preamplifier filter cut off values set at .3 Hz (low) and 100 Hz (high). Each one-second sweep of the raw EEG data was converted into a power spectrum for the frequencies 0 to 63 Hz. The fast fourier transform and power spectral analysis are sub-routines of a PDP-11/10 software package called "Sparta", supplied by Digital Equipment Corporation.

The power spectrum from each one-second sample (the power at 0-63 Hz over time), as well as an average of the 300 individual power spectra for each rat (the average power collapsed across time) were stored on disk for further analysis. Disk storage limitations precluded saving the raw EEG data. Storage limitations also restricted the frequency analysis to the 0-63 Hz band.

Description of the data matrix. To facilitate understanding the rather complex EEG data structure, the data will first be described in terms of a matrix. For each animal there are two \((f,t)\) matrices, one for each day of testing, with 64 values of frequency (0-63 Hz) and 300 values of time (every other second for
The overall data structure can be represented by a six-dimensional matrix: \((A, D, H, n, f, t)\), where \(A = 2\) levels of age (MA, SN), \(D = 2\) levels of day (Day 1, Day 2), \(H = 8\) levels of time of day (Groups 1-8), \(n = four\) subjects/group, \(f = 64\) frequencies (0-63 Hz), \(t = 300\) time points (every other second for 10 minutes). This matrix can be collapsed in different ways to answer different questions about the data. Since the number of subjects per group became uneven, due to hardware problems or deaths, the data was typically collapsed across \(n\) and \(H\). If \(H\) was included as a variable, it was broken into early (12:00 - 20:00, Groups 1-4) and late (20:00 - 04:00, Groups 5-8) times of day, which will be called \(H'\). The choice of these time categories was based on a previous study on circadian cycles of perforant path evoked potentials carried out in this laboratory (Barnes et al., 1977).

The overall matrix, \((A, D, H, n, f, t)\) was collapsed in the following ways for this study:

1) \((A, D, f)\), which gives the four average power spectra collapsed across time for each age group and each day of testing, for 0-63 Hz;
2) \((A, D, H', f)\) which gives the average power spectra collapsed across time for each age group, each day of testing, and for each of the 64 frequencies at early and late times of day;
3) \((A, D, f, t)\) which gives the average power of each of the 64 frequencies over 300 time points, for each age group and each day of testing;
4) \((A, D, H', f, t)\) which gives the average power of each of the 64
frequencies over 300 time points, for each age group, each day of testing, and at early and late times of day.

**EEG analysis.** The data are shown in two ways: The actual power scores were averaged across subjects to give mean power score. To compare groups, ratios of total power at each frequency were calculated. Since the power spectrum represents a breakdown of the variance in the EEG voltage into frequency components, standard statistical methods are not appropriate. The ratio of two power spectra, being the ratio of two variances, should be distributed as $F_{\nu_1, \nu_2}$ where

$$F = \frac{\Sigma \sigma^2_1 \left( \frac{n_1}{n_1-1} \right)}{\Sigma \sigma^2_2 \left( \frac{n_2}{n_2-1} \right)}$$

with $\nu_1 = n_1-1$, $\nu_2 = n_2-1$ degrees of freedom. In this case

$$F = \frac{\Sigma \text{power}_1 \left( \frac{n_1}{n_1-1} \right)}{\Sigma \text{power}_2 \left( \frac{n_2}{n_2-1} \right)}$$

Since the number of observations were 300 times the number of rats in each group, the degrees of freedom are extremely large (ranging from 2700 to 8700 in various cases). This is probably a minimal estimate since it may be argued that the degrees of freedom should also be multiplied by the number of points taken during sampling. Therefore an estimate of a true confidence interval for the power ratio is $F_{\alpha, 01} = 1.20$ (or the inverse .80). This confidence interval is admittedly rather arbitrary, but is nevertheless extremely conser-
Results and Discussion

**Average power spectrum collapsed across time.** The average power spectrum for each rat represents the rat's individual power score at each frequency (0-63 Hz) in the 10-minute period sampled. The means for each age group of these power scores are shown in Figure 8.1 and 8.2, representing the matrix (A,D,f). Only 0 to 30 Hz are shown in the mean power plots because the scale factor obscures the differences above 30 Hz (the scale factors are the same in both plots). Changes in frequencies over 30 Hz are clearly visualized however, in the ratio plots (which continue out to 63 Hz).

On Day 1, the MA rats (n = 28) and SN rats (n = 29) show similar power at the frequencies investigated; however, the power tends to increase substantially on Day 2 in the MA group (n = 24) and to increase a small amount in the SN group (n = 23) (see Figures 8.1 and 8.2). It is easier to visualize these differences when looking at the ratio graphs.

The value of the ratio of Day 2/Day 1 in both age groups is seen in Figure 8.3. In general, there is an increase in power from Day 1 to Day 2, which is particularly apparent in the higher frequencies measured. Since a cycle of an EPSP lasts approximately 25 msec, the power at the frequency components around 40 Hz should reflect contributions from this waveform. These data therefore suggest that either there is more EPSP activity, that the amplitude
Figure 8. 1) Mean power for the frequencies 1-30 Hz for the MA rats on the first and second day of EEG testing. 2) Mean power for the frequencies 1-30 Hz for the SN rats on the first and second day of EEG testing. 3) The ratio of power (Day 2/Day 1) for both age groups and 63 frequencies. Note that the power on Day 2 in the testing room generally remains above that on Day 1. 4) The ratio of power between age groups (MA/SN) on both days of testing for 63 frequencies. Note that on Day 2 the MA rats generally show more power than the SN rats. The broken horizontal lines represent the 99% confidence limits in the last two parts of this figure.
of the EPSPs are greater, or that there is a phase change between the EPSPs on the second day in the recording room. Without modeling this system, there is no way to distinguish between these possibilities. However, the fact remains that there is a change in the frequency band which probably represents EPSP activity.

Figure 8.4 compares the age groups on each day. Here it can be seen that the power of the SN and MA rats are quite similar on Day 1, with very few components lying outside the confidence intervals (with two notable exceptions: the MA rats having more power at 5 Hz, and the SN rats more power at 7 Hz). On Day 2, however, the power for the MA groups is generally higher than for the SN groups. As can be seen from the mean power scores (Figures 8.1, 8.2), this difference is due to a larger increase of power from Day 1 to Day 2 in the young group.

When the SN and MA groups are divided into early (12:00 - 20:00, Groups 1-4) and late (20:00 - 04:00; Groups 5-8) times of day, as in the matrix (A,D,H',f) the ratios for both groups show a similar pattern (see Figure 9). Both the SN and MA "late" groups show more power than the "early" groups. Also, both age groups show a reduction in the theta regions from Day 1 to Day 2, and an increase in the higher frequencies from Day 1 to Day 2. Although the pattern of change is similar in both age groups, the magnitude of this change tends to be greater in the MA than in the SN groups.

**Average power at each frequency over time.** The average power found at each frequency was examined as a function of time,
Figure 9. Power ratios for both age groups comparing 63 frequencies at late and early times of day. Note that the power is typically greater in the late groups for both ages. The broken horizontal lines represent the 99% confidence intervals.
representing the matrix \((A,D,f,t)\). Thus in the following graphs time is on the \(x\) axis (rather than frequency). When viewing the power of individual frequencies over time, two patterns emerged which were consistent throughout age group and day of testing. There appeared to be an abrupt shift after 7 Hz from a pattern which declined with time, to a stationary pattern.

Figure 10 shows the result when the power at 3, 4, 5, 6, and 7 Hz are averaged for both age groups and on both days. The apparent exponential decline in power with time at these frequencies appears to be selective. That is, when the power at 8, 9, 10, 11 and 12 Hz are averaged for the different age groups and different days of testing, there does not appear to be a consistent decline (see Figure 10). Further, for frequencies higher than 12 Hz, the time course of power was also flat.

Most habituation and adaptation processes appear to involve an exponential decline of the dependent variable (Kandel, 1974). While there is as yet no theoretical model of habituation which predicts an exponential function, an exponential does give the best empirical fit to these type of data. Thus the exponential time constant \((\tau)\) is a good objective measure of the rate of decline of a response in an habituation-type paradigm.

The mathematical method of choice for fitting these functions is a method based on the Fourier convolution theorem developed for this application by S. W. Provencher (1976). He provided the Fortran computer program for analysis of these data. With Provencher's
Figure 10. The averaged 3-7 and 8-12 Hz power shown across time for both age groups and both days of testing. Note the decline across time in the 3-7 Hz band. The scale factor is the same for all sections of this figure.
program, it is only necessary to input the raw data (no potentially biased information is required, such as the expected number of components or the baseline constant). The objectivity of this method makes it far superior to the commonly used method of least squares (this is described mathematically in a number of papers by Provencher (1976) and others (Gardner, Gardner, Laush, & Meinke, 1959; Isenberg, Dyson, & Hanson, 1973)).

All the curves in Figure 10 were analyzed with Provencher's program. The parameters of the exponential ($\alpha$, $\lambda$, $c$) as well as the standard error of the fits for the best solution of the data were obtained from the program. This program fits the data to a sum of exponential components given by

$$F(t) = c + \sum_{i=1}^{n} \alpha_i e^{-\lambda_i t}$$

where

$c =$ the baseline constant;

$\alpha_i =$ the initial value of the $i^{th}$ exponential component;

$\lambda_i =$ the rate constant, which is the reciprocal of the time constant (a time constant ($\tau$) is the time in which an exponential falls to $1/e$ of its original value);

and selects the number of components and parameter values for the best fitting solution. For the data discussed here, all optimal solutions contained only one component, and hence are described simply by

$$F(t) = c + \alpha e^{-\lambda t}$$

The parameters from the 3-7 Hz data are seen in Table 14. Although Provencher uses $\lambda$ in his program (the rate constant) it is
perhaps easier to describe the results in terms of $\tau$ (the time constant $\tau = 1/\lambda$). The time constant for the MA group on Day 1 and Day 2 is 55.15 and 48.43 seconds respectively; and for the SN group 95.85 and 67.26 seconds on Day 1 and Day 2 respectively. (Remember that the time constant represents the time in which an exponential falls to 1/e, or approximately 1/3, of its original value.) Thus the MA rats show a more rapid decline on both days than the SN rats, and the decline is faster in both age groups on the second compared with the first day. Although this effect resembles habituation, the experimental criteria, which allow the use of this term, have not been met (Thompson & Spencer, 1966).

It is more difficult to assign meaning to the other components of the exponential, without having an exact physical model; however, $c$ (the constant to which the power was falling) was always higher in the MA than in the SN group. Although this could be seen in the data with power collapsed across time on Day 2 (Figure 8.4) this effect may have been masked by collapsing the data over time on Day 1. Thus the data from $c$ gives more evidence for the notion that the SN rats may generally show less power than the MA rats in the theta frequencies.

The data for 8-12 Hz on both Day 1 and Day 2 and in both age groups showed no reliable tendency to decline over the 10-minute observation period. In summary, it appears that while there is a notable decline over time in the 3-7 Hz components, the 8-12 Hz components do not show this effect.
Table 14

Values of the Exponentials Fit to 3-7 Hz and 8-12 Hz Time Course Data

<table>
<thead>
<tr>
<th></th>
<th>Day 1, 3-7 Hz</th>
<th></th>
<th>Day 2, 8-12 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MA</td>
<td>SN</td>
<td>MA</td>
</tr>
<tr>
<td>( \lambda ) (min)</td>
<td>1.088</td>
<td>0.626</td>
<td>( \lambda ) (min)</td>
</tr>
<tr>
<td>SE</td>
<td>.23</td>
<td>10</td>
<td>SE</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>234.0</td>
<td>231.5</td>
<td>( \alpha )</td>
</tr>
<tr>
<td>SE</td>
<td>31.0</td>
<td>18.0</td>
<td>SE</td>
</tr>
<tr>
<td>( c )</td>
<td>494.5</td>
<td>405.7</td>
<td>( c )</td>
</tr>
<tr>
<td>SE</td>
<td>6.1</td>
<td>6.9</td>
<td>SE</td>
</tr>
</tbody>
</table>

This dissociation of low-frequency components has been previously noted in behavioral and pharmacological terms. It approximates Vanderwolf's and others (Vanderwolf, 1969, 1971, 1975; Klemm, 1976; Kramis, et al., 1975; Winson, 1974) two categories of theta. Thus it appears that the atropine sensitive theta (4-7.5 Hz) shows an exponential decline over time, which may be likened to habituation. This time course provides a further dissociation between the two types of theta activity, suggesting that they reflect different physiological processes.

The time course data for the two bands of low-frequency theta were also broken into early and late times of day (representing the matrix \((A,D,H',f,t)\) and were analyzed with the exponential program. The fits for the data separated into early and late times of day were not as good as those for the data collapsed across time of day. This may be related to the necessary reduction in number of subjects per group. The 8-12 Hz data will not be discussed.
further, since the fits were extremely unreliable. The average power of 3-7 Hz over time (10 minutes) at early and late times of day for both age groups are seen in Figure 11. The values of the parameters fit to the average power scores can be seen in Table 15. There was a particularly large amount of error in the estimate of \( \alpha \) (the initial value of the exponential) in the early Day 1 young group data. However, two interesting features of these data should be noted. There was a striking difference in the magnitude of the time constant when the MA group was separated into early and late times of day:

- early, Day 1 = 367.20 seconds,
- late, Day 1 = 54.20 seconds,
- early, Day 2 = 252.63 seconds,
- late, Day 2 = 29.85 seconds.

On both days, the early MA groups were substantially slower to decline than the late MA groups. However, separating the SN rats into early and late times of day did not produce a similar effect:

- early, Day 1 = 113.10 seconds,
- late, Day 1 = 101.37 seconds,
- early, Day 2 = 64.30 seconds,
- late, Day 2 = 68.70 seconds.

In fact, the value of the time constants from both the early and late times of day in the SN group, tended to be intermediate between the corresponding time constants in the MA groups.
Figure 11. The averaged 3-7 Hz power shown across time when broken into early and late times of day. This is shown for both age groups and both days of testing. Note the apparent difference in decline between these times of day in the MA group. The scale factor is the same for all sections of this figure.
A second point of interest in these data breakdown is the values of $c$ at early and late times of day. In both age groups and on both days of testing, the power to which the frequencies from 3-7 Hz decayed was higher in the late groups than in the early groups (see Table 15).

### Table 15

Values of the Exponentials Fit to the 3-7 Hz Time of Day Data

#### DAY 1

<table>
<thead>
<tr>
<th></th>
<th>MA (3-7 Hz)</th>
<th>SN (3-7 Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>$\lambda$ (min)</td>
<td>.1634</td>
<td>1.107</td>
</tr>
<tr>
<td>SE</td>
<td>.22</td>
<td>.35</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>-99.92</td>
<td>276.1</td>
</tr>
<tr>
<td>SE</td>
<td>55.0</td>
<td>53.0</td>
</tr>
<tr>
<td>$c$</td>
<td>318.4</td>
<td>516.7</td>
</tr>
<tr>
<td>SE</td>
<td>65.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

#### DAY 2

<table>
<thead>
<tr>
<th></th>
<th>MA (3-7 Hz)</th>
<th>SN (3-7 Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>$\lambda$ (min)</td>
<td>.2375</td>
<td>.2010</td>
</tr>
<tr>
<td>SE</td>
<td>.01</td>
<td>.49</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>292.4</td>
<td>160.4</td>
</tr>
<tr>
<td>SE</td>
<td>46.0</td>
<td>39.0</td>
</tr>
<tr>
<td>$c$</td>
<td>478.5</td>
<td>514.1</td>
</tr>
<tr>
<td>SE</td>
<td>6.2</td>
<td>46.0</td>
</tr>
</tbody>
</table>

**Summary of EEG Data**

The overall power ($A,D,f$) (particularly at the higher frequencies) increases from the rat's first experience in the testing room (Day 1) to the second experience in that room (Day 2). Although
the average power spectra are quite similar between age groups on Day 1, the power measured at most frequencies from the MA rats on Day 2 was higher than the corresponding measures from the SN rats (Figure 8). Further, if the day was broken up into "early" and "late" (A,D,H',f) the rats measured at night in both age groups on both days, showed higher power than the rats measured earlier in the day (Figure 9). The late groups in both age groups show a reduction in power in the theta regions from Day 1 to Day 2, and an increase in the higher frequencies from Day 1 to Day 2.

When the power at each frequency is viewed across time (A,D,f,t), the frequencies from 3 to 7 Hz show an exponential decline in both age groups and on both days of testing (Figure 10). The other frequencies which were measured did not show such a decline. The SN rats declined more slowly than the MA rats, but both groups declined more quickly on the second than on the first day of testing. When the 3-7 Hz data was broken into early and late times of day (A,D,H',f,t) the MA rats tended to show a slower decline at night than in the day, whereas the SN rats showed no time of day effect, declining at a rate between the early MA group and the late MA group (Figure 11). The final power in both age groups at 3-7 Hz was higher in the late than in the early groups.

Two features emerge in this section of the experiment which may be important age differences. The first is a possible difference in circadian rhythmicity between age groups, seen both in the total power (the MA rats generally showed a greater difference in
total power when parcellled into early and late times of day; and in the power versus time data (the SN rats did not show the separation in rate of decline in the 3-7 Hz components, when viewed at early and late times of day).

Further, the SN rats showed less capacity for modification through this experience (i.e., being placed in a new environment for 10 minutes on two consecutive days). This is supported by two observations: first, the increase in total power from the first to the second day was greater in the MA than in the SN rats (especially at the higher frequencies); also, when the power over time is examined, the MA rats appear to habituate or adapt more quickly than the SN rats.

