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BEHAVIORAL, ELECTROPHYSIOLOGICAL, AND NEUROANATOMICAL PLASTICITY IN THE RAT, AS A RESULT OF COMPLEX ENVIRONMENT HOUSING

by

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Department of Psychology

Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Ontario
August, 1993

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ABSTRACT

Long-term potentiation (LTP) is an electrophysiological phenomenon of neuroplasticity, whereby synaptic transmission of specific brain pathways become enhanced, or potentiated, after the delivery of a series of brief, high-frequency electrical trains. The increase in synaptic efficacy is of substantial duration, and therefore LTP has been likened to the theoretical reverberatory circuits that lead to permanent memory traces, as originally hypothesized by Hebb (1949). To further examine the direct relations between LTP and learning this thesis focused on the paradigm of complex environments, which have been shown to enhance learning, neocortical thickness, and hippocampal evoked potentials. It was decided to examine the effects of this manipulation upon a wide variety of behavioral, electrophysiological, and neuroanatomical measures, which prior to this thesis had not been examined on such a broad scale, nor within the same preparation. Additionally, the effects of complex environments upon LTP induction were studied, an experiment, which surprisingly had not yet been performed.

Results from the complex environment studies indicated that rats housed in complex environments differed from their individually housed littermates in many ways, including learning, LTP, and cortical thickness, with rats that had been housed in the complex environments outperforming their individually housed littermates on all aspects. However, when the inter-relatedness of LTP, learning and neocortical thickness were examined it was found that both learning and LTP independently had relations to neocortical thickness at separate locations, but that LTP and learning did not relate to each other directly. Thus overall, this thesis provided mixed support for the link between

LTP and learning, but in doing so, may have clarified a number of issues, and indicated some valuable directions for future research. Finally, this thesis stands out, as the most comprehensive examination of the effects of complex environments completed to date.

ACKNOWLEDGEMENTS

Although in the end the completed thesis belongs to a single individual, it is rare that the process of creating it was endured alone. As such, I wish to acknowledge a number of individuals, who have ensured safe passage of my thesis, myself, and my sanity through graduate school. First and foremost, I would like to thank my advisor, Dr. Peter Cain, for tolerating my many meanderings off the well defined pathway of my thesis, and for knowing the right time to direct me back to that path, before I disappeared into the woods forever. Second, I would like to thank Francis Boon for his work on my thesis and the things he taught me about the surgery that I originally taught him. I would also like to acknowledge the contribution of Dr. Bryan Kolb to this project by doing the majority of the neuroanatomical analyses for this thesis. Next I would like to thank the members of my advisory committee Drs. Case Vanderwolf, and Peter Ossenkopp for enduring my thesis, its mutations, spin-offs, and side-effects. I would like to give a special thanks to Drs. Martin Kavaliers, and Doreen Kimura for their support of side projects, finances, and shelter. I would also like to acknowledge the classical advice of Richard Cooley, and the help of Lynn Mitchell, Peter Moore, Danny Pullham, and John Orphan throughout my graduate career.

There are also a number of students past and present, Drs. and Drs. to be, who as colleagues and friends have made graduate school all the more endurable and learned... David Carey, Cam Teskey, Phillip Servos, Neil Watson, Zoe Dennison, Liisa Galea, Barbara Peck, Deb Saucier, and Jeff Hall.

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
AD	Afterdischarge
AEP	Average Evoked Potential
AP	Anterior-posterior
APV	Amino-phosphonovaleric acid
Ca^{2+}	Calcium ions
CA1 - CA4	Cornu Ammonis
ChAT	Choline-acetyltransferase
cm	centimetres
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CNS	Central Nervous System
CS	Conditioning Stimulus
Dep	Depth
EBS	Electrical Brain Stimulation
EC	Environmentally complex and training condition
EP	Evoked Potential
EPSP	Excitatory post-synaptic Potential
g	grams
GABA	Gamma-aminobuytric acid
IC	Impoverished condition
i.p.	intraperitoneal
kg	kilograms
KIP	Kindling Induced Potentiation
Lat	Lateral
LTP	Long-term Potentiation
Mg^{2+}	Magnesium ions
m	metre
mg	miligrams
min	minutes
mV	milivolts
ms	miliseconds
MSG	Monosodium Glutamate
Ne	Noradrenaline
NM	Nictitating Membrane
NMDA	N-methyl-D-aspartate
pop-spike	Extracellular population-spike
s	seconds
SC	Social condition
UCS	Unconditioned Stimulus
α-CaMKII	type II calmodulin-dependent protein kinase
μm	micrometres
μa	microampres

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CHAPTER 1 - LONG-TERM-POTENTIATION AND LEARNING: THEORETICAL AND HISTORICAL CONSIDERATIONS, AND A SUMMARY AND CRITIQUE OF THE RELEVANT LITERATURE WITH SPECIAL EMPHASIS ON COMPLEX ENVIRONMENTS

1.1 Introduction:

In the early 1950s a young man suffering from intractable epilepsy underwent a bilateral resection of the medial temporal lobes. Although usually reserved for unmanageable psychotics it was thought that this operation in the young man's case would potentially reduce the severity of his seizures. Ultimately successful in reducing the seizures, the surgical resection however, left the young man with a profound anterograde amnesia. First reported by Scoville (1954) as 1 of 2 cases out of 230 that developed memory problems after bilateral resection of the temporal regions, the case of H.M. did not have its full impact until Scoville and Milner (1957) found evidence of amnesia in 8 testable pyschotics, who had also received this operation and found identical results. Scoville and Milner (1957) attributed H.M.'s severe amnesia to the removal of both hippocampi and amygdali. The now famous case of H.M. has had a major influence in the field of memory, focusing much of the work since, on the structure and function of the hippocampus.

Accordingly, this thesis is primarily concerned with the hippocampal formation and its electrophysiology. In particular, the phenomenon known as Long-Term Potentiation (LTP) was examined since it has been used to model theoretically postulated

memory mechanisms, and may potentially have one or more of underlying mechanisms held in common with learning and memory. Thus, this introduction will first discuss the theoretical memory mechanisms as put forth by Hebb (1949). Then the basic anatomy, slow wave activity, and functional synaptic connectivity of the hippocampal formation will be covered. The neuroplasticity phenomena of LTP and Kindling then will be described, as well as a brief summary of the neural basis of LTP. The implications of LTP for learning mechanisms will be discussed, and the literature assessing the relationship between LTP and learning will then be critically reviewed and evaluated within the framework that was outlined by Morris and Baker (1984). Finally, a series of experiments involving the effects of complex environment housing upon the rat will be outlined, which will form the basis and focus for the remainder of this thesis.

1.2 The Hebbian Synapse:

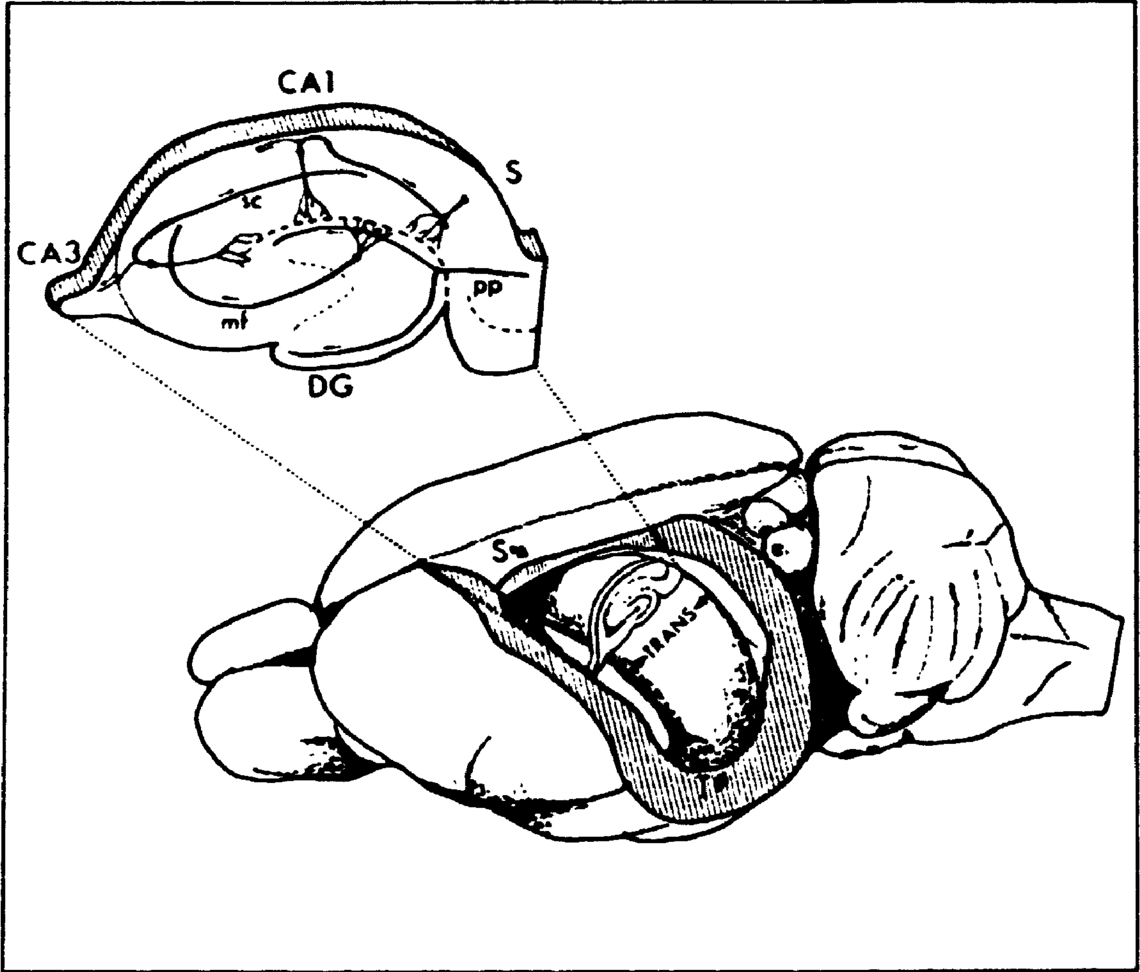
In his 1949 seminal work, Hebb tried to reconcile the "field" and "switchboard" theories of memory formation of his time into a single approach, where flexible memory circuits could be developed throughout the brain. This synthesis was formulated as the "dual trace" neural mechanism of memory formation (Hebb, 1949). The dual trace theory posited that stimuli, if repeated sufficiently, would lead to the development of a neural circuit or "cell-assembly", which did not require specific loci within the brain, but could be widely distributed throughout a number of structures. The cell-assembly would then act as a closed loop system, sustaining the activation pattern induced by the stimuli, yet, be wholely contained within the "reverberating" circuit. Hebb (1949) considered this sustained neural activity as the first trace, holding the information while the second or

permanent trace was laid down. It was this second trace that would store the information. The second trace was suggested to form from a series of structural changes within the cell-assembly that would facilitate the subsequent triggering of the encoded pattern by the reappearance of the original stimuli. The distinction between the short-lived, labile first trace and the enduring second trace corresponded to "short" and "long" term memory stores, in that the first trace was considered far more easily disrupted than the second. The cellular mechanism that underlies the second or more permanent trace and possibly encodes and stores memories has come to be called the "Hebbian" synapse (Morris, Halliwell, and Bowery, 1989).