It is, of course, difficult to specify what part of the manipulation in this section of the experiment was producing the observed changes. That is, the age differences may reflect differences in ability to process environmental information as the rat acquires a new spatial map, or it may simply reflect differences in the response to the stress of being placed into a novel situation. The data from this study cannot distinguish between the effects of stress and the effects of acquiring new information.
SECTION IV: LOW-FREQUENCY STIMULATION

This section of the study was designed to determine whether low-frequency stimulation would have any effect on the size of the evoked potentials in awake chronically implanted animals. This was included as a control section, since test pulses had to be used to monitor the effects of HF stimulation in a later section. Test pulse frequencies of .2 Hz or higher have been used by a number of workers (Douglas & Goddard, 1975; Racine, Newberry, & Burnham, 1975); however, no systematic study of the effects of this kind of low-frequency stimulation in awake animals has been reported.

Procedure

The day after all EEG was collected, stimulus-response relationships were determined for each rat (II in Table 2). This consisted of collecting an averaged evoked response (ten sweeps per average) from a number of stimulus intensities, in a fixed ascending series of stimulus amplitudes. The intensity at which a reliable (but small) population spike was first recorded was subsequently used as the individual rat's stimulation parameter. A more detailed description of the manner in which the stimulation parameters were determined for this section is elaborated in Section VI.

On the two consecutive days (P1 and P2 in Table 2) following the determination of stimulus-response relationships, each rat was stimulated with its individual stimulation parameters once every five seconds for five minutes (60 evoked responses on both P1
and P2). As in the EEG recording sessions, each rat was placed singly into the recording room, and measurements were begun immediately after the rat's head cap was connected to the recording and stimulating system (this system is as described in Section III). P1 and P2 sessions were the fourth and fifth time, respectively, that the rats had been placed into the shielded room. The number of SN rats in this section was 26, and the number of MA rats was 23.

Measurement of the Evoked Response

Measurements of the evoked responses in this and the following sections were made as described below. The slope of the EPSP was measured between two fixed latency points, 2.50 and 3.25 msec after stimulus onset (approximately .50 to 1.25 msec after EPSP onset). These latencies lie within the range of values accepted as reflecting EPSP amplitudes with little contamination from the population spike component or polysynaptic events (Lomo, 1971a, b; Steward, White, Cotman & Lynch, 1976). An automatic analysis program was used to measure the population spike component. It will be seen that this latter component will be the less important measure for this thesis.

Results and Discussion

The ERP slope and population spike area were measured from each of the 60 potentials collected on both days of testing in all rats. Linear regression was performed on the 60 values obtained from the EPSP and population spike for each animal (one regression line for each day of testing). The regression parameters
(the slope $b$, and intercept $a$) from the individual rat's regression coefficients were then averaged for each age group and day of testing.

The population spike area. The data for the population spike was extremely variable in all animals. This was to be expected since the intensity of stimulation was set close to spike threshold; hence the granule cell discharge was much less reliable than it would have been in a case where the stimulus intensity was well above threshold. This variability may have obscured any trends which might have been present in the data if higher intensities were used. The population spike is therefore not considered further in this analysis.

The slope of the EPSP. The mean regression lines of the EPSP slope for both groups on both days of testing are shown in Figure 12. The regression coefficient $b$ (slope) was statistically different from zero in each case ($p < .05$, using a one sample test statistic for the mean). Thus, the EPSP increased significantly over time in both MA and SN animals, when the interpulse interval was .2 Hz. This increase, however, is small compared with the increases normally seen with high-frequency stimulation.

While the mean data tends to suggest that the amplitude of the EPSP remained elevated for 24 hours (relative to beginning of P1) a test for differences between the intercept values within groups (on P1 and P2) came very close to, but did not reach the $\alpha .05$ level. A similar result was obtained in the comparison of
Figure 12. The mean regression lines for each age group from the first (P1) and second (P2) day of low-frequency stimulation. Test pulses were delivered at a rate of .2 Hz. A linear regression line was fit to the 60 values obtained from the EPSP analysis, and the individual coefficients of regression were averaged for each age group. There was a statistically significant positive-going slope ($b$) in all cases. The mean amplitude of the EPSP (the intercept $a$) was different between age groups.
slopes between age groups; the MA rats tended to show a steeper slope on both days than the SN rats, but this failed to reach significance.

A noticeable aspect of Figure 12 is the large difference between the MA and SN y intercept (the y intercept reflecting the initial amplitude of the EPSP). This difference was statistically significant (p < .05). Since the stimulus intensity was set at the population spike threshold for each animal, and since the intensities were not different between age groups (see Table 16), it is apparent that the threshold for granule cell discharge was reached at a lower value of extracellular EPSP in the old rats.

Table 16

<table>
<thead>
<tr>
<th></th>
<th>SN (µ amps)</th>
<th>MA (µ amps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>X</td>
<td>482.9</td>
<td>402.2</td>
</tr>
<tr>
<td>SE</td>
<td>52.9</td>
<td>48.3</td>
</tr>
</tbody>
</table>

There are a variety of factors which could have led to the observed difference. Since the data from Section VI contributes to the dissociation of these possibilities, further discussion will be deferred until those data have been presented.

Time of day. The values of the regression parameters were partitioned into early and late times of day (corresponding to the breakdown in time of day used in the EEG section), for each age group on both days of testing. The means and standard errors are shown in
Table 17. For the MA rats, the intercept and slope tended to be larger at the late times of day on both P1 and P2. These differences in time of day, although in the anticipated direction (Barnes, et al., 1977), are very small ($p > .05$), compared with the striking differences seen in the MA EEG data (Section III). The SN rats, on the other hand, show the opposite result. The intercept and slope tend to be larger at the early times of day ($p > .05$). Because of the high degree of variability, conclusions are tenuous.

Table 17
Low-Frequency Stimulation Regression Coefficients for Early and Late Times of Day

<table>
<thead>
<tr>
<th></th>
<th>SN</th>
<th>MA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1</td>
<td>P2</td>
</tr>
<tr>
<td>Intercept</td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>$\bar{x}$</td>
<td>1.714</td>
<td>0.803</td>
</tr>
<tr>
<td>SE</td>
<td>0.559</td>
<td>0.227</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

Slope X10

<table>
<thead>
<tr>
<th></th>
<th>SN</th>
<th>MA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1</td>
<td>P2</td>
</tr>
<tr>
<td>$\bar{x}$</td>
<td>4.646</td>
<td>1.497</td>
</tr>
<tr>
<td>SE</td>
<td>2.004</td>
<td>.912</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

Summary. A smaller EPSP was recorded from the SN rats than from the MA when the stimulation intensity was set at spike threshold. This may indicate important differences in the physiological properties of the SN rats' granule cells (such as a lower threshold for granule cell discharge) which will be discussed more fully in Section VI.

The amplitude of the EPSP increased during the 60 test pulses on both P1 and P2 in each age group. This finding stands in sharp
contrast to reports of habituation-like responses to low-frequency stimulation in chloralose-urethane anesthetized rats (Harris & Steward, 1976) and in the hippocampal slice preparation (Teyler, et al., 1977). There are numerous factors which could have lead to this apparent discrepancy. These include effects of anesthetic, the various disruptions in neuronal function caused by removing a slice of tissue from the brain, and differences in stimulation procedures (the studies mentioned above used very few test pulses over a shorter period of time and were generally carried out with stimulation parameters well above spike threshold, Harris, personal communication). However, another reasonable interpretation may be that the habituation-like effects are directly related to the actual stimulation in animals which have most likely been set up in the recording apparatus for at least 1/2 hour, whereas the increasing effects in the awake animals from this thesis may have occurred independently of the stimulation due to some ongoing neuronal process.

The increase in the amplitude of the EPSP from the first to the second day of testing is quite reminiscent of the increases noted in the power of the EEG (in these same animals) from the first to the second day of EEG testing (particularly at the frequency components which would include EPSP waveforms). Thus, if the kind of increase in the power of the EEG were still occurring during P1 and P2, this might well account for the recorded increases in EPSP amplitude.

The increase due to low-frequency stimulation, using the procedure of recording from the rat immediately after it was placed into the testing room, has now been replicated using five awake rats
prepared for another experiment (McNaughton & Douglas, personal communication). More importantly, in collaboration with McNaughton we found that the same magnitude of increase occurred when test pulses were given at a rate of once per minute (1/12 the rate used in the main study), which supports the notion that these changes may occur independently of the stimulation.

These increases might go unnoticed in experiments using chronic animals to measure the large changes that occur with high-frequency stimulation. However, the increases seen in the EEG and low-frequency stimulation sections may be a reflection of normal learning-memory processes in the dentate gyrus occurring in spite of the experimental intervention of low-frequency stimulation.
SECTION V: HIGH FREQUENCY STIMULATION

Each rat was stimulated with HF bursts (Racine, et al., 1975; Douglas, 1977) five times during this experiment. The increased evoked response resulting from this kind of stimulation (Bliss & Gardner-Medwin, 1971, 1973; Douglas & Goddard, 1975) was of particular interest to this thesis, as described in the introduction, and as will briefly be reiterated below. The hypothesis was that this neurophysiological test could potentially detect age differences in one type of synaptic modifiability which might arise from an increase in the amount of stored information gained through a longer life. Since the brain is a physically limited system, it must have a finite information storage capacity. It is conceivable that the limit of this capacity may be approached during the lifetime of the organism. It was therefore anticipated that the SN rats' synapses would be more powerful, and thus nearer to their maximum possible amount of potentiation. Since the SN rats would be expected to have more stored events, and since the probability of one stored event being distinguished from another decreases as the number of stored events increase, retrieval and therefore durability of the elevated evoked response were anticipated to be altered.

It must be recognized however, that the foregoing rests on at least three assumptions: that this measure is a sensitive enough indicator of synaptic modifiability, that synaptic modifiability is an important component of the memory process, and that long-lasting
memories are not prone to slow decay. The experimental goal thus can only be met to the degree that these assumptions are valid.

General Procedure

The procedure was identical for all eight groups. During the appropriate time period all rats, in a particular group were moved into an eight-compartment cart designed to hold rat cages (see Appendix G). The cart was then wheeled into the recording room and each rat's head cap was connected to an individual recording cable. The cable was attached in such a way that the rat was essentially free to move within its cage. The stimulating and recording equipment were the same as that described in Section II. The HF stimulation episodes were divided into three parts, each part intending to supplement the information of the others. The three phases of this section will be described separately.

Phase I - Time Course of Decline After a Single HF Episode

The first HF stimulation (T1 in Table 2) provides information about the time course of decline (over a week) of the elevated evoked response after a single bout of HF stimulation. Averages of the evoked response were collected 48 hours (B1) and 24 hours (B2) before T1, and 24 hours (B3), 48 hours (B4) and seven days (B5) after T1. These baseline responses (B1-B5) are all averages of 10 evoked potentials collected at a rate of one every five seconds. The stimulation intensity used in this phase of the experiment was determined by the stimulus-response relationships obtained in Section VI. This was the intensity necessary to elicit a reliable but small
population spike (e.g., the same intensity as used in the low-frequency stimulation section).

On the day of the HF stimulation (T1) five averaged evoked responses were collected. The first was taken as baseline before the HF stimulation, the second was sampled during the HF stimulation itself (and consists of an average of 15 rather than 10 waveforms), and the subsequent three averages were taken at two-minute (± 30 sec), 10-minute (± 30 sec), and one-hour (± 2 min) intervals after the HF stimulation. The HF stimulation consisted of 16, 20 msec bursts of 400 Hz, delivered once every five seconds. A burst of that duration and frequency consists of eight stimulus pulses. Thus the rats received a total of 120 single pulses during this stimulation episode. After-discharge was not observed in any of the rats in this study, using the above parameters for HF stimulation.

In summary, this first phase of HF stimulation consisted of three baseline averages before the tetani (B1, B2 and the average collected immediately before T1), the tetanic stimulation (T1), and the test averages collected two minutes, 10 minutes, one hour, 24 hours (B3), 48 hours (B4), and seven days (B5) after T1.

Results and Discussion of Phase I

The EPSP slope. The slope of the averaged EPSP was measured between two fixed latency points (2.5 msec and 3.25 msec after stimulus onset) as described in Section IV. The fractional change of the evoked response was then calculated. The fractional difference is defined as \((V_t - V_o)/V_o\), where \(V_o\) is the amplitude measure.
before HF stimulation, and $V_t$ is the amplitude measure after (or during) HF stimulation. The three baseline measures obtained from each rat before HF stimulation were averaged and this value was used as the individual rat's $V_0$ (see Table 18). After the fractional difference was calculated for each rat, the mean fractional change of the EPSP for each group was obtained. This can be seen in Figure 13.

Table 18

Values of Baseline EPSP Slope Measurements Used to Determine $V_0$ for T1-T4

<table>
<thead>
<tr>
<th></th>
<th>MA (mV/msec)</th>
<th>SN (mV/msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{X}$</td>
<td>2.95</td>
<td>1.10</td>
</tr>
<tr>
<td>SE</td>
<td>.59</td>
<td>.31</td>
</tr>
<tr>
<td>n</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>B2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{X}$</td>
<td>2.15</td>
<td>.91</td>
</tr>
<tr>
<td>SE</td>
<td>.63</td>
<td>.17</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>Before T1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{X}$</td>
<td>2.55</td>
<td>1.10</td>
</tr>
<tr>
<td>SE</td>
<td>.55</td>
<td>.26</td>
</tr>
<tr>
<td>n</td>
<td>24</td>
<td>23</td>
</tr>
</tbody>
</table>

$V_0$: 2.55  1.04

Although the MA rats tended to show larger increases than the SN rats, there were no statistically significant differences between age groups in this phase (see Table 19). The evoked responses in both groups remained substantially elevated for at least 48 hours after T1, but declined to near initial baseline levels by seven days.
Figure 13. The mean fractional change of the slope of the EPSP over its value at $V_0$. T1, T2, T3, and T4 represent the first through fourth times respectively that these rats were given HF stimulation. The time points shown after HF stimulation (2 min, 10 min, 1 hr, etc.) represent the time elapsed between the end of the HF episode and the beginning of the test pulse measurements for that given time point.
MEAN FRACTIONAL CHANGE OF EPS P OVER INITIAL BASELINE
Table 19

The Number of Rats Included (n) and Values from Statistical Tests (t) for Age Differences in the Time-Points Measured from T1-T5 (MA minus SN)

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>2 min</th>
<th>10 min</th>
<th>1 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN n</td>
<td>21</td>
<td>21</td>
<td>20</td>
<td>18</td>
<td>20</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>MA n</td>
<td>20</td>
<td>19</td>
<td>20</td>
<td>16</td>
<td>16</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>t</td>
<td>.497</td>
<td>.764</td>
<td>.203</td>
<td>-.854</td>
<td>.642</td>
<td>.422</td>
<td>.109</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>8 days</th>
<th>T2</th>
<th>2 min</th>
<th>10 min</th>
<th>1 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN n</td>
<td>19</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>MA n</td>
<td>20</td>
<td>20</td>
<td>19</td>
<td>19</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>t</td>
<td>-.200</td>
<td>.749</td>
<td>.511</td>
<td>.361</td>
<td>.381</td>
<td>.425</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>T3</th>
<th>2 min</th>
<th>10 min</th>
<th>1 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN n</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>MA n</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>t</td>
<td>.396</td>
<td>.717</td>
<td>.638</td>
<td>.513</td>
<td>2.022*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>T4</th>
<th>2 min</th>
<th>10 min</th>
<th>1 hr</th>
<th>24 hr</th>
<th>7 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN n</td>
<td>19</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>18</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>MA n</td>
<td>19</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>18</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>t</td>
<td>2.357*</td>
<td>2.206*</td>
<td>2.031*</td>
<td>2.180*</td>
<td>2.701*</td>
<td>3.217*</td>
<td>3.058*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>T5</th>
<th>2 min</th>
<th>10 min</th>
<th>1 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN n</td>
<td>23</td>
<td>20</td>
<td>22</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>MA n</td>
<td>24</td>
<td>25</td>
<td>24</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>t</td>
<td>-.039</td>
<td>.551</td>
<td>.105</td>
<td>.786</td>
<td>-.160</td>
</tr>
</tbody>
</table>

*p < .05
Examples of the averaged evoked responses from the first HF episode, with two standard errors on either side of the mean (representing the 95% confidence limits), are seen in Figures 14-17. When the averages after HF stimulation are overlapped with the average collected before, the confidence intervals do not overlap in critical regions of the EPSP and population spike, thereby demonstrating a reliable increase in the evoked response (see Douglas & Goddard, 1975).

The data stored on magnetic disk from this section of the experiment was largely destroyed due to a programming error. Fortunately print outs of most of the individual EPSP and spike magnitudes were collected at the time of data sampling. However, the data from the population spike are missing after the baselines (B1 and B2) before T1 until 24 hours after T1 (as well as the measurements during T2, T3 and T4 from Phase II). Because the only time points available from this phase are the 24-hour, 48-hour and 7-day measurements, the population spike will be discussed more thoroughly in Phase II.