1.3.1 Gross Anatomy of the Hippocampus:

Figure 1 depicts a schematic of the rat brain with a cut-away section revealing the location and orientation of the hippocampal formation (after Amaral and Witter, 1989). Highlighted and enlarged is a transverse hippocampal slice displaying the trisynaptic circuit, described below. The hippocampal formation is a bilateral limbic structure with an overall *W*-shape. The centre peak of the *W* indicates the septal pole of each hippocampus located dorsally at the midline of the brain, immediately below the neocortex and corpus callosum. The two outside peaks of the *W* indicate the placement of the ventrally located temporal poles. The septal and temporal poles of the left hippocampus are indicated in Figure 1. The course of each hippocampal structure follows along the medial aspects of the ventral floor of the lateral ventricles. Its internal structure is consistent throughout its length and consists of an infolding of the evolutionarily older, and more simple, archicortex or allocortex in the form of two *C*s with one *C* reversed

Figure 1. Three dimensional schematic of the rat brain exhibited in an oblique position. The overlying neo-cortical tissue in the left hemisphere has been excised to reveal the hippocampus. Within the hippocampus an individual lamella, taken as a tranverse hippocampal slice has been shown in the upper left of the figure revealing the trisynaptic circuit (taken from Amaral and Witter, 1989). S) subiculum, pp) perforant-path, DG) dentate gyrus, mf) mossy fiber, CA3) cornu Ammonis region 3, sc) Schaffer collateral, CA1) cornu Ammonis region 1, S→) septal pole, T→) temporal pole. Note the bifurcation of the perforant-path to the upper and lower blades of the dentate-gyrus, once it has crossed the hippocampal fissure. Also note the perforant-path fibers synapsing on the middle third of the dendrites of the granule cells.


CA1
S
CA3
sc
pp
mf
DG
TRANS

in relation to the second, interlocked with each other. In addition to the allocortex the hippocampal formation is made up of surrounding periallocortex (Chronister and White, 1975). Cross sections taken perpendicular to the hippocampal axis reveal the clear intrinsic connections. So clear were these major intrinsic connections that the neuroanatomist Ramon y Cajal was able in 1911 to determine the major direction of synaptic flow of the trisynaptic circuit by examining Golgi stained normal material (Andersen, 1975). Recently however, more emphasis has been placed on the associational fibres that connect the transverse circuitry, and the integrated three-dimensional functioning of the hippocampus as a whole (Amaral and Witter, 1989; Witter, 1989).

1.3.2 Delineation of Hippocampal Fields:

The archicortical infolding that primarily makes up the hippocampal formation consists of the cornu Ammonis or Ammon's horn, the fascia dentata or the dentate gyrus, and nearly all of the subiculum. The periallocortex is made up of the remaining portion of the subiculum and its associated presubiculum and parasubiculum, and the adjoining area retrosplenialis and area entorhinalis (Chronister and White, 1975). The interlocking *C*s of the allocortex each consist of a single cell layer, with the hippocampal pyramidal cells, plus associated interneurons making up the first of these layers and the dentate granule cells, plus interneurons making up the second. Of the hippocampal formation only Ammon's horn is considered to be the hippocampus proper. However, for the remainder of this thesis the term hippocampus will refer to the archicortex infolding, which includes Ammon's horn, the dentate gyrus, and that portion of the subiculum, which is not considered part of the periallocortex.

Ammon's horn has been subdivided different ways by a number of different authors. Initially, Ramon y Cajal (1893; as cited by Lorente de No, 1934) distinguished the regio superior and the regio inferior, which corresponds with the upper and lower blades of the first *C*. Subsequent to this division the regio superior and the regio inferior of the cornu Ammonis have been further broken down by Lorente de No (1934) into 4 subfields (CA1-CA4), based on the size, density, and projection characteristics of the pyramidal cell layer. The subfield CA1 consists of smaller and more tightly packed pyramidal cells than does the CA3 subfield, which contains the giant pyramidal cells of Ammon's horn. The CA2 subfield acts as a transition region between CA1 and CA3 subfields, where the different sized pyramidal cells overlap. The CA1 subfield is wholely contained within the regio superior, while the CA2 and CA3 subfields are contained within the regio inferior (Blackstad, 1956; Lorente de No, 1934). The pyramidal cells of subfield CA4, placed in the hilus of the dentate gyrus, are relatively scattered and diffuse, only becoming a distinct lamina upon shifting into the CA3 subfield. Although the CA4 subfield was originally classified as part of Ammon's horn (Lorente do No, 1934), it is now thought to be part of the fascia dentata (Blackstad, 1956), or an intermediate zone between the fascia dentata and the hippocampus proper (Amaral, 1978; Bayer, 1985).

The dentate gyrus forms the basis of the second *C*, and comprises the lamina of the dentate granule cells and associated interneurons, and the enclosed region, known as the hilus of the fascia dentata (Blackstad, 1956). The hilus consists largely of scattered interneurons of many types that typically project back onto the granule cell bodies or

onto specific strata of the granule cell dendritic tree (Amaral, 1978). Inhibitory internerurons are embedded within, and adjacent to the cell layers of Ammon's horn and the dentate gyrus (Bayer, 1985; Lacaille, Kunkel, Schwartzkroin, 1989; Lorente de No, 1934).

1.3.3 The Trisynaptic Circuit:

Extrinsically the hippocampal formation receives most of its afferent input directly from the entorhinal cortex (Blackstad, 1958; Hjorth-Simonsen, 1972; Hjorth-Simonsen and Jeune, 1972; Lynch, Rose, and Gall, 1978; Steward, 1976). Second to the entorhinal projections, the hippocampus receives afferents from the basal forebrain, largely from fibres originating in the septal region or coursing through it from deeper brainstem nuclei. Collectively these afferents are known as the septo-hippocampal projection (Lynch et al., 1978; Swanson, 1978). These fibres enter the hippocampal formation through the fimbria/fornix or traverse dorsally through the supracallosal striae and enter through the entorhinal cortex.

The dentate gyrus receives its primary afferents from the entorhinal cortex. Fibres from the entorhinal pyramidal layers II and III, form a bundle called the perforant-path. These fibers penetrate the subicular complex, turn and run along the medial-temporal axis of the hippocampal formation in the angular bundle. Individual fibres separate from the bundle, cross the hippocampal fissure, enter the hippocampus, bifurcate, and send axons to both blades of the dentate gyrus, where they synapse on the dendrites of the granule cells in the molecular layer (Figure 1). Recent evidence has suggested that the entorhinal projection neurons give rise to a number of collaterals, indicating that such cells may

have a more diffuse projection than envisaged by the original lamellar hypothesis, which suggested that the hippocampus was organized transversely, as a series of parallel slices (Andersen, Bliss, and Skrede, 1971b; Amaral and Witter, 1989). The perforant-path is composed of a medial and lateral division, arising from medial and lateral portions of the entorhinal cortex (Blackstad, 1956). The medial division synapses in the middle third of the molecular layer of the dentate gyrus and the lateral division synapses in the distal third of the molecular layer, which has been shown anatomically (Hjorth-Simonsen and Jeune, 1972; Hjorth-Simosen, 1972; Steward, 1976) and electrophysiologically (McNaughton, 1980; McNaughton and Barnes, 1977). The perforant-path also directly send fibres to the CA3 and CA1 regions of Ammon's horn travelling through the subicular complex and lacunosum molecure (Hjorth-Simonsen and Jeune, 1972; Lorente de No, 1934; Steward, 1976). The axons of the dentate gyrus granule cells, referred to as the mossy fibres, run parallel in the CA3 stratum radiatum and project both infra- and supra- pyramidally to the dendrites of the CA3 pyramidal cells. The latter cells in turn, synapse on the apical dendrites in the lacunosum-moleculare of the CA1 pyramidal cells via the Schaffer collateral system (Blackstad, 1956; Lorente de No, 1934).

This multisynaptic pathway, originating in the entorhinal cortex and passing successively to the dentate granule cells, the CA3 pyramidal cells, and finally to the CA1 pyramidal cells is collectively known as the "trisynaptic" circuit or loop (Andersen, Holmqvist, and Voorhoeve, 1966b; Swanson, 1982; Witter, 1989). Secondary to this main pathway of afferent flow within the hippocampus are a number of local feedback circuits based on the inhibitory interneurons (Amaral, 1978; Lacaille, et al., 1989).

Additionally, a number of these interneuronal circuits are of a feedforward nature, with afferents originating in the entorhinal cortex (Buhl, Schwerdtfeger, and Germroth, 1989; Buzsaki, 1984; Buzsaki and Eidelberg, 1983). Recent evidence also suggests that a number of these non-pyramidal/non-granule cells project out of the hippocampus to the septum (Buhl, et al., 1989).

1.3.4 Excitatory and Inhibitory Amino Acid Systems:

Work on the spinal cord in the 1970s suggested that the excitatory amino acids of glutamate and aspartate might function as neurotransmitters (Headley and Grillner, 1990; Johnson, 1978; Phillis, 1970). However, the widespread distribution of these excitatory amino acids in non-neural tissue, and their participation in a number of basic cellular metabolic processes, such as the tricarboxylic acid cycle (Armstrong, 1989; White, Handler, Smith, Hill, Lehman, 1978) initially led to doubts as to their role as neurotransmitters within the CNS (Watkins and Evans, 1981). Nevertheless, excitatory amino acid subreceptors were originally divided into three types: 1) N-methyl-D-aspartate (NMDA) preferring, 2) quisqualate preferring, and 3) kainate preferring (Watkins and Evans, 1981). However, recent work using more selective antagonists has shown a need to develop a revised scheme (Lodge and Collingridge, 1991). Thus, kainate has been down played since it only uniquely appears on the dorsal root C fibres of the spinal cord, quisqualate has been replaced by the more selective competitive antagonist α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). *L*-2-amino-4-phosphonobutanoic acid (*L*-AP4), a potential presynaptic glutaminergic autoreceptor has been added along with a much slower acting metabotropic receptor activated by glutamate, quisqualate, and

ibotenate which is potentially linked to the formation of inositol 1,4,5-triphosphate and diacylglycerol (Watkins, Krogsgaard-Larsen and Honore, 1991).

The stratified distribution of glutamate and aspartate within the hippocampus indicated that this structure would be worth examining in order to identify the role(s) of these putative transmitters (Iverson and Storm-Mathisen, 1976; Storm-Mathisen, 1978; Straughn, 1975). Iontophoretic application of the excitatory amino acids and their analogues in hippocampal slices were found to excite spontaneous and evoked responses of the CA1 pyramidal cell layer and the dentate gyrus granule cell layer (Collingridge, Kehl, and McLennan, 1983a; 1983b; 1983c; 1984). Further, selective lesions of specific intrinsic hippocampal pathways and afferents led to changes in hippocampal release and uptake of excitatory amino acids (as reviewed by Wieraszko, 1983). Together these two lines of research contributed heavily to the current understanding of the participation of the excitatory amino acids in two of the pathways of the trisynaptic ciruit (Cotman, Monaghan, Ottersen, Storm-Mathisen, 1987; Lester, Herron, Coan, and Collingridge, 1988; Ottersen and Storm-Mathisen, 1989). In particular both the medial perforant path to granule cell synapses and the CA3 pyramidal cell Schaffer collaterals to the CA1 pyramidal cells use the excitatory amino acids glutamate and aspartate as transmitters (Ottersen and Storm-Mathisen, 1989).

The majority of interneurons within the hippocampus appear to use gamma-aminobuytric acid (GABA) as a transmitter (Curtis, Felix, and McLellan, 1970; Fonnum and Storm-Mathisen, 1978; Ottersen and Storm-Mathisen, 1989; Storm-Mathisen and Fonnum, 1971; Storm-Mathisen, 1972), and accordingly have been deemed to be largely

inhibitory in function (Biscoe and Straughan, 1966; Ottersen and Storm-Mathisen, 1989). Anatomical evidence indicates that interneurons located in the polymorph layer of the dentate gyrus and in the supra- and infra- pyramidal layers make contact with the principal cells (Amaral, 1978; Amaral and Witter, 1989; Frotscher, Leranth, Lubbers, and Oertel, 1984). Additionally, a large portion of the hippocampal afferents from the medial septal nuclei and nuclei of the diagonal band are also GABAergic, and similar to the commissural fibres make contact with GABAergic interneurons (Freund and Antal, 1988; Kohler, Chan-Palay, and Wu, 1984; Peterson, Williams, Varon, and Gage, 1987). Electrophysiological work in the hippocampal slice also suggests that some of the GABA inhibitory interneurons are activated by excitatory amino acid transmission (Davies and Collingridge, 1989; Davies, Davies and Collingridge, 1990).

1.3.5 Endogenous Opioid Systems:

The remaining pathway of the trisynaptic circuit, that of the granule cell mossy fibre synapses on the CA3 pyramidal cells appear to use endogenous opioids as their transmitter (Chavkin, Shoemaker, McGinty, Bayon, and Bloom, 1985; Gahwiler, 1983; Gall and White, 1989; Henriksen, Chouvet, McGinty and Bloom, 1982; Stengaard-Pedersen, Fredens, and Larsson, 1981; 1983; Storm-Mathisen, 1978). Recent electrophysiological and pharmacological evidence suggests that a significant proportion of the lateral perforant-path fibres may also be opioid in nature (Bramham, Errington, and Bliss, 1988; Gall and White, 1989; Stengaard-Pedersen et al., 1983). Further, Met- and Leu- enkephalin immunoreactive staining after enhancement through intraventricular application of colchicine has suggested that some of the scattered interneurons throughout

the hippocampal formation are also opioid in nature (Stengaard-Pedersen et al., 1983).

1.3.6 Evoked Potential Analysis of the Functional Connectivity of the Hippocampus:

A functional analysis of the hippocampus circuitry was undertaken by a group of Norwegian researchers in Oslo (Andersen, Bliss, Lomo, Olsen, and Skrede, 1969; Andersen, Bliss, and Skrede, 1971a; 1971b; Andersen, Holmqvist, and Voorhoeve, 1966a; 1966b; Lomo, 1971a; 1971b). Here it was found that brief pulses of electrical stimulation delivered to the entorhinal cortex and through the perforant-path produced reliable waveforms or evoked potentials (EPs) in the hippocampus. In this series of studies, electrodes were vertically passed through the layers of the hippocampus, all the while recording EPs (Andersen et al., 1966a; 1966b).

The resulting depth profiles enabled them to match the amplitude of evoked potential events at specific depths with the structural anatomy of the hippocampus. Thus, they were able to confirm that the middle third of the granule cells dendritic tree received the strongest perforant-path input, and that this input was excitatory in nature, discharging the granule cell population on both blades of the dentate gyrus (Andersen et al., 1966a).

Subsequent studies enabled them to show . strength of the functional connectivity within the transverse lamellae of the hippocampus (Andersen et al., 1969). This was done by recording antidromic and orthodromic CA3 EPs from two different electrodes, displaced along the medial/lateral axis, in response to 10 different stimulation locations also displaced along the medial/lateral axis. Results indicated that evoked effects were maximal when records were taken from a narrow lamella runing transversely

across the hippocampus, or "on beam". Effects were less than maximal when stimulation was displaced medially or laterally from the transverse lamellae, or "off beam" (Andersen et al., 1969). This lamellar organization was consistently found throughout the trisynaptic circuit (Andersen et al., 1971b).

In another set of studies, specific EP events at different depths were compared with intracellular (Lomo, 1971a) and extracellular unit recordings (Andersen et al., 1971a). The perforant-path/dentate-gyrus EP at the level of the synaptic layer was found to have a short latency negative wave, the extracellular post-synaptic potential (EPSP), upon which a positive diphasic spike potential was superimposed, the extracellular population-spike (pop-spike). When the recording electrode was lowered to the granule cell body layer these EP events reversed themselves such that a negative going pop-spike was superimposed on a positive going EPSP. The EP maintained this configuration throughout the hilar region and into the granule cell layer on the lower blade of the dentate gyrus. The EPSP was found to be strongest in the middle third of the molecular layer, where the medial perforant-path made contact and the pop-spike was found to be strongest at the level of the granule cells (Lomo, 1971a). Further, when the EPSP and pop-spike were matched with the intracellular recordings it was found that 1) the pop-spike correlated temporally with the firing of granule cells (Lomo, 1971a), 2) the amplitude of the pop-spike correlated with the number of unit discharges (Andersen et al., 1971b), and 3) both the negative EPSP wave recorded in the synaptic layer and the positive EPSP wave recorded in the granular/hilar layers correlated with each other and the EPSP recorded intracellularly (Lomo, 1971a). However, single unit recordings have

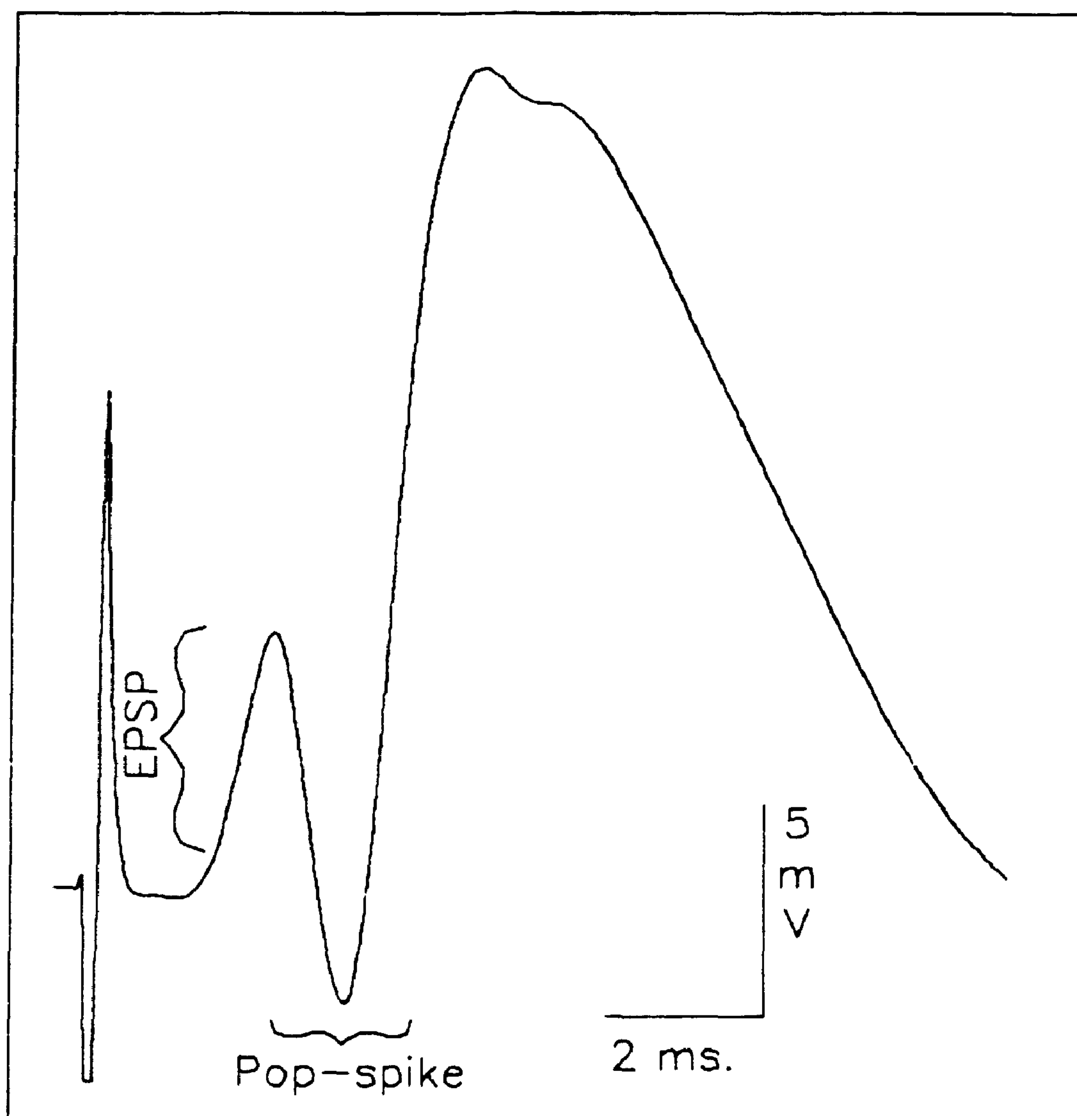
shown that interneurons in the granular layer fire during the positive going EPSP recorded extracellularly at the granular/hilar layers, and thus only EPs recorded at the synaptic layer represent true, uncontaminated EPSPs (Buszaki and Eidelberg, 1982). Figure 2 depicts an averaged EP recorded from the granular/hilar region of the dentate gyrus in response to a brief single pulse applied to the perforant-path. Both the EPSP and the pop-spike in this panel are labelled.

Throughout the course of their examinations the Oslo group found a number of conditions during which the EPs became potentiated or depressed. A short acting facilitation or inhibition could be observed in EPs immediately preceded by others, with the resulting appearance of facilitation or inhibition dependent upon the interval between the paired pulses (Andersen et al., 1966a; Lomo, 1971b). If repetitive electrical stimulation pulses were continually applied, an initial depression occurred, which lasted a few seconds, after which, if the stimulation were continued, the EP would become potentiated (Andersen et al., 1966a; Lomo, 1966). The latter of these potentiation phenomena was subsequently studied in both anaesthetized and unanaesthetized preparations, and what had earlier been called frequency potentiation was now called long-lasting potentiation, or as it is called today Long-Term Potentiation or LTP (Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973).

1.4.1 Long-Term Potentiation:

LTP is an enduring increase in synaptic efficacy in certain monosynaptic pathways, after the application of a series of brief high frequency electrical trains. LTP is typically assessed through the measurement of EPs responding to an unchanging

Figure 2. Depiction of an averaged evoked potential (AEP) recorded from the dentate gyrus of the hippocampus in response to perforant-path stimulation in the same animal. The AEP was recorded from the granular or hilar region. Both the EPSP and the pop-spike are indicated in the figure. The horizontal bar represents 2 ms and the vertical bar represents 5 mV.


EPSP
Pop-spike
5
m
V
2 ms.

afferent volley and it is not uncommon to record 200% increases after LTP (Hargreaves, Cain, and Vanderwolf, 1990). LTP typically exhibits a half life decay constant of approximately 17 days (Racine, Milgram and Hafner, 1983). LTP occurs throughout the hippocampal trisynaptic circuit, and in a large portion of the surrounding limbic structures (Racine et al., 1983; Teyler and Discenna, 1987). LTP has also been shown to occur in nervous system structures as diverse as the deep cerebellar nuclei (Racine, Wilson, Gingell, and Sunderland, 1986) and the superior cervical ganglion (Brown and McAfee, 1982). However, the most robust and enduring effects of LTP are still found within the hippocampal circuitry (Racine et al., 1983; Teyler and Discenna, 1987).