Phase II—Repeated HF Stimulation Episodes

On the eighth day after T1, the second phase of high-frequency stimulation was begun (seen in Table 2 as T2, T3, and T4). In this phase of the experiment, tetanic stimulation was delivered three times at 24-hour intervals, providing information about the size and duration of the increase in the evoked response after repetitive HF stimulation. The test stimulation and HF stimulation parameters were the same as in Phase I.
Figure 14. Examples of averaged evoked responses (with the 95% confidence limits) collected for T1 through T4 for one SN rat. (Calibration: 4mV, 4 msec)

1) The averaged response collected immediately before T1 is seen as "A", the HF stimulation (T1) as "B" (with the mean only), two minutes after as "C", 10 minutes after as "D", and one hour after as "E".

2) The mean responses for the Day 7 and 8 time points after T1 are shown over-lapped in "A". The HF stimulation (T2) is shown as "B". The "C", "D", and "E" time points are the same as described in "1" above.

3) The average response collected for T3 is shown as "B", the "A", "C", "D", and "E" time points are as in "1" above.

4) The average response collected for T4 is shown as "B", the "A", "C", "D", and "E" time points are as in "1" above.
Figure 15. Examples of averaged evoked responses (with the 95% confidence limits) collected for T1 through T4 for one SN rat. (Calibration: 4 mV, 4 msec)

1) The average response collected immediately before T1 is seen as "A", the HF stimulation (T1) as "B" (with the mean only), two minutes after as "C", 10 minutes after as "D", and one hour after as "E".

2) The mean responses for the Day 7 and 8 time points after T1 are shown overlapped in "A". The HF stimulation (T2) is shown as "B". The "C", "D", and "E" time points are the same as described in "1" above.

3) The average response collected for T3 is shown as "B", the "A", "C", "D", and "E" time points are as in "1" above.

4) The average response collected for T4 is shown as "B", the "A", "C", "D", and "E" time points are as in "1" above.
Figure 16. Examples of averaged evoked responses (with the 95% confidence limits) collected for T1 through T4 for one MA rat. (Calibration: 4 mV, 4 msec)

1) The average response collected immediately before T1 is seen as "A", the HF stimulation (T1) as "B" (with the mean only), two minutes after as "C", 10 minutes after as "D", and one hour after as "E".

2) The means and standard errors of the responses for the Day 7 and 8 time points after T1 are shown overlapped in "A". The HF stimulation (T2) is shown as "B". The "C", "D", and "E" time points are the same as described in "1" above.

3) The average response collected for T3 is shown as "B", the "A", "C", "D", and "E" time points are as in "1" above.

4) The average response collected for T4 is shown as "B", the "A", "C", "D", and "E" time points are as in "1" above.
Figure 17. Examples of averaged evoked responses (with the 95% confidence limits) collected for T1 through T4 for one MA rat. (Calibration: 4 mV, 4 msec)

1) The average response collected immediately before T1 is seen as "A", the HF stimulation (T1) as "B" (with mean only), two minutes after as "C", 10 minutes after as "D", and one hour after as "E".

2) The means and standard errors of the responses for the Day 7 and 8 time points after T1 are shown overlapped in "A". The HF stimulation (T2) is shown as "B". The "C", "D" and "E" time points are the same as described in "1" above.

3) The average response collected for T3 is shown as "B", the "A", "C", "D", and "E" time points are as in "1" above.

4) The average response collected for T4 is shown as "B", the "A", "C", "D", and "E" time points are as in "1" above.
The value of $V_0$ for each rat was the value calculated for the initial baseline before T1 (see Table 18). Averaged responses were collected before and during each HF stimulation episode, and after intervals of two minutes, 10 minutes, one hour and 24 hours. In addition, averages were collected at seven days and 14 days after T4.

**Results and Discussion of Phase II**

The EPSP slope. The mean fractional change of the EPSP slope was determined for each age group and is shown in Figure 13. The 7th and 8th day after T1 were quite similar. Although the mean fractional increase of the MA rats' EPSP was consistently higher than that for the SN rats after T2 and T3, the means between age groups were not statistically different until the 24-hour time point after T3 (see Table 19). After this point, the fractional change for the MA group remained statistically above the fractional change of the SN group (see Table 19). Examples of the evoked responses measured during T2 to T4 from individual SN and MA rats are shown in Figures 14-17.

The population spike area. Although the changes in the population spike can be clearly viewed in the averaged evoked responses shown in Figures 14-17, the enormous variability in the amount of change objectively measurable makes the demonstration of reliable differences between groups difficult (there were no statistically significant differences between age groups, $p > .05$). For example, fractional changes in the population spike area
easily ranged from -.5 to 1500 (the fractional difference was calculated in the same way as for the EPSP). The data from both groups are collapsed to show the time course of increase and decline in the spike area with repeated trials, and can be seen in Figure 18. This figure begins at the 24-hour time point after T1, and does not show the area during T2, T3, and T4 because of the missing data described in Phase I. The decay of increase in the population spike area (over 24 hours) is similar to the time course of decline in EPSP enhancement. However, the relative decline is greater in the population spike than in the EPSP after the HF stimulation was completed (compare the seven-day and 14-day time points in the two data sets, Figures 13 and 17). Bliss and Lomo (1975) and Bliss and Gardner-Medwin (1973) first recognized the possible independent variation of the size of the population spike and EPSP after tetanic stimulation in rabbits. This was again noticed by Douglas (1975) in rats. At least in the MA animals in this study, the spike tended to decay, whereas the EPSP tended to remain elevated out to 14 days. In the SN animals, both components tended to decay similarly.

**Time of day.** Each group received HF stimulation during the time period designated to the group number, from T1 through T4. The question of interest here, concerns the influence of the time of day on the modifiability of perforant path synapses. Each age group was broken into "early" (12:00 - 22:00) and "late" (22:00 - 04:00) times of day. The mean fractional change of the EPSP was calculated for each age group at these two times of day. Finer time
Figure 18. The mean and standard errors of the fractional change in the population spike area for the SN and MA groups combined. The time points from which the population spike measures were obtained from T1 to T4 are shown on the x axis.
breakdowns were also made, but had too few animals for reasonable comparisons to be made. The results from the first and second phase for the MA and SN groups are seen in Figure 19.

The mean fractional change in the EPSP slope for T1 and T4 shows that the MA rats tend to have larger increases in the late than in the early groups (20 out of 25 cases). The time points that were statistically different in the MA group (p < .05) include the two-minute and 48-hour time points after T1, T2 and one hour after T2, one hour and 24 hours after T3, and 10 minutes after T4. Although the larger responses from the late groups were not consistently significant, the general features of the data suggest that the time of day influenced the amount of increases seen after HF stimulation in the MA rats. The time of day effects in synaptic excitability are known to be rather subtle (Barnes, et al., 1977). The fact that in seven cases the late groups reached a statistically higher value than the early groups, and that the reversals of this effect were all very small, encourages further investigation of circadian influences in a more robust design.

The mean fractional change in the EPSP for the SN rats (Figure 19) was higher in the late groups in 11 out of 25 cases. In one instance (48 hours after T1) the late group was statistically higher than the early group (p < .05). Unlike the MA rats, the SN rats showed at least five instances where the early groups increased substantially more than the late groups. Since the variability in the SN groups, when broken into late and early times of day, is
Figure 19. The mean fractional change of the EPSP slope when broken into early (12:00 - 22:00) and late (22:00 - 04:00) times of day for each age group. The $n$ for each histogram is approximately $9 \pm 2$ in each case.
Clearly greater than the MA groups, this supports the notion that there may be a period or phase disturbance of circadian rhythmicity in the older animals. There was similar evidence for an alteration in the rhythmicity of SN rat EEG (Section III), response to low-frequency stimulation (Section IV) and spontaneous alternation behavior (Section VII).

Phase III: High-Intensity HF Stimulation

The last HF stimulation is seen in Table 2 as "T5".

For this section of the experiment, the stimulation intensity given to all rats was the same: 1 mA for 50 μsec. This intensity was greater than that generally used on the rats in the experiment up to this point. It was reasoned that such an intense level of stimulation, held constant over age groups, might further distinguish differences in synaptic modifiability between the MA and SN animals. Further, it might be expected to reduce the variability from that obtained with lower level stimulation. The purpose of this section was to maximally activate the perforant path fibers, including those for which the stimulus intensity in T1-T4 was below threshold, so that the modifiability of this residual population of synapses could be examined. These results would then serve to corroborate the results of T1.

The EPSP slope. In this phase the baseline before T5 was used as $V_0$ and the fractional changes of the EPSP are seen in Figure 20. The increases are smaller, but reminiscent of the pattern of increase and decline in T1 and T2. There was a lasting
Figure 20. The mean fractional change of the EPSP slope for the high-intensity (T5) HF stimulation episode, and time points after.
increase over the initial baseline out to 24 hours in both age groups, but no statistically significant differences between age groups (see Table 19). Part of the reason for the smaller fractional changes observed in this phase is undoubtedly that a significant fraction of the synapses were already closer to their maximum potentiation due to the HF stimulation given in Phase I and II. The finding that the SN and MA rats did not differ with respect to the fractional increase in the size of the EPSP, might be explained in terms of there being only one occurrence of this stimulation episode. This, in essence, makes TS a replication of the first HF stimulation (T1), where there was no difference between age groups after a single HF stimulation episode. Perhaps if repeated HF bursts were given at this intensity, differences would have emerged as they did in Phase II.

Representative examples from the evoked responses collected during TS are seen in Figures 21 and 22.

Summary

Since the brain is a physically limited system it was hypothesized that the limit of this capacity may be approached during the lifetime of the organism. It was expected that either the amount of potentiation and perhaps its decline after T1 would have been different between age groups. Neither of those predictions were correct. The initial changes were not different between groups. This was seen both in Phase I and III where there was only a single HF stimulation episode given. The most striking feature of these
Figure 21. The mean and 95% confidence limits of the evoked responses from four different SN animals (shown as 1-4). The response collected immediately before T5 is shown as "A" in all examples, T5 is shown as "B" (with the mean only), two minutes after T5 as "C", 10 minutes after as "D", one hour after as "E", and 24 hours after as "F".
Figure 22. The mean and 95% confidence limits of the evoked responses from four different MA animals (shown as 1-4). The response collected immediately before T5 is shown as "A" in all examples, T5 is shown as "B" (with the mean only), two minutes after T5 as "C", 10 minutes after as "D", one hour as "E", and 24 hours after as "F".
data appears to be the large difference in retention that became apparent after repeated HF input (as shown in Phase II). These data suggest that although the absolute magnitude of increase might not change dramatically from one HF episode to the next (with the parameters used here), good retention requires a certain amount of repeated HF input. This "amount" was not reached after T4 for the SN rats.

It is unfortunate that the effects of repetition were not anticipated so that either Phase II or Phase III could have been extended. For instance, the SN rats may have approached the same level of retention as the MA rats if more than three repeated HF episodes had been included in Phase II. The way in which the time course of increase in the evoked response had typically been viewed (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973; Douglas & Goddard, 1975) was that the largest changes relative to baseline responses were seen after one HF episode. This feature plus the maximum capacity notion led to the design of this section. The aspect that had not been anticipated (the requirement for repeated episodes for good retention) was actually the more important finding of this experiment.

The change in amplitude of the evoked response after HF stimulation tended to be larger when administered and measured at later than at earlier times of day for the MA rats. This time of day difference was not noted for the SN rats, suggesting a disturbance of circadian rhythmicity in the older animals.
SECTION VI: STIMULUS-RESPONSE RELATIONSHIPS

The relationship between stimulus intensity and the magnitude of the synaptic response was determined for each rat twice during the study: immediately after the EEG data were collected (I1), and after T4 (I2). From these data, input/output curves could be plotted with the response magnitude on the y axis and the ascending stimulus strengths on the x axis. The various uses for this information will be discussed below.

Rational for Determining Stimulus-Response Relationships

**Systematic determination of stimulation parameters.** The intensity at which a small reliable population spike was first recorded in each rat was determined with the stimulus-response relationship procedure. This particular intensity was used for P1 and P2, the baseline measures, and the HF stimulation episodes T1-T4. The use of the population spike threshold intensity was preferable to using fixed stimulus intensity across rats since it resulted in a more uniform population of evoked responses.

**Advantage of low-intensity stimulation.** Besides providing a rather homogeneous population of evoked responses (at least in each age group), there are certain theoretical reasons for choosing sub-maximal stimulation strengths. The mechanism of long-term potentiation is unknown. However, a possible (and not unlikely) mechanism may involve an increase in synaptic conductance, such as would occur if either more transmitter was released, or the post-synaptic
membrane became more sensitive (as suggested by Lomo, 1971b and Bliss & Lomo; 1973). The amount that the synaptic potential can be increased by increasing synaptic conductance is limited by the reversal potential of the transmitter (de Castillo & Katz, 1956; Jack, Nobel & Tsien, 1975). With infinite synaptic conductance, the synaptic voltage change is exactly equal to the absolute value of the difference between the resting and reversal potentials. Multiple synapses on a cell can be represented approximately as parallel synaptic conductances, for which an equivalent series conductance can be derived. Therefore as the stimulus intensity is increased, and more perforant path fibers are activated, the equivalent series synaptic conductance is increased, and the resulting synaptic potential is closer to its theoretical limit. It follows that with high-intensity stimulation there may be less relative change in the evoked response for a given increase in the conductance of individual synapses. Since the stimulus amplitude was set at a value that can be considered moderate in terms of EPSP size, this should have tended to optimize the chances of observing good potentiation in these animals (see Section V).

**Stimulus-response determinations before and after HF stimulation.** The first stimulus-response determination may detect differences between age groups in the granule cell response to stimulation of the perforant path at different intensities. The second stimulus-response determination was made so that the magnitude of the post-synaptic response and stimulus strength after HF
stimulation could be compared with the values before HF stimulation.

Procedure

The procedure was identical for all eight groups: During the appropriate time period, all rats in a particular group were moved into the testing cart which was then wheeled into the recording room. Each rat's head cap was connected to a recording cable as in Section V.

The stimulus values ranged from 75 µamps X 50 µsec to 1000 µamps X 90 µsec (see Table 20). The lowest intensity stimulus was delivered to the rat once; if there was no response to that intensity, a single test pulse at the next highest intensity was given, until the first clear response was evoked. At this point 10 responses at that intensity were measured and averaged. Averages were subsequently taken at each intensity above this point until the maximum was reached.

Table 20

The Fixed Ascending Series of Stimulus Amplitudes and Pulse Widths Used in I1 and I2

<table>
<thead>
<tr>
<th>Intensity (µamps)</th>
<th>Duration (usec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>105</td>
<td>50</td>
</tr>
<tr>
<td>150</td>
<td>50</td>
</tr>
<tr>
<td>200</td>
<td>50</td>
</tr>
<tr>
<td>250</td>
<td>50</td>
</tr>
<tr>
<td>300</td>
<td>50</td>
</tr>
<tr>
<td>360</td>
<td>50</td>
</tr>
<tr>
<td>430</td>
<td>50</td>
</tr>
<tr>
<td>510</td>
<td>50</td>
</tr>
<tr>
<td>600</td>
<td>50</td>
</tr>
<tr>
<td>1000</td>
<td>50</td>
</tr>
<tr>
<td>1000</td>
<td>70</td>
</tr>
</tbody>
</table>

Maximum

1000               90
Results and Discussion

The fractional change after HF stimulation. The EPSP amplitude and the population spike area were measured for both age groups at three different intensities from I1 and I2. The "low" intensity was the lowest intensity used for each animal, the "med" intensity was the intensity at which each animal had been stimulated throughout the study; the "high" intensity was the maximum intensity used in the intensity series (see Table 21 for the mean values of stimulus strength in the three categories for each age group).

Table 21

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th></th>
<th>Med</th>
<th></th>
<th>High</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µamps</td>
<td>µsec</td>
<td>µamps</td>
<td>µsec</td>
<td>µamps</td>
<td>µsec</td>
</tr>
<tr>
<td>X SN</td>
<td>191</td>
<td>50</td>
<td>288</td>
<td>50</td>
<td>1000</td>
<td>90</td>
</tr>
<tr>
<td>X MA</td>
<td>168</td>
<td>50</td>
<td>339</td>
<td>50</td>
<td>1000</td>
<td>90</td>
</tr>
</tbody>
</table>

The fractional increase for each rat at each of these intensities was then calculated. In this case, $V_t$ is the amplitude measure after HF stimulation (I2), and $V_0$ is the amplitude measure before HF stimulation (I1). The means are shown in Figure 23. There is no consistent difference between the low and medium intensities in the EPSP magnitude within age groups.

Between groups, however, the MA rats show larger increases after HF stimulation than the SN rats. These differences are statistically significant for the EPSP ($p < .05$) at the low and medium levels. Since these rats were not given HF stimulation at the high
Figure 23. The mean fractional change in the EPSP slope when measured before (I1) and after (I2) HF stimulation, at three stimulus intensities for both age groups.
intensity (prior to I2), the lack of a difference in this measure was not surprising. These data therefore corroborate the results obtained in Section V. The variability in the population spike data was again a problem (as in Section IV and V). Although the means for the population spike data show a pattern similar to that for the EPSP, these differences were not statistically significant. Examples of evoked responses collected from I1 and I2 from both age groups are seen in Figure 24.

Time of day. The fractional change in the EPSP at the low, medium and high intensities, discussed above, was broken into early (Groups 1-4) and late (Groups 5-8) times of day. The MA rats showed a reliably larger fractional increase at the late time of day with the low-intensity stimulation (p < .05). The time of day differences at the medium and high intensities, however, did not reach statistical significance. The SN rats showed a statistically larger increase at the early time of day at the low intensity (p < .05), but no difference at the medium or high intensity. This finding is interesting in terms of the time of day results in Section V, where there were quite a few points after HF stimulation where the SN rats showed much higher increases in the early than in the late groups (although none of these differences were statistically reliable). These data are consistent with the notion that circadian rhythmicity is altered in the SN rats (at least compared with these MA rats, and with the cyclicity anticipated from other experiments in this laboratory, Barnes, et al., 1977).
Figure 24. Examples of evoked responses collected at different stimulus intensities before and after HF stimulation. Each waveform represents the mean of 10 evoked responses. Two SN rats are shown as "A" and "B", and two MA rats as "C" and "D". (Calibration: 4 mV, 4 msec)
Initial stimulus-response determinations. The relationship between stimulus strength and EPSP slope prior to HF stimulation is shown in Figure 25. The lines through the data points are least squares fits to a rectangular hyperbola. At all stimulus intensities the MA rats showed a larger EPSP response. There are at least three possible explanations of these data: 1) the MA animals had more powerful synapses; 2) the driving force for the synaptic potential (resting potential minus reversal potential) was smaller in the SN animals; 3) the number of perforant path fibers activated by a given stimulus intensity was smaller in the SN rats. These possibilities cannot be discriminated on the basis of these data alone, and it is possible that more than one of these factors may be operating simultaneously.

Recent evidence suggests that there are actually 30% fewer synapses on old than on young rat granule cell dendrites, in the region of termination of the perforant path (Bondareff & Geinisman, 1976). The ages of the rats used in this study were three months (young, n = 5) and 25 months (old, n = 5). Although the MA and SN rats in the present study were older than those used in Bondareff's study, it is very likely that there is a difference in the number of synapses between age groups.

Some support for this observation is obtained in the present study from the relationship between EPSP size and fiber potential size in the few animals that had measurable fiber responses, of which there were five MA and five SN rats. Table 22 shows the
Figure 25. The relationship between stimulus strength and EPSP magnitude for both age groups. The solid lines are rectangular hyperbola fit to the data by least squares.
mean and standard errors of the EPSP slope/fiber potential area ratios for the MA and SN rats at the three intensities shown in Table 21. Although the ratio for the SN rats is consistently larger at each intensity than those for the MA rats, none of these differences were statistically significant. Since fiber potentials are observed only with the most favorable of recording electrode positions, the number of animals with such responses was quite low. Thus there was little power to detect an effect. The direction of these results nevertheless support the contention that there are fewer perforant path fibers activated for a given stimulus strength in the SN animals.

Table 22

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Med</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>6.04</td>
<td>3.95</td>
<td>5.34</td>
</tr>
<tr>
<td>SE</td>
<td>2.70</td>
<td>.80</td>
<td>2.27</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>MA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>2.64</td>
<td>3.85</td>
<td>3.02</td>
</tr>
<tr>
<td>SE</td>
<td>.99</td>
<td>2.23</td>
<td>.98</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

In addition, the population spike was elicited at a lower value of the EPSP in the SN rats with equivalent stimulus intensities (see Section IV). If the SN rats' granule cells were more depolarized, the difference between the resting and reversal potentials would be smaller, making the recorded extracellular
current flow less. Also the SN rats' granule cells would be closer to discharge threshold. Although the above can account for the observed results, the possibility remains that the spike generating mechanism becomes more sensitive, or that the "trigger zone" moves further up the soma-dendrite in the old animals. These lines of evidence tend to contradict the conclusion from the stimulus-response curves (Figure 25) that the young rats' synapses were more powerful. In fact the evidence that the EPSP to fiber potential ratio tended to be larger in the SN rats suggests the opposite, i.e., that the SN animals had more powerful synapses. This is particularly true if the notion that the SN rats' granule cells were more depolarized is accepted. The effect of this would be to bias the EPSP to fiber potential ratio in the opposite direction (the recorded extracellular EPSP's being smaller in the SN rats). This suggests that the SN rats had fewer but substantially more powerful synapses.

Summary. The MA rats showed a larger fractional change in EPSP amplitude than the SN rats when measured after HF stimulation. This held at lower intensities as well as at the intensity of test stimulation. These data duplicate the finding in Section V, that the SN rats did not retain the elevated evoked response as well as the MA rats. Further, it appears that the SN rats' granule cells may be more depolarized than the MA rats' granule cells, and that the SN rats may also have fewer but more powerful synapses. The latter conclusions, however, must be regarded as tentative.
Before a discussion of spontaneous alternation trials, the ancillary electrophysiological measures will be presented, which will aid in the interpretation of a number of the foregoing sections.
SECTION VII: DOUBLE PULSE INHIBITION AND FACILITATION

When the perforant path is stimulated twice in rapid succession, two quite different processes can be observed which depend upon the nature of the response first elicited, and upon the interpulse interval (IPI). If the first pulse elicits a response which is above threshold for granule cell discharge, the population spike on the second pulse is reduced compared with the first over a variable range of intervals (Lomo, 1971b; Steward, et al., 1976). This is a well established process thought to be due to recurrent inhibition. At longer interpulse intervals, however, there is an excitatory effect (Lomo, 1971b; Steward, et al., 1976). That is, the second population spike is larger than the first. This experiment was designed to determine whether the effect of double pulse stimulation at variable IPI's differed between the MA and SN rats.

Procedure

The intensity of perforant path stimulation was kept constant for all rats (1 mA for 50 µsec), and was above threshold for a population spike in all animals. Each rat was tested individually with 89 double stimuli which were delivered in ascending steps of 1 msec, beginning at 7 msec and ending at 95 msec. The intervals between pairs was 20 seconds. Examples of the double responses from one MA and one SN rat are shown in Figure 26. The individual testing session was of such a length that the time of day variable had to be eliminated in this portion of the experiment. Measurements of the
Figure 26. Examples of double responses from one MA ("1") and one SN ("2") rat. The intervals (in msec) between the double stimuli are shown down the center. (Calibration: 4 mV, 8 msec)
EPSP slope and population spike area were made as described in Section IV.

Results and Discussion

Averages of the fractional change of the EPSP slope and population spike area \((\text{Pulse 2} - \text{Pulse 1})/(\text{Pulse 1})\) were calculated as a function of interpulse interval for both age groups. These data can most conveniently be divided into the effects of the interpulse interval on the population spike and the effects of the IPI on the EPSP.

The population spike. The mean fractional change in the population spike for the SN animals \((n = 15)\) is nearly identical with that of the MA animals \((n = 17)\) (see Figure 27). In both age groups, when the first pulse of a double pulse series elicited granule cell discharge, the second population spike was inhibited provided that the IPI was between 7 to 45 msec (the lowest fractional change for the SN rats = -.34, and for the MA rats = -.36). As the IPI's were extended further, the fractional change of the population spike became positive and remained elevated (between 15-30% above the first pulse) to the longest interval tested here (95 msec).

The area of the population spike on the first pulse was quite stable in both MA and SN age groups, so that the changes seen in the fractional difference data were not due to the changing size of the first population spike.

These results are basically similar to those described for urethane-chloralose anesthetized preparations (by Lomo, 1971b
Figure 27. The mean fractional change in the EPSP slope and population spike area for both age groups, over a range of interpulse intervals from 7 to 95 msec (in steps of 1 msec). The mean is shown as a solid line, and the 95% confidence limits as broken lines. Note that values below the zero line represent cases where the amplitude of the second pulse is smaller than the first, and above the line cases where the amplitude of the second pulse is larger.
and Steward, et al., 1976) although the time course of inhibition is somewhat extended in the chronic animals used here (about 45 msec compared with about 25 msec). This may be due to a combination of procedural differences: the stimulation intensity used, the absence of anesthetic, differences in the animals used (Lomo worked with rabbits), or less likely, but possible, strain differences (Steward, et al., used Wistar rats).

Since other laboratories (both Lomo and Steward) use spike height to describe the amplitude of granule cell discharge during double pulse stimulation, rather than area (as was used here), it was of interest to determine whether the area and height measurements gave consistent results. When these measures were compared, the correlation between the spike area and spike height in the MA rats was $r^2 = .979$, and in the SN rats was $r^2 = .992$. Thus it appears that spike area is a comparable measure with the spike height, which rules out the possibility that differences between laboratories might be due to different kinds of measurements of the population spike.

Although the population spike was larger at longer IPI's measured by area or by height, the latency of the spike peak of the second pulse was longer than the first. The fractional difference remained above zero (except in the very initial IPI's where the latency measures are 0 because the spike is often completely inhibited). The fractional change was consistently between .15 and .30 with IPI's from 15-95 msec. This indicates that the spike latency was greater
Both while the area and height were inhibited, and while the area and height were facilitated. This was not anticipated, because when the population spike is increased by applying a greater intensity of stimulation or by increasing the response through post-tetanic potentiation, the latency becomes shorter. This result has not been reported before, (perhaps because it was not measured) and raises problems for current interpretations of spike facilitation. This will be discussed after the effects on the EPSP have been described.

The EPSP. The mean fractional change of the EPSP slope versus IPI was very similar in the SN and MA rats (see Figure 27). At an interval of 7 msec, the EPSP was drastically reduced. As the interval was increased to about 20 msec, the EPSP returned sharply to a level which continued to be below the amplitude of the first pulse, where it remained without appreciable change out to 95 msec.

The nature of the effects due to the first pulse is obviously complex. The pronounced suppression of the EPSP during the first 20 msec (lowest mean fractional change for the SN rats = −.50, and for MA rats = −.60) may be due simply to the fact that the granule cells have not repolarized, so that the response recorded on pulse 2 would appear depressed. The rational for this is as follows. If the cell is depolarized, the difference between the resting potential and the reversal potential is smaller, making the recorded extracellular potential smaller. Further, it is well known that EPSP's decay exponentially, which may account for the exponential-like character of the early portion of the curve. Granule cell
depolarization, however, cannot account for the population spike blockage during this initial period. If the cell is actually depolarized, it should be easier to fire an action potential, unless the action potential mechanism was refractory, or if there was recurrent inhibition present. The latter is a favored interpretation, since population spikes can be elicited in rapid succession in the hippocampal slice preparation where basket cell inhibition is known to be disrupted (Schwartzkroin, 1975). The sustained depression of the EPSP on the second pulse is more difficult to interpret, particularly since one might expect EPSP facilitation when the population spike area is facilitated (although the fact that the spike latency is increased is congruent with a decline in the EPSP).

These apparently paradoxical results cannot be explained by currently accepted neurophysiological concepts of facilitation.

This study did not include a series of double pulses delivered at intensities that are below threshold for a spike. In urethane-chloralose anesthetized preparations (Lomo, Steward) there is clear evidence that the EPSP is facilitated, maintaining this elevated second response for at least 100 msec. This kind of facilitation may well be due to an increased probability of release of transmitter on the second pulse, as in the well-documented case of facilitation in the neuromuscular junction (del Castillo & Katz, 1954; Mallart & Martin, 1967; Magleby, 1973). However, in the case where the evoked response includes a population spike, the same explanation does not fit the data obtained from the chronic animals
in this study. Since this finding is not directly relevant to the primary question of this thesis, a more thorough discussion of these data along with a description of some pilot work on this topic will be presented in Appendix H.

Summary

The major finding of importance to this thesis from the foregoing data is that the extent and time course of double pulse inhibition and facilitation (when above threshold for a population spike) were the same in the SN and MA rats. Although the underlying processes of these effects are not as yet fully understood, the probability is quite high that none of the processes involved differ between age groups, since the result of their combined effects are the same.

The findings which are more important to the field of hippocampal neurophysiology include the fact that, in awake rats, the second pulse EPSP is suppressed and the spike latency is increased when the first pulse is above threshold for a population spike. Furthermore facilitation of EPSP's, where there was no population spike present, was not observed in awake animals (see Appendix H).
SECTION VIII: ANESTHETIC DOUBLE PULSE SERIES

In the hippocampus (Nicol, Eccles, Oshima & Rubia, 1975), and undoubtedly throughout the central nervous system (Bowery & Dray, 1976), pentobarbital produces a general depression of neuronal excitability. Intracellularly recorded IPSP's are prolonged, which presumably leads to more effective summation of repetitive inhibitory activity. It was of interest to determine how a barbiturate anesthetic would influence the evoked granule cell response elicited by single and double perforant path stimulation.

The two most current theories concerning pentobarbital action suggest that 1) it prolongs synaptic inhibition by delaying the removal of GABA from the synaptic cleft (Nicol, et al., 1975); 2) or that it has a direct action on GABA (or inhibitory amino acid) receptors (Bowery & Dray, 1976). The most recent paper tends to favor the latter interpretation, but is by no means conclusive. From the above, it could be predicted that the general amplitude of the evoked granule cell response should decline, and that double pulse inhibition might become more effective since the inhibitory inter-neurons would presumably be more effective. Of more direct importance to this thesis, was to determine whether Nembutal had a differential effect between age groups. Furthermore, knowing the nature of the change in evoked response produced by Nembutal might allow a more accurate interpretation of the results from acute preparations in relation to the awake state.
Procedure

The stimulation intensity was kept constant for all animals (mA for 50 µsec), and was above threshold for a population spike in all cases. One hundred and thirty-five pulse pairs were delivered at a fixed IPI of 15 msec, and at a rate of one pair per 20 seconds. At this IPI, the amplitude of the EPSP and population spike on the second pulse was always smaller than the amplitude on the first pulse. As was discussed in Section VII at this interval the second population spike is thought to be decreased by recurrent inhibition produced by inhibitory interneurons (basket cells) in the vicinity of the granule cell bodies. The decrease in the amplitude of the EPSP on the second pulse at this IPI is probably due to a combination of processes, (see Appendix G). The double evoked responses were collected continuously for 15 minutes, at which time the rat was picked up and injected (without discontinuing sampling), and responses were collected for 30 minutes further. The results can be broken into the following sections: 1) the amplitude of the EPSP and population spike of Pulse 1; 2) the amplitude of the EPSP and population spike of Pulse 2; and 3) the fractional change between the first and second pulse over time.

Results and Discussion

Examples of double evoked responses before and after Nembutal injection are shown in Figure 28.

Pulse 1. The mean slope of the EPSP versus time (0 to 45 minutes) is shown for the SN rats (n = 11) and MA rats (n = 13) in
Figure 28. Examples of double evoked responses (IPI = 15 msec) before and after Nembutal injection in one SN ("1") and one MA ("2") rat. The time points shown are: before injection 14 minutes ("a"), 7.5 minutes ("b"), and 1 minute ("c"); and after injection 1 minute ("d"), 15 minutes ("e") and 30 minutes ("f"). Calibration: 4 mV, 4 msec
Figure 29.1. After the injection both groups rise and then begin to decline. Regression lines were fit to the last 30 minutes of the data (the time points after the injection) for each group, and the means were then calculated. The mean slope (parameter b) of the regression lines were statistically different between age groups (see Table 23), whereas the intercepts (parameter a) did not differ. This indicates that the synaptic response of the SN rats declines more sharply after Nembutal administration than did the evoked response of the MA rats. The initial increasing phase after Nembutal injection, however, was not different between age groups (there was no difference in the intercept).

Although there is a clear age effect in these data, with the SN rats showing a much steeper decline in the amplitude of the evoked response, it was of interest to obtain exponential fits to these data using the exponential program discussed in Section III (Provencher, 1976). The questions of interest here included the number of exponential components in these curves, and an estimate of the value of the constant to which the exponential was falling. This latter question is of particular importance to the interpretation of the evoked responses collected at surgery (Section II) from these animals, since the peak response obtained from each rat during surgery was measured while the animals were deeply anesthetized with Nembutal. If the depth of anesthetic (given by the constant) could be predicted reliably for each group, the surgery potentials could be corrected for the differential influence of Nembutal between age groups.
Table 23

Pulse 1 Regression Parameters and t Values of the EPSP Slope and Spike Latency after Injection of Anesthetic

<table>
<thead>
<tr>
<th></th>
<th>Slope (b) (arbitrary units)</th>
<th>Intercept (a) (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SN</td>
<td>MA</td>
</tr>
<tr>
<td>EPSP</td>
<td>X</td>
<td>-13.11</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>2.19</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>-2.91*</td>
</tr>
<tr>
<td>Spike</td>
<td>X</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>.013</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>2.10*</td>
</tr>
</tbody>
</table>

*P < .05

The mean raw data from 0-14 minutes (pre-anesthetic) and 15-45 minutes (post-anesthetic) were analyzed with the exponential decay program for each age group. In both the MA and SN age groups, one component was fit to the 0-14 minute time period, with an extremely low variance measure and no difference between age groups. This indicated that the baseline tests had reached a constant level before injection, so that differences in the effect of the injection cannot be accounted for by differences in the state of the baseline responses. Two components were fit to the 15-45 minute time period in each age group. These two components (one a positive going exponential and the other a negative going exponential) appear to separate an excitatory and inhibitory phase after injection. These exponentials plus the fit from 0-14 minutes were derived from the mean data and are super-imposed on the mean raw data in Figure 29.2
Figure 29. 1) The mean slopes of the EPSP of the first pulse versus time are shown for both age groups. The time of injection is indicated by the arrow. 2) Mean EPSP data from SN rats along with the fit obtained from the exponential program. 3) Mean EPSP data from MA rats with exponential fit.
(SN) and 29.3 (MA). Unfortunately, the estimate of the final baseline constant was extremely noisy and did not produce reliable fits. This may in part be due to the fact that the program was attempting to decay to a flat baseline, rather than to consider the possible influence of an additional recovery function. Thirty minutes more recording may have been able to improve the chances to statistically resolve the projected baseline.