The original Bliss and Lomo (1973) and Bliss and Gardner-Medwin (1973) studies were soon followed by replications from other groups, using both *in vivo* (Douglas, 1977; Douglas and Goddard, 1975) and *in vitro* slice preparations (Alger and Teyler, 1976; Schwartzkroin and Wester, 1975). Both methods continue to be fully utilized in current research (Teyler and Discenna, 1987).

1.4.2 Kindling:

Another neuroplasticity phenomenon developed at approximately the same time as LTP was that of kindling. Kindling was first extensively examined by Goddard, McIntyre, and Leech (1969), who demonstrated that behavioral seizure activity, once evoked by electrical stimulation at specific brain foci, would progressively develop and spread to other brain structures, along with concomitant behavioral convulsions, in a variety of species. Kindling occurs, as a result of the repeated application of afterdischarge (AD) evoking trains, given at regular intervals, usually 24-48 hours

(Corcoran, 1988; Racine, 1978).

1.4.3 Relations between LTP and Kindling:

Some researchers have suggested a similarity between the underlying mechanisms of LTP and kindling (Baudry, 1987). Some of the early LTP and kindling papers even intermixed their terminology (Douglas and Goddard, 1975). However, it has also been argued that the underlying mechanisms of these two neuroplasticity phenomena are different from each other (Cain, 1989; Racine, Burnham, Gilbert and Kairiss, 1986). Further, Cain, Boon and Hargreaves (1990; 1992) have shown a pharmacological double dissociation between kindling and LTP, and between kindling induced potentiation (KIP) and LTP. In these studies urethane anaesthesia prevented kindling and KIP, but not LTP. Conversely, N-methyl-D-aspartate (NMDA) blockade with amino-phosphonovaleric acid (APV) prevented LTP, but not KIP or kindling. The relevance of kindling phenomena as briefly discussed here, will be expanded upon in sections 1.7.4.2 and 1.7.4.3 of this thesis.

1.5 Underlying Mechanisms of LTP:

Since the original reports of LTP in 1973, much has been done to elucidate the underlying mechanisms by which LTP is induced and maintained. As such, the rapidly accumulating results have been the subject of periodic reviews over the last decade (Bliss and Collingridge, 1993; Lynch, 1989; Sarvey, 1988; Teyler and DiScenna, 1984; 1987). Much of the understanding of what mechanisms underlie hippocampal LTP has come from the *in vitro* slice preparation and the examination of the CA3 pyramidal cell Schaffer collateral to CA1 pyramidal cell monosynaptic pathway (Collingridge and

Singer, 1991).

The transmitter system in the CA3 Schaffer collateral to CA1 pyramidal cell pathway depends on the excitatory amino acid glutamate (Collingridge, Kehl, and McLennan, 1983b; Olverman, Jones, and Watkins, 1984; Storm-Mathisen, 1978). In keeping with Watkins and Evans (1981) classification scheme there are NMDA, kainate, and quisqualate excitatory amino acid receptor subtypes. Functionally however, this classification scheme appears to cluster into NMDA and non-NMDA excitatory amino acid subreceptor types, based on the specificity of available antagonists (Lester et al., 1988; McLennan, 1989). As such, non-NMDA receptors appear to be activated during normal synaptic transmission, while NMDA receptors appear to be activated under special circumstances, in particular those that occur during the induction of LTP (Bliss and Collingridge, 1993; Collingridge et al., 1983b; 1983c; 1984; Harris, Ganong, and Cotman, 1984). However, it has been shown that a small NMDA mediated component of low frequency synaptic transmission does occur (Davies and Collingridge, 1989).

Thus, in those hippocampal pathways that utilize the excitatory amino acids, LTP induction can be blocked by competive NMDA antagonists, most notably APV (Bliss and Collingridge, 1993; Collingridge et al., 1983c; Errington, Lynch and Bliss, 1987; Harris, Ganong, and Cotman, 1984; Morris, Andersen, Lynch, and Baudry, 1986), but not by blockade of non-NMDA receptors antagonists such as 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Davies and Collingridge, 1989).

The special circumstances that occur during the tetanic stimulation that activate the NMDA subreceptor are the sufficient depolarization of the membrane, which may in

part occur due to the depression of inhibitory responses (Davies et al., 1990; Collingridge, 1989). As a result, LTP can be affected by the intracellular injection of depolarizing or hyperpolarizing current (Collingridge, Herron, and Lester, 1988a; Malinow and Miller, 1986; Wigstrom, Gustafsson, Huang, and Abraham, 1986), or by reductions in inhibition through the application of GABA antagonists (Davies and Collingridge, 1989; Dingledine, Hynes, and King, 1986; Herron, Williamson, and Collingridge, 1985).

Occupation of the NMDA receptor complex by glutamate following afferent fibre stimulation results in results in expulsion of Mg^{2+} from the NMDA ion channel making it permeable to Ca^{2+} (Herron, Lester, Coan and Collingridge, 1985; MacDermott, Mayer, Westbrook, Smith and Barker, 1986; Mayer, MacDermott, Westbrook, Smith, and Barker, 1987; Mayer, Westbrook, and Guthrie, 1984; Mayer and Westbrook, 1985; 1987; Nowak, Bregestovski, Ascher, Herbet, and Prochiantz, 1984). Therefore, alterations in Mg^{2+} concentration also affect LTP-induction/NMDA-activation (Coan and Collingridge, 1985; Herron et al., 1985; 1986; Huang, Wigstrom, and Gustafsson, 1987), as do alterations in Ca^{2+} concentration, either extracellular or intracellular (Dunwiddie and Lynch, 1979; Harvey and Collingridge, 1992; Lynch, Larson, Kelso, Barrionuevo, and Schottler, 1983, Obenaus, Mody, and Baimbridge, 1989; Turner, Baimbridge, and Miller, 1982).

The intracellular influx and release of Ca^{2+} signals an enzymatic cascade, in which calpain, protein kinase C, type II calmodulin-dependent protein kinase (α-CaMKII), and tyrosine kinase have all been implicated (Bliss and Collingridge, 1993).

Electron microscopy had revealed structural changes after LTP, in the number of synaptic-spine contacts, and in the shape of the spine heads (Greenough, and Chang, 1985; Lee, Oliver, Schottler, Creager, and Lynch, 1979; Lee, Schottler, Oliver and Lynch, 1980). These structural changes after LTP were found to be accompanied by protein synthesis (Duffy, Teyler, and Shashoua, 1981). Thus, incubation of hippocampal slices with protein synthesis inhibitors prevented LTP (Stanton and Sarvey, 1984) and *in vivo* administration of anisomycin prevented a late phase of LTP (Krug, Lossner, and Ott, 1984). Recently it has been shown that the late phase of LTP requires newly synthesized proteins (Frey, Krug, Brodemann, Reymann, and Matthies, 1989; Otani, Marshall, Tate, Goddard, and Abraham, 1989).

1.6.1 Theoretical Relationship of LTP and Learning:

The value of LTP as an electrophysiological phenomenon worthy of study was clearly stated at the end of the original publications. LTP was postulated to relate potentially to learning phenomena (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973). The assumption that substantial overlap exists between the underlying mechanisms of LTP and a number of forms of "hippocampally-dependent" learning is present in much of the current literature concerning LTP and learning (Alkon, Amaral, Bear, Black, Carew, Cohen, Disterhoft, Eichenbaum, Golski, Gorman, Lynch, McNaughton, Mishkin, Moyer, Olds, Olton, Otto, Squire, Staubli, Thompson, Wible, 1991; McNaughton and Morris, 1987; Morris and Whilshaw, 1989; Teyler and Discenna, 1984; 1987). LTP is conceptually attractive for its similarity to Hebb's (1949) postulation of memory formation at the cellular level. In a now famous passage, Hebb (1949) described this

mechanism, a statement which could have just as easily applied to LTP...

> When an axon of cell **A** is near enough to excite a cell **B** and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing **B**, is increased. (Hebb, 1949, p.62).

1.6.2 Evaluation of the Relationship Between LTP and Learning:

Morris and Baker (1984) outlined and assessed four different strategies for evaluating the relationship between LTP and learning. These four approaches were labelled by Morris and Baker (1984) as the "correlational", "prior-enhancement", "prior-learning", and "blockade/facilitation" strategies. Since Morris and Baker's (1984) assessment, much work evaluating the relationship between LTP and learning has been done. However, virtually all of it can be classified under Morris and Baker's (1984) rubrics.

1.6.3 Spatial Tasks Employed in Examining the Relationship Between LTP and Learning:

Although a variety of tasks have been employed in the examination of LTP and learning, most of the crucial studies have focused on tasks that are "spatial" in nature, such that they require the learning of a location or locations that are independent of any proximal or associational cues. A second feature of these spatial tasks is that solving them requires an intact hippocampus. For this reason they have also been characterized

as hippocampally-dependent (Hargreaves, Cain and Vanderwolf, 1990). The association between spatial deficits and hippocampal lesions has been well established and forms the basis of one of the major theories of hippocampal function (Barnes, 1979; McNaughton and Morris, 1987; O'Keefe and Nadel, 1978; Olton, 1990). The three tasks that much of the LTP and learning literature has focused on are the radial-arm maze (Olton and Samuelson, 1976), Barnes'(1979) circular platform task, and the Morris water-maze (Morris, 1981). These three tasks however, do not exhaust the total number of spatial tasks available, nor do they exhaust the number of tasks that have been employed in the examination of LTP and learning. Yet, most of the studies reviewed below rely heavily upon these three tasks.

The radial-arm maze developed by Olton and Samuelson (1976), was one of the first maze tasks that attempted to test memory of locations, instead of testing memory of routes. In its original form, eight-arms projected out radially from a central location. The end of each arm was baited with a small food reward and rats were placed in the centre of the maze and allowed to enter any of the arms until all the food was consumed. An optimal strategy for obtaining all the food with the least amount of maze running was to visit each arm only once during a trial. Arms that were re-entered after being cleared of food were scored as errors, and increased the time required to complete the maze (Olton, 1977). Rats with lesions that effectively isolated both hippocampi from their afferent pathways resulted in the inability to perform the radial-arm maze without errors (Olton, 1983).

Barnes (1979) employed a spatial task, in which a series of 18 holes were located

around the perimeter of a brightly lit circular platform. Beneath one of the holes was located a darkened "safe" box, which could be entered. Rats were placed in the center of this platform and upon its discovery allowed to escape to the safe box. This task was deemed to have reinforcement advantages over previous maze tasks in that it did not require food deprivation (Barnes, 1979). Although animals with experimentally induced hippocampal lesions have not been formally tested on this apparatus, comparisons have been made with aged rats, which have been shown to possess a number of neuroanatomical deficiencies, as discussed below. Results from these comparisions indicate that the senescent rats do not perform as well as younger animals (Barnes, 1979; Barnes and McNaughton, 1985). These studies in particular will be discussed in greater detail below.

Morris (1981) developed the water-maze in order to demonstrate the ability of rats to locate an object without visual, auditory or olfactory cues proximal to the object itself that would aid in the rat's approach. In this task animals are introduced into a circular pool of water and expected to find a platform submerged just beneath the surface. The water is usually made opaque by a substance such as powdered milk. Over a number of trials in which the animals are released from various points around the circumference of the pool, they learn the location of the hidden platform based on distal cues not directly associated with the platform. Strength of the subsequent learning is tested by a probe trial, in which the hidden platform is removed and the amount of time spent in the former region of the platform, is measured as the strength of the learning (Morris, 1981; Sutherland and Dyck, 1982). The Morris water-maze task claims to assess spatial

navigation and learning, and has been found to be particularly sensitive to hippocampal lesions (Morris et al., 1982; Sutherland, Whishaw and Kolb, 1983). However, medial frontal lesions and parietal neocortex lesionsdisrupt this task in a way similar to that of hippocampal lesions (Di Mattia and Kesner, 1988; Sutherland, Kolb, and Whishaw, 1982).

1.6.4 Correlational Strategy:

The correlational strategy involved correlating various parameters common to LTP and learning. The correlations that have been used fall into one of two types: 1) mere convergences, and 2) true correlations. The drawback of this strategy of course is the inability to draw causal inferences about the relationships that are found, and the presence of uncontrolled factors that may account for the systematic variance in both the learning and LTP phenomena. However, despite these problems the correlational strategy has provided some interesting data. Some of the more obvious relations that have been demonstrated by this strategy are the convergence of learning and LTP phenomena upon the hippocampus, the enduring time courses of both phenomena, and of course the similarity between the method for inducing LTP and Hebb's (1949: p. 62) description of the cellular basis of his postulated memory mechanism, or the "Hebbian" synapse. Many investigators have also used this strategy to study more specific parameters of LTP and learning, using a true correlational approach.

One of the more thorough studies of this nature was conducted by Barnes (1979), in which senescent (28-34 mo.) and young rats (10-16 mo.) were trained on the circular platform task and subsequently tested for LTP. The senescent animals in the later stages

of the acquisition phase performed more poorly than their younger counterparts. Similarly, evoked potential measures recorded from a range of stimulation intensities indicated that the younger animals displayed greater baseline synaptic efficacy than did the senescent animals. Further, after repeated LTP sessions, EP data from the senescent animals decayed to baseline levels more rapidly than the younger rats. Additionally, specific correlations indicated that greater amounts of plasticity were related with decreased error scores, measured as the number of holes (in 20° angle increments) away from the safe box and search distance, measured as the distance travelled by the rat, from the release box to the safe box (Barnes, 1979).

In a replication of the above findings, old and young rats were either run in an LTP protocol or a learning protocol (Barnes and McNaughton, 1985). Animals either received daily LTP sessions until the measures asymptoted and thus saturated, at which point decay rates were traced, or acquired the spatial task discussed above and were tested for retention at subsequent post-acquisition intervals. The senescent animals in both cases took longer in acquiring the task or in asymptoting after daily LTP treatments. Similarly, the senescent rats retained less of the task after shorter periods, and decayed back towards their pre-LTP baseline more rapidly than the younger animals (Barnes and McNaughton, 1985).

The age group differences in behavior and plasticity observed in these two studies may be related to the anatomical changes that occurr in the dentate gyrus with age, specifically, reductions in the volume of dendritic tree branching of the molecular layer of the dentate gyrus (Barnes, 1983). Although there remains a relatively constant number

of granule cell bodies and associated mossy fibres, there is a 27% reduction of synapses per unit area in the middle third of the molecular layer, where the medial division of the perforant-path synapses onto the granule cells, when comparing 3 mo. old rats to 25 mo. old rats (Geninisman and Bondareff, 1976; Geinisman, deToledo-Morrell, Morrell, Persina, and Rossi, 1992a; 1992b). These anatomical data agree nicely with the physiological results reported by Barnes in that the senescent rats are slower to achieve their maximal performance and slower to reach LTP saturation, but that the levels of learning and LTP, once achieved are the same as those achieved by the younger animals (Barnes, 1979; Barnes and McNaughton , 1985).

This however, still leaves the within group correlations between repeated LTP sessions and error levels reported by Barnes (1979) unexplained.

Similar correlational analyses have been run after the acquisition of the water-maze, which was followed two weeks later by LTP induction (Cain, Hargreaves, Boon, and Dennison, 1993). The results of these analyses revealed no relationship between learning and LTP. Admittedly, the correlational analyses attempted to relate a single day of training to a single session of LTP, whereas Barnes (1979) found her significant relationships after repeated LTP sessions matched against the final trials of the platform task. Thus, more similar to Barnes (1979) correlational analyses, were those run by Jeffery and Morris (1993), who found significant correlations between asymptotic levels of LTP and water-maze performance. Specifically, the amount of time spent in the vicinity of the platform in a post-acquisition probe trial correlated positively with the asymptotic levels of LTP of the EPSP (Jeffery and Morris, 1993). These correlations

were replicated across 2 experiments and respectively accounted for 90% and 64% of the variance in these studies. Thus, these correlations support those of Barnes (1979) and tentatively suggest that relations to learning may be found at, or near capacity levels.

Another correlational study performed by Ramirez and Carrer (1989) found that shuttle box avoidance acquisition was correlated with the frequency threshold for inducing subsequent LTP in hippocampal slices. In this study, rats were trained in a simple oneway active avoidance task, and sacrificed three days later for slice work. LTP was induced in the slices by applying a series of trains progressively increasing in their frequency, with EPs assessing the induction of LTP 15 minutes after the delivery of every train. The lowest frequency that induced LTP was identified as the threshold frequency. The threshold frequency was negatively correlated with the percentage of successfully conditioned trials during training, such that better performance was related to a lower LTP threshold. The subsequent amount of LTP induced however, was not related to performance in the avoidance task. Although the results of this study are intriguing, a close examination of their Figure 2, which depicts the scatterplot and regression line, reveals an uneven distribution of the data. In particular, one tight clustering of 5 animals that performed quite poorly, between approximately 10% and 15% successful trials out of 50, indicate that the LTP frequency threshold may have been above the highest frequency used (200 Hz), and thus may actually have been a failure to induce LTP altogether. As such, a clean linear association may not exist between LTP frequency threshold and percentage of correctly performed avoidance trials. In the case of the 5 animals it is possible that some non-specific stress related factor affected both

the animals' performance and the ability to induce LTP (Diamond, Bennett, Fleshner, and Rose, 1992; Dubrovsky, Liquornik, Noble, and Gijsbers, 1987; Mana, Zigmond, and Berger, 1992).

As can be seen above there have been a number of studies using the correlation strategy. However results from this approach, although suggestive are far from definitive. Most of the data from this approach remain as vague similarities or mere convergences of various phenomena. Further, the linear relationships that do hold up, and are replicable commonly can be explained by intervening uncontrolled factors, such as the decreased size of dendritic trees in senescent animals. As such, the initial drawback to this approach, stated at the beginning of this section as the inability to draw causality remains. However, some evidence of stronger linear relationships between learning and plasticity at or near saturation levels, have been found and tentatively replicated, and therefore this strategy is not without its merits.

1.6.5 Prior Enhancement Strategy:

The prior enhancement strategy involves assessing the influence of prior LTP upon learning. Several different appoaches within this strategy have been employed. First, LTP-like trains have been used as conditioning stimuli (CS) for a footshock avoidance task (Ott, Ruthrich, Reymann, Lindenau, and Matthies, 1982) and an operant bar pressing task (Skelton, Miller and Phillips, 1985). Second, LTP induction has been used to improve subsequent acquisition of the conditioned nictitating membrane (NM) response (Berger, 1984). Finally, asymptotic LTP induction has been used to "saturate" and therefore impair the acquisition of spatial information (Castro, Silbert, McNaughton,

and Barnes, 1989; McNaughton, Barnes, Rao, Baldwin, and Rasmussen, 1986). Slightly different assumptions underlie each of these approaches. The first assumes that most of the information required for hippocampally dependent learning must flow from the entorhinal cortex through the perforant-path to the dentate gyrus of the hippocampus. Therefore if LTP is like learning, then electrical stimuli delivered to the perforant-path may be able to take on discriminative properties, such that they can be used as a CS. The second approach assumes that if both LTP and learning are based on increases in synaptic efficacy, then prior LTP induction may assist in the passage of information required during subsequent learning. The final approach assumes that if similar mechanisms underlie both LTP and learning, then given a finite amount of plasticity in the hippocampus the two phenomena compete with each other for that amount of plasticity. That is, if repeated LTP sessions are given then eventually all the plasticity will be used up and the amount of LTP will asymptote. At this saturation point there would be no available plasticity through which learning could occur.

The first two experiments mentioned above were actually deemed to use more of a "detection" paradigm then a true prior enhancement paradigm (Morris and Baker, 1984). As such these studies indicate only that animals can detect perforant-path stimulation and use its presence to produce behavioral responses. Also, the conditioning trains used by Ott et al. (1982) more than likely induced some form of synaptic disruption or AD, given the stimulation parameters of the CS trains (15 Hz delivered in 660 msec bursts every second for up to a maximum of 9 seconds per trial, for 40 training trials per session). Further, their reports of an increased EPSP slope and a depressed

pop-spike following conditioning, although seemingly contradictory, are actually supportive of a post-ictal state. First, Ott et al. (1982) recorded EPs in the molecular layer of the dentate gyrus, where the components are reversed in comparison to recording in the hilus, such that they recorded a negative going EPSP and a positive going pop-spike. However, the EPSP measurement employed by Ott et al. (1982) was not adjusted for this reversal. Thus, the change in the EPSP after training was mistakenly reported as an increase, when in fact it was actually a suppression. If interpretated as a decrease then the EPSP data become consistent with the observed decrease in pop-spike amplitude, of which both phenomena occur following AD, but precede KIP. Seen in this light, Ott et al.'s (1982) further finding that EPSP slope increases were associated with good learners and not associated with bad learners in this paradigm only reflects the ability of these animals to detect the more severe electrical brain stimulation (EBS). Similarly, Skelton et al.'s (1985) report of increased acquisition rates of EBS as a CS, after LTP induction, probably also reflects only an increase in sensitivity of the rats to detect such EBS.

Berger (1984) implanted rabbits with stimulating and recording electrodes in the perforant-path/dentate-gyrus system. Upon recovery, the rabbits were given daily LTP sessions over a four day period (8 trains, 400 Hz, 20 msec duration) or were given control stimuli. The rabbits were subsequently trained on the standard classical conditioning paradigm, during which the NM was conditioned to respond to a tone (CS) paired with an airpuff (UCS). Results indicated that the LTPed animals acquired the conditioned NM response much sooner than the control animals, even when more

stringent learning criteria were used (Berger, 1984). The meaning of these results becomes less clear given that bilateral hippocampal lesions do not affect the acquisition of this task (Solomon and Moore, 1975) or alternately facillitate its acquisition (Schmaltz and Thieos, 1972). However, hippocampal lesions do affect the acquisition of trace conditioning, in which there is no period of overlap between the CS and UCS (Weisz, Solomon, and Thompson, 1980). Hippocampal lesions also affect reversal learning of the NM response. In this paradigm one tone is initially paired with the air puff (CS^+/UCS), while another tone remains unpaired (CS^-). After an initial learning criterion is reached, the CS^+ and CS^- are reversed in their meaning. Hippocampally lesioned rabbits take approximately three times as long to learn this new or reversed discrimination as do normal controls (Berger and Orr, 1983). Yet, in this latter paradigm Rioux and Robinson (1993) have recently shown that LTP induction immediately prior to each training session had no effect on the initial acquisition of the NM response nor on the reversal phase.

Contrary to the results of Berger (1984), McNaughton and Barnes and their colleagues (Castro et al., 1989; McNaughton et al., 1986) reported impaired performance after asymptotic LTP induction on the brightly lit platform task described above, and on the water-maze, developed by Morris (1981).