The area, height and latency of the population spike for the first pulse was similar to the data collected for the EPSP. The spike latency data (measured from EPSP onset to the peak of the population spike) was the least variable, and was thus used in this analysis. Regression lines were fit to the last 30 minutes for each rat in both age groups. The mean regression parameters are shown in Table 23. Again the slope \(b\) was statistically steeper in the SN than in the MA rats, while the intercept \(a\) was not different, indicating that the SN rats were influenced to a greater extent than the MA rats by this anesthetic.

**Pulse 2.** Regression lines were fit to the individual rat's EPSP slopes and population spike parameters from Pulse 2. Although the mean EPSP slope and population spike amplitude in both age groups declined after administration of the anesthetic (the mean decline was steeper in the SN than in the MA rats) the regression parameters were not statistically different between age groups.

**Fractional change.** The mean fractional change, \((\text{Pulse 2 - Pulse 1})/\text{(Pulse 1)}\), of the EPSP and population spike versus time was
calculated for each age group, and are seen in Figure 30. A decline in the fractional difference represents more effective inhibition, whereas an increase represents less effective inhibition. Therefore, the inhibition of the population spike is seen to become more effective in both age groups after the anesthetic was injected. However, the inhibition of the EPSP appears to increase at first, after which the strength of inhibition declines. This decrease in inhibition may be due to the size of the first pulse decreasing, the cells being more hyperpolarized, or a combination of these factors.

**Summary.** The decline in the amplitude of the EPSP and the increase in the latency of the population spike on Pulse 1 supports the prediction of a general decline in the amplitude of the evoked granule cell response after Nembutal anesthesia. Further, this decline was shown to be more pronounced in the SN than in the MA rats. Since Nembutal had a differential effect on the evoked response recorded between age groups, this indicates that the size of the response measured during surgery (see Section II) was biased. Thus, even though the SN rats' peak responses were not larger than the MA rats' responses, (as was predicted if their synapses were more powerful) this may have been partly due to a relatively larger decline in the amplitude of the evoked response after Nembutal administration. Although there was assuredly a bias, the exact amount of differential influence was not possible to determine from these data (using Provencher's exponential fit program).

The fractional change data from the population spike
Figure 30. The mean fractional change, \((\text{Pulse 2} - \text{Pulse 1})/\text{(Pulse 1)}\), of the EPSP slope and population spike area before and after Nembutal injection. The injection is indicated by the arrow. Since the second pulse is inhibited at this time (15 msec), the fractional change is negative; thus, a decline represents more effective inhibition and an increase less effective inhibition.
indicates that inhibition of the spike becomes more effective after Nembutal injection. The change in the effectiveness of the spike inhibition appears similar in both age groups. This recurrent inhibition becomes more effective in spite of the fact that the first population spike has diminished. The effect of Nembutal on the EPSP appears to be more complex, showing an initially more effective reduction of the second pulse, and then an eventual lessening of this effectiveness.

In general the data from this section indicate that extrapolation of electrophysiological data in pentobarbital anesthetized preparations to the awake state is, at best, tenuous, particularly when comparisons of young and old rats are to be made. It is clear, however, that the EPSP magnitudes during surgery were likely to have been depressed more in the SN than the MA rats.
SECTION IX: BLOOD PRESSURE

In a longitudinal study of non-institutionalized adults, it has recently been established that old individuals who are hypertensive perform much more poorly as measured by the Wechsler Memory Scale, than old individuals who are not hypertensive (Wilkie, Eisdorfer & Nowlin, 1976). Although there were no differences between the hypertensive and normotensive or mild blood pressure elevation groups at the outset of the study, the hypertensive group became statistically inferior on the memory tests during the 6.5-year follow-up studies. It appears that hypertensive people may not be impaired because of memory failure per se, but as they grow older they become impaired on a wide variety of performance factors, that may in part be related to high levels of anxiety interacting with the test situation.

It was thus considered important to determine whether there were differences in blood pressure between the two groups of rats in this study. If there was a difference, then the results of the behavioral sections could be confounded. Of peripheral interest in this regard, was the response of the blood pressure in these two population of rats to adrenalin. It has been demonstrated that learning in older persons can be improved by administering drugs which decrease autonomic arousal (e.g., propranolol) (Eisdorfer, et al., 1970). Eisdorfer suggests that old individuals may have an increased autonomic end-organ sensitivity, causing them to be on the
over-aroused poor-performance end of the autonomic arousal-performance curve. If this were true for rats (i.e., if the sensitivity were increased), one might expect a larger elevation in blood pressure after adrenalin injection in the SN than in the MA group.

Procedure

The descending aorta was cannulated allowing the blood to be led through a tube against a column of fluid. The fluid in the column consisted of 7 cc of heparin (700 IU) in 150 ml of saline, plus .1 gram neutral red (a vital stain which was added to color the solution). The needle which was inserted into the aorta was connected to tubing (which had an inner diameter of .085") and was mounted vertically on the wall, reaching a height of 3.05 meters. Adjacent to the tubing was a calibrated piece of tape, from which measurements of the height of the fluid in the column could be taken.

The precise procedure was as follows:
1) After the anesthetic double pulse testing was completed, the rat was removed from the recording room, was photographed, and then was taken into the room where the blood pressure apparatus was set up.
2) The body cavity was opened and the descending aorta was dissected away from the connective tissue.
3) The fluid in the column was set at 90 cm, and the column was clamped:
4) Heparin (.05 cc) was injected intrarenally,
5) The aorta was clamped and the needle inserted upstream from the clamp, after which the aorta and fluid column were unclamped.
6) Measurements were collected at an interval of 1 per 30 seconds, for a period of five minutes.

7) After the reading taken at five minutes, .2 cc adrenalin chloride (a $2 \times 10^{-5}$ gram dose in a physiological salt solution) was injected into the renal vein.

8) Measurements were taken every 30 seconds for five minutes, after which the rat was perfused.

Results and Discussion

The mean arterial blood pressure results are seen in Figure 51 (the time of injection of adrenalin is indicated by the arrow). The scores were converted from cm of water solution to mm Hg with the formula: $(((n H_2O) \cdot \text{density H}_2O) = ((m Hg) \cdot \text{density Hg})$. The MA ($n = 17$) and the SN ($n = 11$) rats did not differ statistically at any of the time points measured ($p > .05$).

The critical feature of these results is that the MA and SN rats do not differ statistically over the first five minutes of blood pressure measurements. Thus, even if the SN rats did tend to show a higher level of blood pressure, it is unlikely that it is sufficiently higher to put them into a different category (hypertensive). Normal blood pressure levels measured from the carotid artery in rats is around 100 mm Hg (Nobel, 1973). The lower levels measured here (70-80 mm Hg) may be due to a combination of procedural differences: a lower pressure is measured from the aorta than from the carotid (which may account for most of the difference); the preparation used here may cause more bleeding, which is known to
Figure 31. Mean and standard errors of arterial blood pressure measured from both age groups. Injection with adrenalin is indicated with the arrow.
lower measured blood pressure (Nishino, Kiyomi & Brooks, 1976); and generally, the technology used here was less sophisticated than that used by Noble.

The maximal responses to adrenalin were around 120 mm Hg for this concentration (10^{-5} g/ml). This is very close to the responses Noble measured to a weaker concentration of adrenalin (10^{-7}), where the blood pressure rose to approximately 115 mm Hg. Thus, it is likely that the dose given here was producing a response close to the limit of adrenalin's ability to raise blood pressure. Clearly, the SN rats were not more "sensitive" to adrenalin in that their blood pressure was not elevated more quickly than the blood pressure of the MA rats. An increase in autonomic end organ sensitivity in old organisms (Eisdorfer, 1975) is thus not consistent with these results.
SECTION X: SPONTANEOUS ALTERNATION

The chronic implantation of electrodes in the angular bundle and fascia dentata (see Section II) could conceivably result in significant damage both to the granule cells and to the perforant path fibers. It is known that unilateral lesions of the entorhinal cortex (the source of the perforant path fibers) result in expansion of the terminal field of fibers from the contralateral hippocampus to fill the vacated synaptic sites (Steward, Cotman & Lynch, 1973, 1976). This reinnervation is accompanied by recovery of a variety of behavioral indices (Loesche & Steward, 1977; Smith, Steward, Cotman & Lynch, 1973; Steward, Loesche & Horton, 1977). If the SN and MA rats' ability to recover from the trauma due to electrode implantation differed, this would tend to confound the interpretation of subsequent electrophysiological data.

When an animal is placed into a two-choice situation, the probability is high that its second choice will differ from its first choice (Dember & Fowler, 1958; Dennis, 1939; Montgomery, 1952). This behavior is termed spontaneous alternation. Since normal behavior on a spontaneous alternation task is thought to be a good index of the completion of synaptic rearrangement after brain damage (Smith, et al., 1973), this task was included at selected intervals before and after surgery. Spontaneous alternation is also thought to be sensitive to the general working of the hippocampus (Kirkby, et al, 1967; Roberts, et al., 1962). For this reason, alternation trials were included after HIF stimulation to
determine whether altering the properties of the granule cell synapses influenced this behavior.

Apparatus and Procedure

The apparatus used was a simple T maze with a wood floor and sides and a plexiglass top. The start area and each arm of the T maze had a slot in which a guillotine door could be inserted (see Figure 32). The maze was placed on top of a table in a small room which was dimly and uniformly illuminated.

The spontaneous alternation trials were given seven times during the study (each trial is seen as A in Table 2). Alternation was measured before surgery, and twice (at eight and 16 days) after surgery. Four additional trials were given after HF stimulation. There were at least eight days between any two alternation trials.

All rats were tested during the two hours designated by their group numbers. Since all testing had to be complete within these two hours, the maximum time allotted for any choice was seven minutes. The rats were placed into the start box with the door down for 10 seconds. After this time, the door was removed and the animal was allowed to move freely until its body was completely within one arm of the maze. The appropriate door was then lowered, and the rat was confined in that arm for 10 seconds. The same procedure was repeated for the second test, after which the rat was returned to its home cage. If the rat did not make a choice before the seven-minute time limit, it was removed from the maze and given another test (if it was the first run) or placed in its home cage (if it was
Figure 32. Diagram of the T-maze used for the spontaneous alternation trials.
the second run).

In summary, each alternation trial consisted of two tests. The rat "alternated" if its second test response choice was different from the first. The percent alternation was calculated as follows: (number of alternation responses/number of rats in group) X (100).

Results and Discussion

Failure to enter either arm became more frequent as more alternation tests were given. Table 24 shows that the frequency of "no-choice" responses increased from Trial 1 to Trial 7 for both age groups. The behavior of the rats on the no-choice response trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>SN</th>
<th>MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>

typically consisted of a series of half body movements or head entries into one or the other arm of the maze. Since the no-choice response is an entirely different category of response, and since rats in both age groups sporadically showed this behavior, eliminating these trials from the data was considered appropriate. Thus, all the data reported below only include active alternating or perseverating res-
penses. It is interesting to note that the increase in no-choice responding rose sharply after HF stimulation (Trial 4) in both groups.

Alternation behavior after surgery. Both MA and SN rats maintained a high level of alternation (above 70%) on the first three trials. It was thus concluded that the surgical treatment did not cause sufficient trauma to produce a marked change in this behavior after eight or 16 days of recovery, nor is there any indication that surgery differentially effected the MA and SN animals. The data from Trials 1, 2 and 3 were thus pooled together and are seen in Figure 33.1 as the "before HF stimulation" measure.

Alternation behavior before and after high-frequency stimulation. Since there were no apparent response biases in either the MA or SN population, the expected random frequency of alternation is 50%. Alternation levels therefore exceeded chance for both the MA and the SN age groups on Trials 1-3 ($\chi^2 = 16.06$ and 9.10 respectively, $p < .05$). Trials 4-7 were also pooled and are seen in Figure 33.1 as the "after HF stimulation" measures. For these trials, the MA group exceeded chance alternation, but the SN group did not ($\chi^2 = 10.40, p < .05$ for the MA; $\chi^2 = 1.14, p > .05$ for the SN).

Assuming that the HF stimulation of the angular bundle was the variable which produced the difference between the MA and SN rats seen in Figure 33.1, it can be seen that this treatment had a greater effect on the SN rats than on the MA rats. If it is also assumed that the only significant result of the HF stimulation is the modification of the perforant synapses, then these results can be
interpreted as suggesting that the deficit on the alternation task is related to the degree of saturation of the perforant path synapses. Since there was not a group which was given alternation tests without being stimulated or a group which was stimulated in another area, necessary control groups for a clear interpretation were not included. However, from an examination of the curves from Trial 1 to 7, the data suggests that it was an abrupt effect after HF stimulation and not merely a progressive decline which might have suggested an effect of repeated trials. The data from the no-choice responses is also in general agreement with this conclusion.

**Time of day.** The mean percentage alternation data from early and late times of day for the MA and SN rats are shown in Figure 33.2 and 33.3 respectively. In the MA rats there is a tendency for the late groups to have higher percentage alternation scores than the early groups. However, there does not appear to be a consistent time of day effect on the alternation behavior of the SN rats. This points to the possibility that circadian cycles in the SN rats are altered in some way.

**Summary.** There is no apparent effect of surgery in either the MA or SN animals as measured on this task. An age difference is apparent in the change in alternation behavior after HF stimulation. Further, the time of day at which the MA rats were tested influenced the extent to which they alternated their responses. A much less pronounced time of day effect was noted in the SN animals.
Figure 33. 1) Mean percent alternation before and after HF stimulation in both age groups. 2) Mean percent alternation broken into early (12:00-22:00) and late (22:00-04:00) times of day for the MA rats. 3) Mean percent alternation broken into early and late times of day for the SN rats.
Although there are a number of possible interpretations of these data, the preferred interpretation will be presented below, since it is consistent with earlier conclusions concerning the SN rats. The age difference on this task is most likely an effect of HF stimulation rather than repeated trials. Even though the SN rats did not retain the elevated evoked response as well as the MA rats, they were relatively more influenced by HF stimulation, perhaps because their synapses were more modified and this treatment therefore more disruptive. This indicates that saturation of synaptic efficacy may have a significant influence on general exploratory behavior.
SECTION XI: CORRELATIONS BETWEEN PHYSIOLOGICAL AND BEHAVIORAL MEASURES

Since the statistical treatment of the data has not provided comparisons of the relationships between treatments, product-moment correlation coefficients and tests for their significance (Freund, 1971) were determined for selected treatments. Of primary interest, was the relationship between the individual rat's behavior on the circular maze and the fractional change of its evoked response after HF stimulation. If a significant correlation existed, it would be evidence in favor of the notion that retention of spatial information was related to retention of the elevated hippocampal synaptic response. It was also considered important to determine whether the increases found in the low-frequency stimulation sessions (P1, P2) were related to the fractional increases after HF stimulation or to the performance on the spatial discrimination task since these increases may represent the normal manner in which spatial information is acquired. These comparisons will be presented below.

Although it is admittedly a rather selective decision as to which behavioral trials should be compared with which HF stimulation trials, the following choices were adopted. Only the two-minute and the 24-hour time points after HF stimulation were used since the behavioral trials were measured at two-minute and 24-hour intervals. Table 25 shows which HF stimulation trials were compared to which maze trials, and which HF stimulation and maze trials were compared to the first low-frequency stimulation test (P1). Since the number of errors
and the distance measurement were both motor independent and may have been sensitive to slightly different factors, they were both included in the correlation measures.

Table 25

Correlation Comparisons

<table>
<thead>
<tr>
<th>LF Stim</th>
<th>HF Stim</th>
<th>Circular Spatial Maze</th>
<th>LF Stim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tet 1, 2 min</td>
<td>Trial 1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>24 hr</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Tet 2, 2 min</td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>24 hr</td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Tet 3, 2 min</td>
<td></td>
<td>6, 10</td>
<td>P1</td>
</tr>
<tr>
<td>24 hr</td>
<td></td>
<td>7, 11</td>
<td>P1</td>
</tr>
<tr>
<td>P1</td>
<td>Tet 4, 2 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. The arrows indicate which measurements were compared.

There were no statistically significant correlations between the behavior and HF stimulation trials in either group until Trial 6 (the third 24-hour trial on the maze) and the T3 24-hour HF trial. Interestingly, this occurred in both age groups. The correlations which correspond to Trials 6, 7, 10 and 11 are thus the only trials considered below. The results are seen in Table 26 with the variables shown along the top representing those that were correlated with the third HF stimulation at 24 hours, the fourth HF stimulation at two minutes, and low frequency stimulation (P1).

Although the bulk of the variance resulted from other factors, a significant amount of variance in the behavior is predictable from the variance in response to HF stimulation. Since there was a time of day effect in the physiology and not in the circular maze
task, this factor may be assumed to be making a large contribution to the residual variance. The fact that a significant correlation exists between the behavior and the electrophysiology is evidence in favor of the notion that learning ability is related to synaptic plasticity. Furthermore, since the synaptic plasticity after HF stimulation is correlated with the increases observed when the rats were put into the new testing situation (P1), these phenomena may share a common mechanism. Hippocampal synaptic plasticity thus appears to be related to the retention of spatial information; the nature of the relationship depending on the age of the organism.
Table 26

Correlation Coefficients \( (r) \) and Tests for Statistical Significance \( (t) \) of the Relationship Between the Behavioral and Electrophysiological Data

<table>
<thead>
<tr>
<th>Trial 6</th>
<th></th>
<th></th>
<th>Trial 10</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Errors</td>
<td>Distance</td>
<td>Errors</td>
<td>Distance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tet 3, 24 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MA  ( r )</td>
<td>-0.43</td>
<td>-0.18</td>
<td>-0.46</td>
<td>-0.11</td>
<td></td>
</tr>
<tr>
<td>( t )</td>
<td>2.225*</td>
<td>0.659</td>
<td>2.502*</td>
<td>0.428</td>
<td></td>
</tr>
<tr>
<td>SN  ( r )</td>
<td>-0.48</td>
<td>-0.63</td>
<td>-0.13</td>
<td>-0.43</td>
<td></td>
</tr>
<tr>
<td>( t )</td>
<td>2.645*</td>
<td>3.353*</td>
<td>0.557</td>
<td>2.098*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trial 7</th>
<th></th>
<th></th>
<th>Trial 11</th>
<th></th>
<th></th>
<th>P1</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Errors</td>
<td>Distance</td>
<td>Errors</td>
<td>Distance</td>
<td>Errors</td>
<td>Distance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tet 4, 2 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MA  ( r )</td>
<td>-0.16</td>
<td>-0.48</td>
<td>-0.56</td>
<td>-0.53</td>
<td>-0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( t )</td>
<td>0.689</td>
<td>2.119*</td>
<td>3.089*</td>
<td>2.419*</td>
<td>2.209*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SN  ( r )</td>
<td>-0.35</td>
<td>-0.64</td>
<td>-0.43</td>
<td>-0.08</td>
<td>-0.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( t )</td>
<td>1.583</td>
<td>3.535*</td>
<td>2.252</td>
<td>0.362</td>
<td>2.576*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trial 6</th>
<th></th>
<th></th>
<th>Trial 7</th>
<th></th>
<th></th>
<th>Trial 10</th>
<th></th>
<th></th>
<th>Trial 11</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Error</td>
<td>Dist</td>
<td>Error</td>
<td>Dist</td>
<td>Error</td>
<td>Dist</td>
<td>Error</td>
<td>Dist</td>
<td>Error</td>
<td>Dist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MA  ( r )</td>
<td>-0.30</td>
<td>-0.17</td>
<td>-0.53</td>
<td>-0.16</td>
<td>-0.11</td>
<td>-0.54</td>
<td>-0.47</td>
<td>-0.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( t )</td>
<td>1.406</td>
<td>0.669</td>
<td>2.793*</td>
<td>0.629</td>
<td>0.494</td>
<td>2.482*</td>
<td>2.380*</td>
<td>1.893</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SN  ( r )</td>
<td>-0.21</td>
<td>-0.11</td>
<td>-0.19</td>
<td>-0.52</td>
<td>-0.61</td>
<td>-0.59</td>
<td>-0.49</td>
<td>-0.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( t )</td>
<td>1.053</td>
<td>0.456</td>
<td>0.887</td>
<td>2.583*</td>
<td>3.528</td>
<td>3.268*</td>
<td>2.576*</td>
<td>1.360</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* = \( p < .05 \)
SECTION XII: HISTOLOGY

Six SN rats and six MA rats were perfused transcardially with a paraformaldehyde-glutaraldehyde solution (Palay & Chan-Palay, 1974, p. 327). The brains were removed and stored in this fixative for one day. The hemisphere opposite that of electrode implantation was prepared for electron microscopy for future study. The hemisphere with the electrode tracks was stored in a 10% formal-saline solution.

The remainder of the rats were perfused transcardially with 10% formal-saline. Soft tissue was removed from the skull, and the skull with the electrode assembly intact was stored in this solution. After the brains were removed from the skulls, the hemispheres with the electrode tracks were blocked approximately between the level of the anterior commissure and the anterior cerebellum (see the representative sections from these blocks in Figure 34). These pieces of tissue were stored in 30% sucrose formalin for one week, and subsequently embedded in egg yolk using a modified Snodgress-Dorsey procedure (Ebbeson, 1970, p. 157). Serial sagittal sections were cut at 30 µm from the medial to lateral aspect. The sections which contained the electrode tracks were mounted on slides for reconstruction of the electrode tip positions. The tips were localized by a technician who did not know which group was represented by which sections.

The tips of the recording electrodes from the SN and MA rats are shown on a representative sagittal section in Figure 34.
Figure 34. Recording and stimulating electrode placements for the SN and MA rats are shown as dark circles: "1" shows the recording electrode positions for the SN rats; "2" shows the stimulating electrode positions for the SN rats; "3" shows the recording electrode positions for the MA rats; "4" shows the stimulating electrode positions for the MA rats.

Magnification factor $X$ 7.5.
(which was traced from this tissue). The tip distribution was quite consistent between age groups (see Table 27). The electrode tips were traced on graph paper and the means and 95% confidence limits of the $\bar{x}$ and $\bar{y}$ coordinates were calculated. These did not differ significantly.

Table 27

<table>
<thead>
<tr>
<th></th>
<th>SN (# of tips)</th>
<th>MA (# of tips)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular layer</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Hilus</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Fornix</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>CA 1-3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

The stimulating electrode positions were localized using the point midway between the two tips of the electrode. The distribution of these electrode tips are shown in a representative sagital section in Figure 34. In both age groups the majority of stimulating electrodes were located in the angular bundle; the positions did not differ significantly.

Rats whose recording placements were classified as CA 1-3 and thalamic in Table 27 were not used in the electrophysiological sections since their potentials were considered uninterpretable. This decision was made during the first electrophysiological recording sessions, and was not based on knowledge of the actual electrode position from histology. However, the animals with placements in the fornix were used. Interestingly they strongly resembled positive
going perforant path - granule cell potentials. Furthermore, the locations of these electrodes were such that one would expect them to be within the field of the perforant path synaptic response, since the mossy fibers pass through this region. These fibers constitute part of the extracellular current source since they are the axons of the granule cells.

In conclusion, the differences between age groups in the electrophysiological data are not likely to be due to differences in the placements of either the stimulating or recording electrodes.
DISCUSSION

The basic contribution of this thesis to aging research and neuropsychology is as follows. Between very old and middle-aged laboratory rats, a difference was demonstrated in spatial memory. This difference was correlated with a difference in the retention of an elevated synaptic response produced through repeated high-frequency (HF) stimulation of a major afferent to an area of the brain thought to be involved in processing spatial information. In addition, within age groups, individual performance on the maze was significantly correlated with the amount of elevation-in synaptic response. These central findings, along with other supportive evidence, will be discussed below.

General Neurophysiological Age Differences and Similarities

The method of extracellular recording at the perforant path–granule cell synapse is potentially a powerful tool for examining the physiology of this cortical cell type. While this method has its drawbacks, it has many advantages over intracellular recording, most notable of which are the stability of the preparation and the fact that the response is essentially an average from many similar if not identical cells. Specifying the mechanisms involved in producing changes in the extracellular synaptic response is problematic. Recognizing the limitations of the interpretations possible, the nature of the differences observed between the senescent normal (SN) and middle-aged (MA) rat granule cell responses collected in this thesis may be suggested, giving indications for the direction of future research.
The old rats' granule cells may have been more depolarized than the young rats' granule cells. Evidence was found both in the stimulus-response relationship, where the amplitude of the synaptic responses to equivalent stimulus intensities was lower in the SN than in the MA rats; and in the low intensity tests where the threshold for granule cell discharge was reached at a lower value of extracellular EPSP in the SN rats. The amount of recorded extracellular current flow is directly proportional to the difference between resting and reversal potentials. In excitatory synapses, the reversal potential is closer to zero than the resting potential. If the cells are more depolarized, the difference between the resting and reversal potentials will be less; thus a smaller extracellular response would be recorded, as described above for the SN rats. The alternate interpretation is that the synapses are weaker in the SN rats, but the population spike threshold is lower. If fewer active synapses fire the granule cells, however, then this is logically equivalent to the SN rats having more "powerful" synapses. To clarify the interpretation, a good model for extracellular recording in the hippocampus, or intracellular recording techniques would be necessary. The latter, of course, would be the most definitive solution, and suggests an interesting application of hippocampal slice methodology.

The evidence, however, favors the SN rats having more effective synapses. At the time of electrode implantation, the peaked positive evoked response was obtained from each rat. There were no statistically significant differences in the amplitude between age groups. If
the possible reduction in the number of synapses and the more profound effect of the anesthetic (discussed in more detail below) is taken into account, it could be argued that the SN synapses were actually more powerful. Although there were very few rats with measurable pre-synaptic fiber responses, the direction of these results generally supported the above. That is, for a given size of fiber response, a larger ERSP tended to be elicited from the SN rats. It is interesting to speculate that the possible depolarization of the granule cells (discussed above) may be causally related to the presence of more powerful synapses. If it is assumed that there is a significant amount of spontaneous activity in the system, and that on the whole this did not differ between age groups, this could have led to a greater mean depolarization of the SN rats' granule cells.

Another neurophysiological difference between age groups was found in the response of the young and old animals to a barbiturate anesthetic. An equivalent dose of sodium pentobarbital differentially influenced the amplitude of the evoked response of the MA and SN rats in this study. Although a precise model cannot be developed from the data collected in this experiment, it is clear that the decline in the extracellularly recorded synaptic response after pentobarbital injection was much more pronounced in the SN than in the MA rats. The measurements at the time of surgery also suggest a greater effect of pentobarbital on the old rats. The respiration rate of the MA and SN rats did not differ even though the MA rats required more anesthetic to be maintained at a surgical level of anesthesia. Furthermore, with a
lower dose of pentobarbital, the SN rats remained behaviorally
anesthetized longer than the MA rats. Dose response relationships
for many drugs are highly dependent upon the stage of development of
the organism (Triggs & Nation, 1975). When experimental questions
arise which require the use of general anesthesia and comparisons of
young and old animals, it is clear that the interpretation of the
results must take into account age differences in response to
anesthetics.

A recent short communication by Winson and Abzug (1977)
suggests a possible interpretation of the results from surgery and
awake testing presented above. In alert rats, the amplitude of the
perforant path elicited extracellular EPSP was greater, and the popula-
tion spike was smaller than when these rats were sleeping (Winson &
Abzug, 1977). This suggests that in alert animals there is less depo-
larization due to ongoing activity, or alternately, greater tonic
hyperpolarization due to more inhibitory activity. In either case,
the predicted result would be greater extracellular current flow for an
equivalent input volley, but less granule cell discharge (a smaller
population spike). The peak amplitude of the evoked responses collected
from the rats in this thesis during surgery indicated that, after
correction for anesthetic effects and fewer synapses, the SN rats'
evoked responses should be larger than the MA rats' responses. In fact,
during the awake state, they were much smaller. The absence of ongoing
activity (in the anesthetized state) may have removed the tonic depolar-
ization which would have been present in the alert state (as suggested
by the Winson and Abzug study), producing smaller evoked responses. This interpretation favors the notion that the difference in synaptic response in the old animals is due to permanent differences in synaptic efficacy, not simply to differences in behavioral state. It does not, however, rule out the possible existence of state dependent differences between age groups.

There were a number of instances where the SN rats appeared to be less susceptible to change produced through normal environmental input or artificial stimulation. This includes the data from the EEG section where the power in the possible EPSP frequency band increased only a small amount from the first to the second day of testing in the SN rats, compared with large increases in the MA rats' EEG at these frequencies. In addition, the MA rats habituation-like decline over time in the 3-7 Hz EEG was much faster on both days of testing than that of the SN rats. The stimulus-response relationship comparisons after high-frequency stimulation show the same general effect, where the MA rats maintained a greater elevation in the evoked-response than the SN rats. The influence of HF stimulation on spontaneous alternation behavior was also found to be more pronounced in the SN than MA rats. Although the percent alternation was equivalent between age groups before HF stimulation, SN rat performance on this maze changed towards chance alternation after the HF stimulation began. This occurred even though the elevated response was not maintained as well in the SN as in the MA rats, suggesting that the older animals are more vulnerable to smaller alterations in the efficacy of their synapses.
As mentioned briefly above, a striking difference in the maintenance of an elevated synaptic response, produced through repeated high-frequency stimulation, was noted between age groups. This difference in retention of the increased synaptic response was observed even though the initial amount of change and the initial rate of decline was equivalent between age groups. Age differences in the physiology of the hippocampus in anesthetized and in vitro preparations (Landfield, McGaugh & Lynch, 1976) have recently been reported in abstract form. In the Lanfield et al. study, the extracellular synaptic response of hippocampal pyramidal cells was examined in old and young rats. In both the anesthetized and hippocampal slice preparations, frequency potentiation and post-tetanic potentiation was produced by HF stimulation in young rats. Equivalent stimulation produced synaptic depression in the old rats. At the time of writing, a complete report of this study was not available. If the preliminary impressions from the abstract are accepted, however, it is difficult to account for the apparent discrepancy between that study and the data from this thesis: in the HF stimulation section (V), no synaptic depression was observed, and an age difference in long-lasting post-tetanic potentiation was only obtained after repeated HF stimulation.

The acute preparations in the Landfield et al. (1976) study were anesthetized with urethane-chloralose. While there is no evidence that this anesthetic acts differently on different aged rats, it is a possibility. However, the fact that Landfield et al. observed the same results in both anesthetized and slice preparations, tends to argue
against this as a major source of difference. Certainly if they used the procedure of Yamamoto (1972), their animals would not have been anesthetized before being prepared for tissue excision. It is possible that the effects of age on pyramidal cell and granule cell physiology may differ; although it would seem to violate the laws of parsimony to resort to this interpretation.

One plausible explanation for the differences noted between this thesis and the Landfield et al. (1976) study is the stimulation parameters used. The parameters used for the chronic animals in this thesis never produced electrographic seizure activity, whereas it is possible that the parameters used in the Landfield et al. study may have caused seizures. If the older animals had a lower threshold for electrically inducing seizures, synaptic depression in these animals could have been the result. Furthermore, experience in this laboratory suggests that post-tetanic depression is more frequently seen after stimulation with lower stimulus frequencies (below 100 Hz) (McNaughton & Douglas, personal communication), than with the high frequencies (400 Hz) used in this thesis. This depression occurs even when seizures are not triggered, particularly when long stimulus trains are used (i.e., greater than 100 pulses). Bliss and Lomo (1973) and Bliss and Gardner-Medwin (1973) report depression of varying lengths with stimulus trains of 10-15 Hz. If Landfield et al. used such parameters, the older rats may have been more sensitive or more susceptible to the depression phenomenon produced through the lower frequency stimulation. This would be an interesting result in itself, however, it is difficult
to decide which combination of these effects led to the apparent discrepancy. It is likely that the inconsistency between the data from this thesis and the Landfield et al. (1976) study will disappear when procedural comparisons can be examined in detail.

Other physiological measures, besides the amount of initial synaptic increases and decay after HF stimulation, did not distinguish between the age groups. There were no striking differences in mean arterial blood pressure. The lack of difference in this measure indicated that the neurophysiology or behavioral differences cannot be attributed to high blood pressure. At least in the area of behavior, it is known that hypertension is associated with apparent memory failure which may be due to performance factors (Wilkie et al., 1976). Fortunately, this possible confounding influence was not present.

There is also indirect evidence that the SN rats did not suffer from chronic lung disease significantly more than the MA rats. Such pathology in young (4-9 months) and old (approximately 2 years) rats has recently been shown to lower the modal frequency of hippocampal theta to lower bands (closer to 5 Hz) (Cooper, Prinz & Marsh, 1975). The healthy old (n = 1) and young rats (n = 8) in the Cooper et al. study could not be discriminated on the basis of "preferred" modal frequency (Irmis, Lat, & Radil-Weiss, 1975), whereas the EEG from those rats with lung disease (2 old and 2 young rats) was shifted to lower frequencies in the theta band. Since there was no general shift in power of the theta frequencies in the SN rats when compared with the MA rats in this thesis, it is likely that such respiratory disease
(if it existed) was equivalent between age groups.

The time course and extent of double pulse facilitation and inhibition were the same between age groups (using responses above threshold for a population spike). It is interesting that the facilitation of the population spike was not altered in the SN rats whereas the long-lasting process was. Recent evidence indicates that facilitation is not affected by long-term potentiation (McNaughton, 1977) and that these two processes have different underlying mechanisms. Aging therefore appears to selectively affect the long-term mechanism. Since the short-lasting process (facilitation) has a time course analogous to that of iconic memory decay (Sperling, 1960; Haber & Standing, 1969), this suggests that one would not find differences in this type of memory in senescent humans, if this physiological process is the underlying mechanism. This is indeed quite amenable to experimental examination.

Circadian Effects

Several lines of evidence suggest that circadian oscillations in neurophysiological and behavioral states are altered in the SN rats of this study. On the spontaneous alternation task, the MA rats alternated their responses more at the late than at the early times of day, whereas the SN rats showed a markedly reduced tendency to alternate differently at different times of day. An age difference was seen in the EEG data where both groups had more power at late than at early times of day, again being more pronounced in the MA than in the SN rats. The MA rats also showed a steeper decline in the 3-7
Hz EEG at the late times of day, while the SN rats did not show this separation. The amount of fractional change in the EPSP after HF stimulation tended to be larger in the late than in the early groups for the MA rats, whereas the largest changes for the SN rats were distributed in both time periods. These data suggest the possibility that a wide variety of changes in circadian organization may accompany aging. Desynchronization of such cycles could lead to a variety of mental and physical symptoms characteristic of advanced age.