McNaughton et al. (1986), conducted a series of four experiments that explored the effects of repeated LTP induction upon the acquisition and retention of Barnes' (1979) platform task, and the radial-arm maze (Olton and Samuelson, 1976). In the first experiment, rats were trained to find a specific location or tunnel on the platform task. Once asymptotic performance was reached, one group of rats received LTP-inducing

stimulation, while the other group received an equivalent amount of current but in a configuration that would not induce LTP. Both groups were then retested on the platform task 24 hrs following the last LTP or control session, but required to find a new location. Results indicated that both groups made the same error, by returning to the previous hole on the first relocation trial. However, on subsequent trials, the control animals began making fewer errors, while the LTPed rats continued to make entry errors before finding the correct position. The second experiment was similar in its procedure to the first, except that the rats received LTP or control stimulation 5 minutes after the first and second relocation trials. Results again indicated that the LTPed rats were impaired in learning the new location and continued instead to return to the old hole. In the third experiment, asymptotic LTP or saturation was induced over a 12 day period prior to the rats learning the platform task. Results indicated that rats receiving LTP saturation treatments were impaired on trials 15 and 16 of the platform task relative to rats given control stimulation. Both groups of rats were then allowed to rest for a two month period, during which time the LTP had presumably decayed down to baseline levels. The rats were then retrained on the platform task using massed trials (4 per day for 7 days), followed by 6 sessions of LTP or control stimulation over a period of two days. The rats were then retested on the platform task located in a different room. Results from this second part of the third experiment indicated that the rats receiving LTP performed worse in the new room compared to control animals, although there was significant improvement over the 16 trials in the new room. The fourth and final experiment entailed training animals on the radial-arm maze until asymptotic performance was achieved.

These animals were then given identical LTP treatments as given in the second portion of the third experiment, and retested on the radial-arm maze. Results indicated that no differences existed between radial-arm maze performance before or after the LTP treatment, indicating a disruption of neither Olton's working nor reference memory (McNaughton et al., 1986).

The third of the above experiments was then repeated with the Morris water-maze (Castro et al., 1989). Here, two groups of animals received LTP saturation, while one group received low frequency control stimulation for 14 days. The control group and one of the saturation groups were then trained in the water-maze, immediately following the last LTP session. The other group that had received LTP saturation, was allowed to decay for 15 days and then trained in the water-maze. Results indicated that the immediate saturation group was impaired in the water-maze acquisition and retention, whereas both the control group tested at the same time and the saturation group tested 15 days after the last LTP session easily acquired the water-maze task.

The data from these two studies has provided a formidable amount of evidence linking together the mechanisms underlying LTP and learning. However, the evidence is not altogether unassailable. First, the second experiment from McNaughton et al. (1986) is not a true prior-enhancement study in that LTP stimulation is delivered immediately following a learning trial. Although this paradigm may be worth investigating upon its own merit, especially in light of recent developments discussed below, it is more of a disruption paradigm, such as was done during much of the early 1970s using seizure-inducing electrical brain stimulation (EBS). Second, an impairment

in the platform task in the first portion of the third experiment of McNaughton et al. (1986) is only found on trials 15 and 16, (on days 15 and 16 following the last LTP session), and not presumably on the first 14 trials following the last LTP session. In contrast, Castro et al. (1989) found no water-maze impairment in one of the saturation groups of rats, in which the LTP was allowed to decay for 15 days. Third, although the data were available, particularly from McNaughton et al. (1986), the correlational analyses emphasized in Barnes (1979), were conspicuously absent in either of the later saturation works. However, this may partially be explained by Jefferey and Morris' (1993) more recent finding of a positive correlation between degree of saturation and water-maze performance, which was replicated in two different experiments. Fourth, and partially related to the third, is that varying amounts of LTP were induced throughout the various experiments comprising the two studies, which were inconsistently related to varying degrees of behavioral impairment. Thus, the least amount of LTP induced after 12 sessions of LTP produced a subsequent learning impairment 15 days later on the platform task, while the greatest amount of LTP induced after only 6 sessions of LTP produce no impairment upon the radial-arm maze task. Finally, and probably most devastating, has been the current failure to replicate the Castro et al. (1989) results on either the Morris water-maze (Cain, Hargreaves, Boon, and Dennison, 1993; Jeffery and Morris, 1993; Korol, Abel, Church, Barnes, and McNaughton, 1993; Sutherland, Dringenberg, and Hoesing, 1993), or the radial-arm maze (Robinson, 1992). This latest round of replication attempts also includes individuals involved in the original saturation findings, who based on current data willingly question the original findings (Bliss and

Richter-Levin, 1993; Korol et al., 1993).

Epileptiform activity induced by kindling on the other hand, appears to have profound effects on the acquisition of spatial tasks (Cain et al., 1993; McNamara, Kirkby, dePape, and Corcoran, 1993; Robinson, McNeill, and Reed, 1992). However, these effects are related to the strength of the seizure and not to kindling *per se* (Cain et al., 1993; McNamara et al., 1993). Additionally, the disruptive effect is only temporary, dissipating within 24 hours (Cain et al., 1993; McNamara et al., 1993; Robinson et al., in press).

In contrast to the short term effects of kindling upon spatial learning are its effects upon the NM conditioned response discussed earlier. Kindled rabbits acquired the normal NM conditioned response faster than their non-kindled controls, but were severely impaired in acquiring reversal conditioning of the NM response (Robinson, Port, and Berger, 1989). Of particular interest here is that 3 of the 5 kindled rabbits had not reached reversal discrimination learning criterion 30 days following the last seizure (Robinson et al., 1989). Thus, to summarize briefly, both hippocampal LTP and kindling accelerate the acquisition of NM conditioning, whereas hippocampal lesions have no effect. In contrast, hippocampal lesions do impair trace conditioning and reversal learning of NM conditioning. The latter task is also impaired by kindling. Earlier Racine and Kairiss (1987) suggested that the observed differences between the effects of LTP on the different learning paradigms (spatial and NM conditioning) may be due to the differing role of the hippocampus in each task. This explanation may still be of some use, but has become further complicated by the effects of kindling, and in retrospect potential species

differences in response to the two neuroplasticity phenomena.

Returning to the issue of AD in spatial learning, in the case of the earlier saturation studies, McNaughton et al. (1986) explicitly state that AD was not observed throughout their experiments; whether this also applies to Castro et al. (1989) is unknown. Similarly, AD was never observed by Cain et al. (1993) even though hippocampal ECG was continuously monitored throughout the tetanization procedures. Further, it is quite difficult to induce AD using high frequency stimulation (400 Hz) such as that used to induce LTP, requiring high frequency trains of no less than 400-500 ms duration (Cain et al., 1993).

As can be seen from the above review, some of the strongest evidence functionally linking LTP and learning together has come from this approach. However currently, data from the saturation paradigm are in a state of disarray, with some degree of suspicion being cast on the original findings. It is still possible that useful data from this paradigm will emerge, but the current inability to replicate the original findings suggests that a variety of the procedures require a technical precision and/or special conditions that are not usually associated with robust phenomena. As such, the data that finally arises from this paradigm may be of limited application.

1.6.6 Prior Learning Strategy:

The prior learning strategy involves assessing changes in the hippocampal EP as animals acquire a behavioral task. This strategy has also been labelled behavioral-LTP (Teyler and Discenna, 1987). This strategy supposes that if LTP and learning processes are similar then LTP-like processes may be observed after learning. Early studies of this

kind revealed LTP-like increases in the perforant-path/dentate-gyrus EP after conditioning of the nictitating membrane response in rabbits, a footshock motivated avoidance task, and an appetitively motivated operant bar pressing task (Ruthrich, Matthies, and Ott, 1982; Skelton, Scarth, Wilkie, Miller and Phillips, 1987; Weisz, Clark, Yang, and Thompson, 1982).

However, Morris and Baker (1984) warn that when employing this strategy a number of controls must be observed in order to avoid "Vanderwolf's Dilemma". Simply stated, this dilemma suggests that it is very difficult to tease apart neural activity reflecting the behavioral response required to perform the learned task, from the neural activity reflecting the learning itself, since before learning neither activity is present and subsequent to learning, both sets of neural activity are present. This becomes especially critical if one is attempting to record potentially subtle increases in synaptic efficacy associated with learning, for not only does the endogenous slow wave activity of the hippocampus vary with behavior, but so do the EPs.

The relationship between behavior, hippocampal EPs, and the underlying slow wave activity has been shown by numerous researchers (Brankack and Buzsaki, 1986; Buzsaki, Gratyan, Czopf, Kellenyi and Prohaska, 1981; Cain, Hargreaves, and Boon, 1992; Green, McNaughton, and Barnes, 1990; Hargreaves, Cain, and Vanderwolf, 1990; Leung, 1980; Racine and Milgram, 1983; Winson and Abzug, 1978). Specifically, it has been shown that perforant-path/dentate-gyrus EPs are larger during immobility than they are during walking/running (Brankack and Buzsaki, 1986; Hargreaves et al., 1990).

Morris and Baker (1984) do suggest however, that Ruthrich et al. (1982) and Skelton et al. (1987) may have gotten around the problem of ongoing behavior by recording in a location that is temporally and spatially distinct from that of the learning location. These behavioral controls were not deemed sufficient by Hargreaves et al. (1990), who also criticized the previous studies for not employing hippocampally dependent tasks. As such the issue of behavioral-LTP was re-examined by Hargreaves et al. (1990), employing both a radial-arm maze task and the technique of "behavioral clamping" a term suggested by Ranck (1983). Behavioral clamping is analogous to the voltage clamp technique, where the membranes of cells can be held at a constant voltage, while the current is allowed to vary. Applied to behavior, clamping simply means holding behavior constant while recording from awake whole animal preparations, and thus allowing the level of the other factor of interest to vary, which in this case was learning. Thus, Hargreaves et al. (1990) recorded EPs 22 hours after each daily learning session, recorded the EPs in a room different from that in which the learning took place, and most importantly recorded EPs under consistent behavioral conditions. When this control was employed in addition to the examination of a hippocampally dependent task, behavioral-LTP was not observed (Hargreaves et al., 1990). These findings were also replicated for the Morris water-maze task (Cain et al., 1993). Although not discussed in the same context, similar results were found for the low frequency stimulation control groups run in the water-maze task by Castro et al. (1989) and run in the radial-arm maze task by Robinson (1992).

As indicated by the above review, several studies have examined the prior learning strategy. The early positive results from this approach were simply flawed either in the recording techniques or in the employment of non-hippocampally dependent tasks. When both of these factors are controlled for, no positive evidence is forthcoming from these paradigms. Further, there are no compelling theoretical reasons why such a gross change in synaptic efficacy would be observed after the acquisition of a single task.

However, a variant of the behavioral-LTP strategy involves the use of complex environments. This paradigm entails rearing one set of animals together in a large cage filled with ramps, boxes and toys that are changed on a regular basis, and rearing another set of animals in standard laboratory cages. A number of these studies have been performed, some of which have employed the appropriate behavioral controls, in which LTP-like increases were still observed (Green and Greenough, 1986). Theoretically, since many aspects of the rat's environment have been altered, it is plausible that a change in the synaptic efficacy may be recorded. Conversely, this also becomes one of the major drawbacks to this paradigm in that what actually contributes to the LTP-like increases is unknown. This paradigm is worth further exploration and thus forms a substantial portion of the present thesis. As such, the complex environment paradigm and its related studies will be discussed in greater detail later in this chapter.

1.6.7 Blockade/Facilitation Strategy:

The final strategy outlined by Morris and Baker (1984) was that of facilitation and blockade. This strategy seeks experimental manipulations that either enhance or block LTP, with the assumption that if parallel mechanisms underlie both LTP and learning

then these experimental manipulations should similarly affect learning. The converse should also be true, such that any manipulations affecting learning should also affect LTP in the same way. Of the four strategies evaluated, Morris and Baker (1984) conclude that the blockade/facilitation strategy had the most power for delineating the relationship between LTP and learning. Consequently, it is not surprising that Morris and his colleagues have since spent a substantial amount of time and energy exploring this strategy.

Throughout most of this work the Morris water-maze as described earlier was employed (Morris, 1981). This task was first established by Morris and others to be hippocampally dependent, such that aspiration, electrolytic, or chemically-induced hippocampal lesions resulted in deficits in acquiring this task (Morris, Garrud, Rawlins, and O'Keefe, 1982; Morris, Hagins, and Rawlins, 1986; Sutherland, Kolb, and Whishaw, 1982; Sutherland, Whishaw, and Kolb, 1983).

Initially however, the first cluster of studies to use this approach focused on the LTP and learning mechanism proposed by Baudry and Lynch (1980a; 1980b; Lynch and Baudry, 1984) involving the Ca^{2+}-dependent protease calpain, which if inhibited by leupeptin blocked the upregulation or increase in glutamate binding (induced by *in vitro* increases in calcium concentrations) and the development of LTP *in vitro* and *in vivo* (Oliver et al., 1989; Staubli et al., 1988). The observations that similar upregulation of glutamate binding occurred following NM conditioning in rabbits (Mamounas, Thompson, Lynch and Baudry, 1984) and in rats, following daily housing in a complex environment (Staubli, Baudry, and Lynch, unpublished; as cited by Lynch and Baudry,

1984) led to the attempt to block radial-arm maze performance with leupeptin (Staubli, Baudry, and Lynch, 1984). Chronic intraventricular infusion of leupeptin by minipumps induced a greater number of incorrect arm entries after a maximum delay of 4 hrs between the fourth and fifth arm choices (Staubli et al., 1984). Similar deficits were not seen after administration of saline or aprotinin, a serine proteinase inhibitor. Further, the low doses of leupeptin did not alter water and food consumption, change body temperature or affect spontaneous locomotor activity and rearing (Staubli et al., 1984).

This method was next applied to the Morris water-maze (Morris, Hagan, Nadel, Jensen, Baudry, and Lynch, 1987). The results of this study indicated that chronic infusion of leupeptin did not prevent the acquisition of the hidden platform, and the rats treated with the Ca^{2+}-dependent protease inhibitor asymptoted in their performance just as quickly as the saline treated rats. It was noted however, that the saline treated rats consis‸ ıtly had shorter escape latencies on the 1st trial of each day, which was likened to the earlier radial-arm maze result of the impaired arm-entry after the imposed delay (Morris, et al., 1987). Following the failure to prevent acquisition of the water-maze, a subsequent study was run in which an assessment of glutamate binding in normal rats acquiring the water-maze was made (Morris, et al., 1987). These results indicated no difference in hippocampal glutamate binding between animals that had learned the location of the hidden platform and control animals, yoked for the time spent swimming in the maze (Morris, et al., 1987).

In evaluating this set of studies, once again, differences between NM conditioning and spatial tasks are readily apparent. It is also unknown whether acquisition of the

radial-arm maze results in an increased glutamate binding, but it may be supposed that the result would be similar to the acquisition of the water-maze, where no differences were found. In contrast to the spatial tasks, equivalent increases in glutamate binding occurred following complex environment housing and following NM conditioning (Lynch and Baudry, 1984). This finding is odd within itself, since one would suspect that the effects of complex environment housing would be far more encompassing than the acquisition of a single conditioned response. However, similarities in glutamate binding increases, in such disparate behavioral paradigms may be accounted for by species differences. Thus, in summary the data indicating that a Ca^{2+}-dependent protease, potentially calpain, is critical for the development of LTP is consistent, but its critical involvement in various forms of learning, most notably spatial learning, is less than consistent.

After their inital work with the water-maze and hippocampal lesions, Morris and his colleagues then focused upon NMDA mechanisms. Morris, Anderson, Lynch, and Baudry (1986) in two separate behavioral experiments assessed the effect of continuous intraventricular APV infusion on the standard spatial version of the water-maze and on a non-spatial, visual discrimination version of the water-maze (Morris, Hagan, and Rawlins, 1986). In this latter task, two visible platforms, one painted grey and the other painted with black and white stripes were used. One of the platforms was rigid and would provide escape from the water, while the other floated on the surface and did not provide a stable escape surface. The two platforms were continuously moved around the maze from trial to trial, such that a spatial strategy could not be implemented in order

to solve the task. Morris and his colleagues found that the APV infusions retarded the acquisition of the spatial task, but not the visual discrimination task. In the third experiment another set of rats were implanted with the cannulae and mini pumps. Between 6-12 days after implantation and continuous APV infusion, animals underwent an accute LTP procedure during urethane anaesthesia. Results indicated that baseline recordings in the APV versus control groups were not different from each other, but after the application of two tetanizing trains at 20 minute intervals only the APV infusion group remained close to baseline levels (Morris et al., 1986).

Keith and Rudy (1990) have criticized the use of NMDA antagonists in learning and LTP studies for being unable to show a clear absence of sensori-motor impairments in the treated animals. In a detailed critique they suggest that Morris' APV rats were motorically impaired, and did actually acquire some spatial information. As evidence, Keith and Rudy (1990) draw attention to the reversal phase, during which the hidden platform was shifted to the opposite quadrant and point out that if the APV rats had not learned anything about the platform location their initial performance on the reversal phase should have been identical to their asymptotic performance during the acquisition phase. This was not the case, since the APV treated animals initially took longer to find the new platform location during the reversal phase than they had during asymptotic performance of the acquisition phase. Keith and Rudy (1990) concluded that much like the control rats, the APV animals were searching the previous platform location. They further noted Morris et al.'s (1986) observations of a number of the APV treated animals falling off the platform, potentially indicating sensori-motor impairments (Keith and

Rudy, 1990). In support of this interpretation Cain, Saucier, Hargreaves, Boon, Hall, DeZousa, and Wilson (1993) have documented a number of water-maze behaviors that are incompatible with acquisition of the hidden platform, and found a higher incidence of these behaviors in rats repeatedly given intraventricular administration of APV. One of the behaviors, platform deflections/walk-overs, in the animals encountered the platform, but failed to mount or remain on it, is particularly interesting, since hippocampally lesioned rats exhibit this behavior to a significantly lesser degree (unpublished results).

In response to the spatial savings argument Morris (1990) pointed out that hippocampal animals also show some spatial savings when given the reversal phase. In response to the criticism that APV infused rats were motorically impaired, Morris (1990) argued that rats infused with APV after the initial acquisition phase did not exhibit spatial deficits in finding the previously learned location (Butcher, Hendry and Morris, 1989). Similarly, rats given pretraining on the task demands before drug administration did not display the same motor impairments on the platform, as had been originally observed (Morris, 1988). Additionally, Butcher, Hamberger, and Morris (1991) recorded APV levels in a number of brain stuctures using high performance liquid chromatography (HPLC). Results indicated that the intraventricular infusions produced relatively uniform distribution of APV in both hippocampi, the striatum, and portions of the frontal and visual cortex (Butcher et al., 1991). Further, direct infusion of APV onto the visual cortex did not hamper the visual discrimination task. Finally, rats with APV directly infused into the hippocampus were able to compensate for the motor deficits by the

reversal phase, while still exhibiting spatial deficits and LTP blockade (Morris et al., 1989). Autoradigraphs, made of tritiated APV that was intracortically microinfused using identical procedures, indicated that the APV was largely restricted to the hippocampus, although some of the drug flowed back along the cannula tract Morris et al., 1989).

One aspect of Morris' work that has not received criticism is the assessment of normal synaptic transmission during APV administration. Whether or not this transmission is truly normal is difficult to assess since both experimental and control animals were placed under urethane anaesthesia, which itself has been shown to induce changes in synaptic communication (Cain et al., 1992), which may further interact with temperature changes (Moser, Mathisen, and Andersen, 1993; Cain, Hargreaves, and Boon, 1993). Further, other studies have shown that NMDA antagonists can disrupt normal synaptic transmission (Abraham and Mason, 1988; Collingridge, Kehl, and McLennan, 1983c; 1984). However, Lester, Herron, Coan, and Collingridge (1988) have argued that the normal synaptic transmission was affected because these studies used non-competitive antagonists or a racemic mixture (equal parts of both dextrorotatory and laevorotatory molecules) of APV, which are not as NMDA specific. In light of this it is worth noting that the earlier studies of Morris and his colleagues used the racemic mixture of AP5, and in their later studies used only the *D*-isomer. It is also worth noting that Cain et al. (1993) in uncovering the more global water-maze deficits also used the racemic mixture of AP5.

Assuming for the moment that the synaptic transmission under the administration of APV was normal, then the work of Morris and his colleagues has shown parallel

disruption of spatial learning and LTP using the same pharmacological treatment. In summary, Morris' claims that LTP and water-maze learning can be disrupted at the same focal dose, and that the behavioral disruption is not dependent upon visual acuity and that observed motor impairments are secondary to the spatial impairments.

Recently, two groups (headed by Kandel and Soriano, and headed by Tonegawa, respectively) have been able to genetically engineer mutant strains of mice, known as "knockouts", in which the genes coding for some of specific kinases involved in LTP were absent (Baringa, 1992, Grant, O'Dell, Karl, Stein, Soriano, and Kandel, 1992; Silva, Stevens, Tonegawa and Wang 1992; Silva, Paylor, Wehner, and Tonegawa, 1992).

The mice being examined by the Kandel and Soriano group were selectively missing genes for different tyrosine kinases (*Fyn, Yes, Src, Abl*), whose phosporylation had earlier shown to be triggered by glutamate binding (Bading and Greenberg, 1991). If the activity of these kinases were specifically blocked LTP induction was prevented (O'Dell, Kandel and Grant, 1991).

The mice tested by the Tonegawa group were missing the gene coding the α-isoform of calcium-calmodulin-dependent protein kinase type II (α-CaMKII). Earlier α-CaMKII was suggested to be a mechanism underlying LTP due to its CNS concentration and intracellular localization (Miller and Kennedy, 1986) and it was later found that specific blockade of CaMKII also prevented LTP induction (Malinow, Schulman, and Tsien, 1989).

focal in its effects, and more importantly does not hamper normal cell communication, of which a number of the NMDA blockade studies are suspected (Lester et al., 1988). Conversely, this technique has the disadvantage of disrupting every other function dependent upon the mutated gene throughout the animal's life. It does appear however, that this disruption, as of yet, has not been found to have severe consequences, since the mutated mice have a normal life span, and can reproduce (Silva et al., 1992).
Also, the genetic knockout technique still appears to be in its infancy, and therefore shows promise in its application to a wide variety of problems facing neuroscience beyond the restricted field of LTP.