Time of day effects, however, were not observed in all aspects of these experiments: There was no apparent time of day effect in either group on the spatial discrimination task. These findings do not necessarily contradict the hypothesis that the electrophysiological measures (which do show these oscillations) reflect neural processes which underlie spatial information processing. If the performance of the spatial task results in the same type of shift in the electrophysiological state of the hippocampus as occurs during the peak of the electrophysiological circadian cycle, the resultant behavioral measures would not show cyclicity. In other words, novel or stressful environmental input may override normal fluctuations. This hypothesis predicts the abolition of the electrophysiological cycles under certain environmental conditions. It is interesting that behavior on the spontaneous alternation task did show a time-of-day separation, perhaps because of the simpler, less stressful nature of the task (i.e., the rats were in a darkened room, in an enclosed space, rather than on an open maze surface exposed to
bright lights). Since the rate of alternation is thought to be motor independent (Anisman, 1975), differential activity levels between day and night most likely cannot account for the separation.

It is likely that curiosity is a more adaptive behavior at one time of day than at another.

Learning-Memory Alterations with Age

The SN rats did not perform as well on the spatial discrimination task as the MA rats. This deficit was independent of motor factors. On the first few trials the performance measures were essentially equivalent between age groups. On subsequent trials, however, the SN rats made more errors and traveled a greater distance on the maze surface before locating the correct position of the goal. One way in which the age difference on this task may be interpreted is in terms of a retention or memory deficit, since the differences arose only after the initial few trials, where the rats were presumably "learning" the problem.

At the outset of these experiments it was considered inappropriate to take a position on the exact locus of the age deficit in the learning-memory process. Regardless of how the learning-memory process is operationally separated into components, the arguments for actually distinguishing the line where learning is separate from remembering are circular: in order to know whether something is learned it is necessary to test memory, and in order to know whether there is memory something first must be learned. The intuitive contention, perhaps molded by our language, that these processes are
distinguishable by various tests of behavior cannot be logically justified. That is, it is logically impossible to determine from performance measures alone whether a learning-memory deficit is due to the initial strength of the trace, to the durability of the trace, or to interference from other memories.

The interpretation that the age deficit is in retention of spatial information, however, is given some support from neuro-physiological data collected from these rats. If it is assumed that memories are stored through modification of synaptic efficacy as observed at the perforant path - granule cell synapse, then one measure of learning could be defined as the absolute value of the initial change in the synaptic response, and the decay thereafter as a measure of forgetting. This definition is, of course, a minimal one. The neurophysiological data from this experiment suggest two fundamental differences between young and old organisms: 1) the amount of synaptic strength may be greater in the old organisms before any HF stimulation (indirect evidence for this comes from the responses collected at surgery and the EPSP to fiber potential relationships); and 2) the ability to retain the elevated synaptic response is reduced in old organisms (evidence for this comes from the repeated HF stimulation sessions). This latter point is consistent with the behavioral data from the circular maze.

According to Marr's (1971) model of the hippocortex, the accuracy or reliability of recall of a particular event is inversely related to the number of events stored in the system. It may be presumed that
by two years of age, a rat has learned the critical information necessary for survival. One would predict for a limited capacity system that reliability would begin to decline as more traces are stored beyond a certain number. It would be adaptive if, after a certain level of synaptic efficacy was reached, further modifications which might interfere with important events already stored, were less likely to be preserved. If there was no direct link between the amount of synaptic modification already present and the mechanism which regulates modifiability, the same effect would be achieved if the potential for plasticity was genetically programmed to decline after a certain period. There is a direct parallel between this interpretation and the limitation of synaptic plasticity in comparatively lower order (sensory) neural systems, i.e., the so-called "critical period" for visual system development. It is possible that visual system plasticity and hippocampal synaptic modification represent similar phenomena, although the critical period for the hippocampus extends further into adulthood.

This raises the question of whether the decrease in the numbers of synapses (Geinisman & Bondareff, 1976) and general slowing of cellular metabolic functions (McGeer, 1975) can actually be considered "pathology". If these changes serve the useful function of altering the amount of information that can be stored to maintain some optimal signal to noise ratio, this indeed appears to be other than pathological.

The final, perhaps more critical finding for models which relate synaptic change to information storage, is that the synaptic modifiability measured here correlated with the ability to learn a
behavioral task. The correlation with a spatial task is particularly interesting in view of a current model of hippocampal function (O'Keefe & Nadel, 1977) suggesting it is involved in the formation of representations of spatial experience. The data thus provide evidence in favor of Hebb's (1949) initial hypothesis that learning involves a growth in synaptic efficiency through repeated use.
REFERENCES


Alvarez-Leefmans, F. J., & Gardner-Medwin, A. R. Influences of the septum on the hippocampal dentate area which are unaccompanied by field potentials. Journal of Physiology, 1975, 249, 14-16P.


Andersen, P., Bliss, T., & Söreide, K. Lamellar organization of hippocampal excitatory pathways. Experimental Brain Research, 1971, 13, 222-238.


Brizzee, K. Quantitative studies on cell packing density and lipofuscin in the brain of the aged non-human primate. From the NYU post-graduate Medical School Symposium on *The Neurobiology of Aging*, 1975.

Brody, H. An examination of cerebral cortex and brain stem aging. From the NYU post-graduate Medical School Symposium on The Neurobiology of Aging, 1975.


Bliss, T., & Gardner-Medwin, A. Long-lasting increases of synaptic influence in the unanaesthetized hippocampus. *Journal of Physiology*, 1971, 216, 32-33P.


Bondareff, W. Extra-cellular space in the aging cerebrum. From the NYU post-graduate Medical School Symposium on The Neurobiology of Aging, 1975.


Crapper, D. Effect of aluminum on the brain. From the NYU post-graduate Medical School Symposium on the Neurobiology of Aging, 1975.


Eisdorfer, C. Autonomic nervous system activity and learning the aged. From the NYU Post-Graduate Medical School Symposium on The Neurobiology of Aging, 1975.


Feldman, M. Aging changes in the morphology of cortical dendrites. From the NYU post-graduate Medical School Symposium on The Neurobiology of Aging, 1975.


Harris, E., & Steward, O. Habituation-like decrements in transmission through a normal and a lesion induced pathway in the hippocampal formation of the rat. Sixth Annual Meeting of the Society for Neuroscience, Toronto, Ontario, 1976 (Abstract).


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


Hughes, J. Post-tetanic potentiation. Physiological Reviews, 1958, 38, 91-113.

Hughes, R. A statistical analysis on the location of EEG abnormalities. Electroencephalography and Clinical Neurophysiology, 1962, 12, 905-909.


Inukai, T. On the loss of purkinje cells with advancing age, from the cerebellar cortex of the albino rat. *Journal of Comparative Neurology*, 1928, 45, 1-31.


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*, 1971, 12, 18-45. (a)

Lomo, T. Potentiation of monosynaptic EPSP’s in the perforant path-dentate granule cell synapse. *Experimental Brain Research*, 1971, 12, 46-63. (b)


McNaughton, B. L. Dissociation of short- and long-lasting modification of synaptic efficacy at the terminals of the perforant path. Seventh Annual Meetings of the Society for Neuroscience, Anaheim, California, 1977. (Abstract)

McNaughton, B. L., & Barnes, C. A. Physiological identification and analysis of dentate granule cell responses to stimulation of the medial and lateral perforant pathways in the rat. Journal of Comparative Neurology, in press.


Milner, B. Psychological defects produced by temporal-lobe excision. Research Publication of the Association for Research on Nervous and Mental Disorders, 1958, 136, 244-257.


Olton, D. Specific deficits in active avoidance behavior following penicillin injection into the hippocampus. *Physiology and Behavior*, 1968, 3, 719-724.


Raisman, G., Cowan, W., & Powell, T. The extrinsic afferent, commissural and association fibers of the hippocampus. *Brain*, 1965, 88, 963, 996.


Roberts, W., Dember, W., & Brodwick, M. Alternation and exploration in rats with hippocampal lesions. *Journal of Comparative and Physiological Psychology*, 1962, 55, 695-700.


Rosenzweig, M., Moligaard, K., Diamond, M., & Bennett, E. Negative as well as positive synaptic changes may store memory. *Psychological Reviews*, 1972, 79, 93-96.


Schmaltz, L. Deficit in active avoidance learning in rats following penicillin injection into hippocampus. *Physiology and Behavior*, 1971, 6, 667-674.


Stone, C. P. The age factor in animal learning. II. Rats on a multiple light discrimination box and a difficult maze. *Genetic Psychology Monographs*, 1929, 6, 125-202. (b)


Strehler, B. Introduction. From the NYU post-graduate Medical School Symposium on The Neurobiology of Aging, 1975.

Swanson, L. W., & Cowan, W. M. An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. *Journal of Comparative Neurology*, 1977, 172, 49-84.

Swanson, L. W., & Hartmen, B. K. The central adrenergic system: An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine B hydroxylase as a marker. *Journal of Comparative Neurology*, 1975, 163, 467-505.


Thatcher, R. Electrophysiological correlates of animal and human memory. From the NYU post-graduate Medical School Symposium on The Neurobiology of Aging, 1975.


Thompson, L. Cerebral blood flow and EEG in aging. From the NYU post-graduate Medical School Symposium on The Neurobiology of Aging, 1975.


Tryon, H. C. Studies in individual differences in maze ability, II. The determination of individual differences by age, weight, sex and pigmentation. *Journal of Comparative and Physiological Psychology*, 1931, 12, 1-22.


Uttley, A. M. Stimulation studies of learning in an informon network. *Brain Research*, 1976, 102, 37-53. (b)


Vinogradova, O. Registration of information and the limbic system.


Weiskrantz, L. A long-term view of short-term memory in psychology.


Weiskrantz, L., & Warrington, E. A study of forgetting in amnesic patients. Neuropsychologia, 1970, 8, 281-288. (a)

Weiskrantz, L., Warrington, E. Verbal learning and retention by amnesic patients using partial information. Psychonomic Science, 1970, 20, 210-211. (b)
White, W. F., Goldowitz, D., Lynch, G., & Cotman, C. Electro-
physiological analysis of the projection from the contra-
lateral entorhinal cortex to the dentate gyrus in normal

Wickelgren, W. Multitrace strength theory. In D. A. Norman (Ed.),

Wilkie, F. L., Eis dorfer, C., & Nowlin, J. B. Memory and blood
pressure in the aged. *Experimental Aging Research*, 1976,
2, 3-16.

Wimer, R. A supplementary report on age difference in retention
over a 24-hour period. *Journal of Gerontology*, 1960, 15,
417-418.

Wimer, R., & Wig dor, B. Age differences in retention of learning.

Winson, J. Interspecies differences in the occurrence of theta.

Winson, J., & Abzug, C. Gating of neuronal transmission in the
hippocampus: efficacy of transmission varies with behavioral

Wisniewski, H. Ultrastructure of the aging brain. From the NYU
post-graduate Medical School Symposium on The Neurobiology
of Aging, 1975.

Woodruff, M., & Isaacson, R. Discrimination learning in animals
with lesions of hippocampus. *Behavioral Biology*, 1972,
489-501.


Zimmer, J. Ipsilateral afferents to the commissural zone of the fascia dentata, demonstrated in decommisurated rats by silver impregnation. *Journal of Comparative Neurology*, 1971, 142, 393-416.

APPENDIX A: Reasons for Decreased Sample Size

During the Course of the Experiment

During the course of the six-month experiment, a number of factors led to the decrease in the number of rats per group. The major causes of group size fluctuation included programming errors, computer malfunction and accidental erasure of data from the disk. These will not be listed; however, the reasons for decreased group size other than the above are listed in Table A.
<table>
<thead>
<tr>
<th>Group</th>
<th>SN</th>
<th>MA</th>
<th>Date</th>
<th>Reason for Termination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>#60</td>
<td>#48</td>
<td>Feb. 16</td>
<td>evoked response unacceptable, died - respiratory disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Feb. 22</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>#39</td>
<td>#53</td>
<td>Mar. 21</td>
<td>died - bladder disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Apr. 25</td>
<td>died - brain tumor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>#91</td>
<td>Feb. 17</td>
<td>evoked response unacceptable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>#78</td>
<td>Feb. 15</td>
<td>faulty head cap connection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>#104</td>
<td>Jan. 15</td>
<td>died in surgery, replaced by #99</td>
</tr>
<tr>
<td>3</td>
<td>#55</td>
<td>#44</td>
<td>Feb. 2</td>
<td>died - brain tumor, replaced by #37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dec. 20</td>
<td>died - bladder disease</td>
</tr>
<tr>
<td>4</td>
<td>#30</td>
<td>#65</td>
<td>Feb. 24</td>
<td>died - peritonitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Feb. 4</td>
<td>died - bladder disease</td>
</tr>
<tr>
<td>5</td>
<td>#52</td>
<td>#102</td>
<td>Mar. 6</td>
<td>died - respiratory disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>#107</td>
<td>Mar. 23</td>
<td>head cap came off</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Feb. 16</td>
<td>died - respiratory disease</td>
</tr>
<tr>
<td>6</td>
<td>#96</td>
<td>#71</td>
<td>Feb. 18</td>
<td>faulty head cap connection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>#73</td>
<td>Mar. 12</td>
<td>faulty head cap connection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>evoked response changed - brain tumor</td>
</tr>
<tr>
<td>7</td>
<td>#49</td>
<td>#84</td>
<td>Feb. 22</td>
<td>faulty head cap connection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>May 12</td>
<td>evoked response changed - brain tumor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>died - respiratory disease</td>
</tr>
<tr>
<td>8</td>
<td>#45</td>
<td>#69</td>
<td>Jun. 3</td>
<td>evoked response changed - brain tumor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>May 23</td>
<td>evoked response changed</td>
</tr>
</tbody>
</table>

Table A

The Group and Animal Number, Date, and Reasons for Terminating the Use of the Animal
APPENDIX B: Body Weights

Body weights were measured at the beginning and at the end of the experiment: once before surgery and again before the anesthetic double pulse section (December and June respectively). Because of deaths during the experiment, the number is much lower in June than it was in December. The mean and standard error of the weights are given in Table B. The SN animals began the experiment at approximately 50 grams heavier than the MA rats, and lost weight during the study. On the other hand, the MA rats tended to gain weight during the study, reaching a level at this time which was comparable to the initial weights of the SN rats.

Table B

<table>
<thead>
<tr>
<th></th>
<th>SN</th>
<th></th>
<th>MA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{x}$</td>
<td>601.19</td>
<td>505.90</td>
<td>552.09</td>
<td>600.64</td>
</tr>
<tr>
<td>$\bar{s}e$</td>
<td>14.02</td>
<td>19.05</td>
<td>12.53</td>
<td>10.66</td>
</tr>
<tr>
<td>$\bar{n}$</td>
<td>32</td>
<td>20</td>
<td>33</td>
<td>25</td>
</tr>
</tbody>
</table>
APPENDIX C: Tumors

The SN and MA rats in this study showed a notable incidence of body and brain tumors. These can be categorized according to four basic types.

The first type of tumor developed underneath the skin, but did not penetrate through the fascia into the muscle. These tumors were basically spheroid in shape, well vascularized, and in one case grew to a diameter of nearly two inches. Fortunately, they could be surgically removed with minimal trauma. Four such tumors in four SN rats were removed at the time of electrode implantation and two others were present at the time of fixation. There were no recurrences of this type of tumor.

The second type of tumor invaded the more interior aspect of the body. There were two cases of this, both in SN animals, one involved the muscle of the forelimb, and one was found in the abdominal cavity. The muscular tumor developed shortly before fixation was scheduled for the animal, and thus did not interfere with the major experiment. The abdominal tumor was discovered after fixation.

The third type of growth was cyst-like and appeared on the surface of the skin without penetrating into the muscle tissue. These growths were much smaller than the first type, and were easily removed. There were eight of these cysts removed in seven SN animals at the time of surgery, and there were three noted at the time of fixation (one of which was a recurrence). One of these cyst-like growths was noted on an MA rat at the time of fixation.
There were two areas of the brain where tumors were found at the time of histology. In one MA rat a large black mass was found in the area of the septum. The other brain area was that of the pituitary, and, when they were large, caused a deformation of the thalamus and hypothalamus. There were seven incidences of pituitary tumors, all of them in the SN rats: two were large, two were of intermediate size, and three others were very small (perhaps just beginning). It is interesting to note that the two animals that had the second type of tumor (invasion of body tissue) both had pituitary tumors. Two of the SN rats that had a brain tumor of this sort died during the course of the study. There were no obvious external symptoms of the presence of the tumors in the other rats.

It is, of course, impossible to say exactly when the rats developed these tumors; however, the evoked response changes and the deaths outlined in Appendix A give a reasonable estimate of the point at which the tumor became a significant size.
APPENDIX D: Pilot Studies Completed for the Development of the Procedure in the Circular Maze Study

Odor Adaptation Trial

An odor adaptation trial was given to all the rats used in this study. This was initially designed as a precursor to an odor preference test, where the time the rat spent examining female or male urine was to be measured. However, the inclusion of a female was eliminated from the design of the spatial discrimination study, and so the necessity of an odor preference study was obviated. The following is a brief description of the adaptation trial that was given.

A large plastic breeding cage was over-turned on top of unused newsprint and served as the testing cage. At each end of the cage were two wire blocks. The blocks were covered with paper toweling and were later to carry the odor of an estrous female or a colony male urine. Each animal was placed under this cage for five minutes. During these five minutes all rats explored the covered boxes. Clean newsprint and boxes were put under the cage for each rat. Since the odor tests were not pursued, this situation was considered part of the handling experience.

First Pilot Study Using the Circular Maze

Two old rats (pets, aged 20 months) and two middle-aged rats (nine months) were given two trials on the maze to generally determine the suitability of the maze design: whether the holes were large enough; whether it was easy for the rat to descend into the
the tunnel; whether the rat would go down if it found the tunnel; and the approximate amount of time it would take to find the correct hole.

Two trials on two consecutive days were given. On the first day of testing a female was not put into a circular compartment which was adjacent to the end of the tunnel. One old and one middle-aged rat were given 10 minutes in the tunnel before being placed into the start box. The other middle-aged and old rats remained in their home cages for the 10 minutes before being placed into the start box. All rats remained in the start box for between 15 to 25 seconds before it was lifted. A maximum of 15 minutes was planned for each rat on top of the maze before removal to the tunnel for a one-minute period. Latency measures were recorded. All rats went down into the tunnel well before the time limit on this day (see Table C).

Since all four rats went down into the tunnel immediately upon finding the correct hole on the first day, even though there was no female in the center compartment, a female was not used on the second day, to determine whether this variable was likely to be necessary. On this day all rats were placed immediately into the start box and remained there between 15 and 25 seconds before it was lifted. They were left in the tunnel for one minute after finding it. Latency was again recorded (see Table C).

Since the two rats that were given adaptation time in the tunnel had lower scores than the two rats that were not, this factor might have improved the latency scores. It was decided that the maximum time limit of 15 minutes was excessive, and that the
female motivating factor was unnecessary. The above considerations as well as general ideas for scoring the rats were considered in designing the second pilot study.

Table C
Latency to Descend into the Goal Box

<table>
<thead>
<tr>
<th>Rat</th>
<th>Old Day 1 (sec.)</th>
<th>Old Day 2 (sec.)</th>
<th>Middle-aged Day 1 (sec.)</th>
<th>Middle-aged Day 2 (sec.)</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laurence</td>
<td>67</td>
<td>4</td>
<td>35</td>
<td>50</td>
<td>#4</td>
</tr>
<tr>
<td>(adaptation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(adaptation)</td>
</tr>
<tr>
<td>Number 3</td>
<td>205</td>
<td>155</td>
<td>110</td>
<td>72</td>
<td>#6</td>
</tr>
<tr>
<td>(no adaptation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Second Pilot Study Using the Circular Maze

Ten four-month old hooded rats from Quebec breeding farms were used in this study. They were tested successively beginning at 22:00 every day for four days. The procedure was as follows:
1) the rat was brought into the testing room immediately prior to the first trial of the day for the rat;
2) the tunnel was in a fixed position but the maze top was rotated randomly above the tunnel for each trial;
3) the rat was placed in the start box for 30 seconds before it was lifted and the trial begun;
4) when the rat found the correct hole and descended into the tunnel, it was allowed one minute inside the tunnel before being removed to its home cage. If the rat did not find the tunnel in four minutes, it was removed from the maze top and placed into the tunnel.
On Day 1, the rats were each put directly into the tunnel for a four-minute period. They were then removed to their home cage for one minute and put into a regular trial as described above. On Day 2, 3, and 4 the rats were given two regular trials in succession, separated by a one-minute period in the home cage.

During the regular trials the rats were tracked with the video-tracking system described in the main circular maze experiment section, and latency and error scores were recorded.

This procedure worked quite well. The rats, in general, learned the task rapidly, indicating that the discrimination might very likely be reversed successfully, in a reasonable amount of time. The reversal was included in the major experiment. The four-minute time maximum appeared to be a reasonable limit, and was also incorporated into the final design. As well as randomizing the position of the hole over the tunnel, the position the rat would first be facing when put into the start box emerged as a possible important variable. So random assignment of initial position in the start box was included in the final study. The equipment was found to be intermittently unreliable, however, no alternate method for tracking the position of the animal in space was available. Also a number of rats jumped from the maze to terminate the trial, which was an obvious procedural complication. Unfortunately, I did not modify the maze for the main study.

The pilot results in relation to the major study. There were a number of problems with the equipment, particularly on the
first two days of data collection. Thus, there is a good deal of missing data on these days in the distance and velocity measures. Since the latency and error scores were taken at the time of testing, all the data is present on these measures (although it must be kept in mind that the maximum \( n \) in this study is only 10).

The mean error over trials is seen in Table D. The pilot rats tended to make more errors on the "short-term" trials than on the 24-hour trials. This was also found to be true of the MA and SN rats in the main study. Further, the lowest mean error scores are around three errors, which was the same as for the MA rats (the lowest mean error scores the SN rats made was around five errors).

Table D

<table>
<thead>
<tr>
<th>Mean Errors Over Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trials</td>
</tr>
<tr>
<td>( \bar{x} )</td>
</tr>
<tr>
<td>SE</td>
</tr>
<tr>
<td>( n )</td>
</tr>
</tbody>
</table>

The mean latency scores over trials are seen in Table E. These pilot rats tended to show latency scores which were very similar to the MA rats on Trials 4-7, but longer latencies were obtained on the first three trials (somewhat shorter, however, than the SN Rats).
Table E

Mean Latency Over Trials

<table>
<thead>
<tr>
<th>Trials</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>181.2</td>
<td>160.2</td>
<td>122.0</td>
<td>68.2</td>
<td>80.8</td>
<td>47.1</td>
<td>33.9</td>
</tr>
<tr>
<td>SE</td>
<td>25.6</td>
<td>32.1</td>
<td>29.3</td>
<td>29.1</td>
<td>22.7</td>
<td>16.9</td>
<td>6.3</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

The mean speed scores over trials are seen in Table F. Although the speed scores tend to increase over trials, as in the main study, these rats were generally slower on the maze than either the MA or SN rats. This poses some difficulty for interpretation. Since the n is quite small (especially on this measure) these data may be misleading.

Table F

Mean Speed (cm/sec)

<table>
<thead>
<tr>
<th>Trials</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>11.61</td>
<td>10.48</td>
<td>11.15</td>
<td>17.19</td>
<td>19.56</td>
<td>20.30</td>
<td>22.28</td>
</tr>
<tr>
<td>SE</td>
<td>1.50</td>
<td>1.68</td>
<td>0.94</td>
<td>1.63</td>
<td>1.91</td>
<td>1.62</td>
<td>1.35</td>
</tr>
<tr>
<td>n</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

Then mean distance scores are seen in Table G. The mean distance in Trials 4-7 is very similar to that obtained in the main study by the MA rats. The first three trials are substantially lower than those distances measured in the main experiment, however, the n was particularly small in these cases.
### Table G

<table>
<thead>
<tr>
<th>Trials</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{X}$</td>
<td>532.6</td>
<td>316.3</td>
<td>540.5</td>
<td>368.7</td>
<td>508.1</td>
<td>348.5</td>
<td>319.1</td>
</tr>
<tr>
<td>SE</td>
<td>110.7</td>
<td>97.6</td>
<td>151.3</td>
<td>129.6</td>
<td>129.0</td>
<td>103.0</td>
<td>49.3</td>
</tr>
<tr>
<td>$n$</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

In general (except for the speed measure) the data from these young rats corresponded remarkably well to the data collected from the MA rats in the main study. The major value of this pilot study, however, was to verify the usefulness of this maze.
APPENDIX E: Detailed Procedure for Circular Maze

Each of the eighteen holes on the maze top were given a number (marked in letter set) and the hole that would be assigned to the position above the tunnel was randomly drawn before each trial, as was the direction the rat would face when placed into the start box. The start box was lowered and centered around a black spot that marked the middle of the maze. After the maze was ready for the beginning of a trial, the spot on the video screen was positioned over the black spot on the maze by adjusting the potentiometers with the joy stick. At the start of each day, the maze was calibrated by moving the joy stick so that it marked the center and goal hole, and outlined the circumference of the maze. The calibration run was stored on tape so that any drifts in the equipment could be noted and then corrected. All rats were carried downstairs in their home cages to the testing room immediately before their first trial of the day.

Each group was tested on six consecutive days during the groups assigned time period. The testing procedure used on each of these days is described below.

Day 1. The rat was placed in the dark tunnel for four minutes. After this "adaptation" trial, the rat was placed back in its home cage (which was shielded from view of the testing area) for one minute. It was then placed into the start box for Trial 1. After 30 seconds, the start box was lifted and the trial was begun.
throughout this period the position of the rat's nose was followed by moving the joy stick (and oscilloscope spot) in accordance with any movement of the rat's nose on the maze. Concurrently, errors were scored on a hand counter and latency to enter the tunnel was measured with a stop watch. The criterion used for an error score was a deflection of the nose, head, and neck into a hole which was not above the tunnel or jumping from the maze completely. The trial was considered complete (the stop watch stopped, and the trial marked finished on the tape recorder) when the rat's four feet were not on the maze top or after four minutes. The rat remained in the tunnel for one minute, after which it was taken out, placed in its home cage and carried back to the colony room. If the rat did not enter the box before four minutes, it was picked up and placed into the tunnel (by opening the drawer, placing the rat inside and closing it) and was left there for one minute. The maze and tunnel were cleaned with a damp (water) paper towel after each rat. Thus on the second trial of Day 2-6, the rat had his own (randomly positioned) odor trial on the maze.

Day 2, 3, and 4. On the days after Day 1, the adaptation trial was omitted and the rats simply received two trials as described above. The two trials were separated by approximately two minutes. There were therefore 24 hours between Trials 2, 4, and 6; with Trials 3, 5, and 7 beginning approximately two minutes following 2, 4, and 6 respectively.
Day 5 and 6. On the 8th trial, the tunnel was moved to a different position in the room (this being the beginning of the reversal trials). The randomly assigned holes were thereafter placed over this tunnel position. The rest of the procedure for Trials 8, 9, 10, and 11 was identical with that of Days 2-6 (Trials 9 and 11 given approximately two minutes after 8 and 10 respectively.)
APPENDIX F: Critical Dimensions of Circular Maze

Diameter: 4' (1.22 m),

Tunnel length: 17.5" (44.45 cm),
    depth: .413" (10.49 cm),
    ramp: 2.5" to floor (6.35 cm),

Center compartment diameter: 12" (30.48 cm),
    depth: 8" (20.32 cm),

Holes diameter: 3.75" (9.525 cm),

Center to center spacing between holes: 7.15" (18.16 cm),

Start box diameter: 7" (17.78),
    height: 8" (20.32 cm).
APPENDIX G: Diagram of the 8-Compartment Rat Cart

Used During Electrophysiological Recording
APPENDIX H: Double Pulse "Facilitation"

The data from the double pulse inhibition-facilitation section (VII) suggested that in the case where the first pulse included a population spike, the second response had a number of properties which were unanticipated on the basis of previous literature (Lomo, 1971b; Steward, et al., 1976). These effects were noted in both age groups. There are three phenomena concerning the longer IPI's (those after the period of recurrent inhibition), which are of interest and are in need of consistent explanation: 1) an increase in the area and height of the population spike; 2) a decrease in the amplitude of the extracellular EPSP; 3) an increase in the latency of the population spike. Part of the difficulty arises in assigning the correct meaning to these extracellularly recorded phenomena. That is, a reduced extracellular EPSP could be a real reduction in the EPSP size (perhaps due to a decrease in transmitter release), or it could result from a tonic depolarization of the granule cells (i.e., the peak membrane potential reached during the EPSP may be higher even though the extracellular recorded potential is smaller). An increase in the population spike could be an increase in the number of granule cells firing (possibly by reducing spike threshold), or it could result from an increase in the size of the individual granule cell action potentials, such as would occur for example if the granule cells were hyperpolarized. Since relative latency measures are independent of whether the potential is recorded
intracellularly or extracellularly, changes in this measure must reflect true changes in the granule cell firing latencies.

In an attempt to clarify these points, and the apparent paradoxical results in both the EPSP and population spike measures discussed in Section VII, some preliminary experimental work was done to provide a direction for future research. This work is still in progress and is being done in collaboration with B. McNaughton.

Pilot Work

Tests for EPSP facilitation in chronic rats. A major deficiency in the double pulse series given to the SN and MA rats in this study was the lack of comparison of double pulses given at an intensity below threshold for a spike. Five, chronic bilaterally implanted rats prepared for another study were used to test the effect of double pulse stimulation, using parameters set below threshold for a spike. Quite surprisingly, the EPSP facilitation which is quite striking in anesthetized preparations, was absent in these awake animals.

Tests for EPSP increases in acute rats. The effects of double pulse stimulation in chronic animals was then compared to the effects seen in Nembutal anesthetized rats. In the first acute preparation, double pulse stimulation was delivered in 1 msec steps from 8 to 308 msec, at a rate of one pair per five seconds. This was done first with a response below the threshold for a population spike, and then with a response containing a population spike.

The fractional change of the EPSP slope vs IPI when the
evoked response was below threshold for a population spike showed a similar kind of facilitation as that reported for other anesthetized preparations (see Figure 35.1). This facilitation lasted over 100 msec, after which the second pulse EPSP was suppressed out to 308 msec. These data also show the strong depression during very short interpulse intervals (out to 15 msec) which is a very similar time course to that found in the chronic animals in this thesis which had a population spike. This lends support to the notion that the initial depression, and the later suppression of the EPSP are not due to recurrent effects stemming from granule cell discharge (since only pure EPSP's were involved).

This animal was also run in a series with the intensity of stimulation being above threshold for a population spike. In this case, the EPSP amplitude of the second pulse remained steadily below the EPSP amplitude of the first pulse out to 308 msec (see Figure 35.2). This finding is similar to that found in the chronic rats. Furthermore, the EPSP remained depressed throughout both the recurrent inhibition and facilitation of the population spike.

It therefore appears that facilitation of the EPSP in an evoked response below threshold for a population spike occurs only in anesthetized preparations, and that even in these preparations a suppression of the EPSP is seen after approximately 100 msec. These findings were replicated in three other Nembutal anesthetized rats, and the late suppression effect was found to last for at least 520 msec in each of these rats. The suppression of the second
Figure 35. Fractional change of EPSP amplitude, (Pulse 2-Pulse 1)/(Pulse 1), in an anesthetized (Nembutal) preparation, at IPI's from 0 to 308 msec (in steps of 1 msec). This is shown both when the first pulse was below population spike threshold (in "1"), and when the first pulse was above population spike threshold (in "2"). Note that the points above the zero line indicate those IPI's where the second pulse was greater than the first, and below this line the cases where the second pulse was smaller than the first.
Below spike threshold

Fractional change of EPSP \( \frac{(P2-P1)}{P1} \)

Above spike threshold

Fractional change of EPSP \( \frac{(P2-P1)}{P1} \)
pulse EPSP, in an evoked response above threshold for a population spike, occurs in both the chronic and Nembutal anesthetized preparations. The suppression effect was seen to be maintained out to an IPI of 520 msec in the three further preparations mentioned above.

One interesting, and testable, possible mechanism for the population spike facilitation is a change in the threshold for spike initiation. It has been shown by Alvarez-Leefmans and Gardner-Medwin (1975) that stimulation of the projection to the dentate from the medial septum has the effect of increasing the perforant path elicited population spike. This septal stimulation, however, produces no observable field potential in the dentate, and it does not change the size of the perforant path elicited EPSP. The septal input, in effect, lowers the threshold for granule cell discharge. It may thus be postulated that the spike facilitation mechanism may arise from more granule cells firing because of increased septal input after the first pulse on the perforant path. This might arise either from septal feedback resulting from the first population spike, or as a result of direct stimulation of afferents to the septum running in the vicinity of the angular bundle.

To test this, the fornix was cut in the four acute preparations discussed previously, and the response to double stimuli above and below threshold for a population spike was then measured. In two of the animals, the histology revealed that the incisions failed to transect the fornix, but did some damage to the lateral septum. In these animals the results of the below and above threshold
runs were virtually identical with the runs before the incision.

In the two other animals, the fornix was at least partially cut: in one rat, 95% of the septal-fornix ventral-commissure system was transected; and in the other rat 100% of the contralateral fornix was cut but only 25% of the ipsilateral fornix was damaged, with the commissure being spared. The latter rat showed a small, but not a striking change in the time course and extent of the inhibition-facilitation of the population spike. The rat with the good fornix cut, however, showed a large alteration in the responses measured after transection, i.e., no facilitation of the population spike area. Although these data can only be considered very preliminary, they suggest that a good transection of the fornix and commissural connections to the dentate, alters the nature of the effect of an initial pulse on a second pulse. This occurs over a wide range of IPI's and may not be due to general trauma resulting from the incision (i.e., the three cuts that "missed" did not show the large disruption). It thus appears that "facilitation" of the population spike in the dentate may in fact be due to recurrent activity from other inputs, and should be clearly distinguished from facilitation of the pure EPSP in anesthetized mammals which may be due to increased transmitter release (as seen in the neuromuscular junction).

Two separate mechanisms must therefore be postulated for the increase in the EPSP and population spike after double pulse stimulation (as suggested by Lomo, 1971; and Steward, et al., 1976).
It has not been reported, however, that the population spike latency does not decrease as might be expected (e.g., its latency actually increases), or that the EPSP shows a marked suppression under certain double pulse conditions. When the first pulse is a pure EPSP there may either be separate or perhaps a balance between facilitatory and suppression-like processes; EPSP facilitation may occur out to IPI's of 100 msec, after which this gives way to EPSP suppression. When the first pulse is above threshold for a population spike the EPSP is continuously suppressed throughout the period where the population spike is initially inhibited and then "facilitated". Therefore, four separate phenomena are observed: EPSP facilitation, EPSP suppression, population spike inhibition, and population spike "facilitation". Another critical aspect of these data involves the fact that anesthetized preparations show the above increases and decreases in both the EPSP and population spike, whereas awake animals do not show EPSP facilitation (although the other three phenomena described above are seen). The significance of this remains to be determined.