1.8 Interim Summary of the Learning and LTP Literature:

As can be seen from the extensive, but by no means exhaustive review above, there have been an ample number of studies using a variety of approaches examining the relationship between LTP and learning. However *in toto* these results are far from definitive. Most of the data from the correlation strategy remain as mere convergences of various phenomena. However, claims of stronger relationships have been somewhat successful, with the findings of Jeffery and Morris (1993) tentatively backing up those of Barnes (1979). Data from the prior enhancement strategy are either unconvincing as in the stimulus detection paradigm, or nonreplicable, as in the saturation paradigm. The data from the prior learning strategy, with the exception of that derived from the complex environment paradigm, are simply flawed or non-existent in the critical tests of hippocampally dependent tasks. Therefore it does appear that in accordance with Morris and Baker (1984) the strongest evidence linking LTP and learning together derives from

cessation of the jumping behavior.

The second task was the standard hidden platform version of the Morris water-maze. Results from this task indicated an impairment in locating the platform that persisted even after asymptotic performance was reached for both groups. Although the knockout mice were performing better at the end of the task than at the beginning of the task, the probe trial indicated no differences in quadrant search time. The hypothesis that the knockout mice's improved performance was the development of an optimal search strategy was tested by placing the hidden-platform in one of 7 different locations, at the same radius as the original hidden location. Results from this task indicated that the control mice found the platform much quicker if located in the original position than in any of the random locations, while the knockout mice exhibited no difference in finding the platform regardless of its location.

The final swim-maze task was a much simpler "+" maze, where a submerged platform was consistently located at the end of one of four arms. On this task, the knockout mice achieved criterion performance within the same range as their wild type controls (Silva et al., 1992).

In behaviors not associated with spatial learning, the knockout mice were judged as normal, based on weight gain as pups, and the display of whisking (darting away to cover), sniffing, and mating behaviors (Silva et al., 1992). The Tonegawa group did note however that the knockout mice were "jumpy" and would avoid human-touch as much as possible. This aberrant behavior was suggested to be similar to that of hippocampally lesioned animals and therefore reinforced the hippocampal/LTP/learning associations

(Silva et al., 1992). A similar argument was employed to explain the hyperactivity found in the knockout mice's open-field behavior and exploratory behavior of a "Y-maze" (Silva et al., 1992).

Thus, the evidence provided in this set of studies indicate that there is mutual dependence of LTP in the CA1 region of the hippocampus and spatial learning, upon the α-CaMKII kinase (Silva et al., 1992).

The evidence given in these studies, although suggestive of a link between the underlying mechanisms of spatial learning and LTP, is not conclusive. First, the evidence suggesting that the knockout mice are behaviorally normal is weak, with a number of observations, and behavioral assays suggesting the opposite, such as the hyper-reactivity and the hyperactivity. The argument that these behaviors are similar to those occurring after hippocampal lesions is weak and similar claims made for rats treated with MK801 have been shown not to hold up to close scrutiny (Hargreaves and Cain, 1992). Second, the supposed inability of the knockout mice to acquire spatial information was not completely demonstrated. Although the quadrant search times showed no differences, the number of annulus crossings during the probe trial did favour (but not significantly) the original platform position and did not favour (significantly) the symmetrical position located in the opposite quadrant. Additionally, although the "+ maze" was designated as being much simpler to solve, it still required navigation based on distal cues in order to choose the correct arm. Data regarding LTP, although not seriously flawed, was not entirely consistent either. Slices drawn from 9 of 11 control animals exhibited LTP, while only 2 of 16 slices drawn from the mutant strain exhibited LTP, which was defined

as a 20% or greater increase, sustained for a minimum of 30 minutes. An examination of those slices from the knockout mice that exhibited LTP indicated that the LTP was similar to that of normal slices (Silva et al., 1992). As such, although the incidence of LTP in the knockout mice was rare, it did occur, and appeared to be similar to normal LTP.

The Kandel and Soriano group in very similar procedures found no differences between hippocampal slices drawn from their *Fyn* mutant mice and wildtype littermates, on maximal values of the field EPSP, paired-pulse facilitation, or post-tetanic potentiation in the presence of APV. Differences in LTP however, were observed between slices drawn from the mutants and their wildtype littermates, at both low and high stimulation intensities, although the high intensity did produce LTP in the *Fyn* slices. LTP could also be induced equally well in slices drawn from the different littermates if low frequency pulses were paired with strong postsynaptic depolarizing current. These results suggested that the *Fyn* deficit did not affect normal synaptic transmission, nor alter the synaptic depolarization induced by LTP trains, which were of sufficient magnitude to activate the NMDA complex (Grant et al., 1992). These results did suggest that *Fyn*, subsequent to NMDA activation altered the LTP induction threshold, but that the increased threshold could be overcome by high intensity stimulation (used to induce the LTP) or by strong depolarizing current applied to the post-synaptic cell (Grant et al., 1992). However, even in the *Fyn* knockout mice, the LTP impairments depended upon the genetic background of the mice, such that in one strain the *Fyn* knockout impaired LTP induction, while in another strain it did not (Barinaga, 1992).

Behaviorally, the *Fyn* mutant mice showed spatial deficits on the hidden platform task, never really improving beyond initial performance levels. However, the mutant mice were able to master swimming to a platform marked with a flag to the same degree as the wildtype controls (Grant et al., 1992). Additionally, the "jumpiness" observed in the α-CaMKII knockout mice is not present in the Fyn knockout mice (Cain lab, unpublished data).

Neuroanatomically, the arrangement of cellular organization was intact in the neocortex and cerebellum. However, the granule cell and the CA3 pyramidal cell layers both displayed irregularities from their normal *C*-curvature exhibiting what Grant et al. (1992) called "undulations", where the cell layer enfolded into extra spikes. These undulations represent an increase in cell number of approximately 25%, but the mossy-fibre projections appeared normal, and the apical dendrites of CA1 extended all the way through the stratum radiatum. However, these same CA1 dendrites appeared to be less organized, which may have been secondary to the CA1 pyramidal cells being less densely packed in the *Fyn* mutant mice. The disorganization of these pathways may be related to the "LTP-blunting" and the spatial deficits, but still appeared to function physiologically within the normal range, as a variety of the electrophysiological tests demonstrated (Grant et al., 1992).

This series of studies is also not without its problems, but the multi-faceted approach, and numerous control conditions, have produced strong evidence linking the underlying mechanisms of LTP and learning together. Behaviorally, the *Fyn* mutant mice exhibit the same initial deficit in the swim to visible target task, that was observed by

Silva et al. (1992). However, no explanation is given for this result, and the refusal to remain on the platform explanation evoked by the Tonegawa group cannot be applied here. Thus, conversely, the Tonegawa group's explanation of their α-CaMKII kinase mutants' jumpiness resulting in impaired performance due to fatigue is weakened by the similar data from the *Fyn* mutant mice. Further, the fact that the *Fyn* knockout mice were run in the spatial task first may have allowed the exacerbation of the spatial deficit. The order of tasks used by Kandel and his associates differs from that used by Silva et al. (1992), and thus the reverse may be true in the latter case, such that the α-CaMKII mice improvement observed during the hidden platform task may have been enhanced by prior swimming practice during the swim to visible target task. The near ceiling performance of the *Fyn* mutant mice on the hidden platform task may be explained in this way. Additionally, Grant et al. (1992) note that overtraining enables the *Fyn* knockout mice to eventually acquire the location of the hidden platform. However, in their own defense, Grant et al. (1992) cite Morris's (1985) contention that overtraining allows hippocampally lesioned rats to bring other neural circuits to bear on the problem and eventually solve the task. Further, it is not unreasonable to expect some LTP and spatial learning considering that these mutant mice still possess hippocampi that in some regards still function normally.

Regardless of these difficulties, data from both the Tonegawa and Kandel group indicate that the different knockout mice are impaired in their LTP induction and acquisition of spatial information in comparison to wildtype littermates. This blockade has the advantage over NMDA antagonism by being intracellular and therefore more

focal in its effects, and more importantly does not hamper normal cell communication, of which a number of the NMDA blockade studies are suspected (Lester et al., 1988). Conversely, this technique has the disadvantage of disrupting every other function dependent upon the mutated gene throughout the animal's life. It does appear however, that this disruption, as of yet, has not been found to have severe consequences, since the mutated mice have a normal life span, and can reproduce (Silva et al., 1992).

Also, the genetic knockout technique still appears to be in its infancy, and therefore shows promise in its application to a wide variety of problems facing neuroscience beyond the restricted field of LTP.

1.8 Interim Summary of the Learning and LTP Literature:

As can be seen from the extensive, but by no means exhaustive review above, there have been an ample number of studies using a variety of approaches examining the relationship between LTP and learning. However *in toto* these results are far from definitive. Most of the data from the correlation strategy remain as mere convergences of various phenomena. However, claims of stronger relationships have been somewhat successful, with the findings of Jeffery and Morris (1993) tentatively backing up those of Barnes (1979). Data from the prior enhancement strategy are either unconvincing as in the stimulus detection paradigm, or nonreplicable, as in the saturation paradigm. The data from the prior learning strategy, with the exception of that derived from the complex environment paradigm, are simply flawed or non-existent in the critical tests of hippocampally dependent tasks. Therefore it does appear that in accordance with Morris and Baker (1984) the strongest evidence linking LTP and learning together derives from

the blockade/facilitation strategy, specifically from the work of Morris and his colleagues, and from the incoming data on the knockout mice. Yet, this work is not without its criticisms, and in the case of the work on the calcium-dependent proteinases, inconsistencies abound.

It is noteworthy that within the blockade/facilitation strategy there have been many attempts to disrupt both learning and LTP, but that there have been few attempts, if any, to facilitate both learning and LTP. Consequently, the facilitation aspect of the blockade/facilitation strategy is the primary focus of this thesis.

As discussed briefly above, complex environment housing has been shown to produce reliable behavioral-LTP results, and this has been shown by a variety of laboratories (Sharp, McNaughton and Barnes, 1985; Sharp, Barnes and McNaughton, 1987; Green and Greenough, 1986; Hoesing, Skelton Evanson, and Sutherland, 1991). These results occur even when ongoing behavior is appropriately controlled for (Green and Greenough, 1986; Hoesing et al., 1991). Apart from the examination of behavioral-LTP, there is also a wealth of literature concerning the effects of complex environments on maze training and neuroanatomical changes (Diamond, 1988; Greenough and Bailey, 1988; Hymovitch, 1952; Forgays and Forgays, 1952; Park, Pappas, Murtha, and Ally, 1991; Renner and Rosenzweig, 1987; Rosenzweig, Krech, Bennett and Diamond, 1962).

Thus, independent of the behavioral-LTP paradigm there appears to be a convergence of evidence suggesting that complex environment housing enhances both learning and neuroanatomical features in the neocortex that may be involved in LTP-like

processes in the hippocampal formation (Greenough, 1984). Taken together then, complex environment housing as a treatment may facilitate both spatial learning and some forms of hippocampal LTP and therefore this paradigm is worth reviewing in its entirety.

1.9.1 Introduction to Complex Environments:

The use of complex or enriched environments has a long history that is thoroughly steeped in brain science. Over the decades, the focus of this paradigm has shifted between the areas of behavior, neuroanatomy, and electrophysiology. The paradigm of complex environments entails rearing littermates in one of two housing conditions. In one housing condition the animals are reared individually in standard laboratory cages, while in the other condition they are reared together in a large cage filled with ramps, toys, and other objects exchanged and/or re-arranged on a daily basis. This treatment has been found to result in improvements in maze learning, thickening of the neocortex, and increased synaptic efficacy of the hippocampus (Diamond, 1988; Forgays and Forgays, 1952; Green and Greenough, 1986).

The idea that brain tissue would increase as a result of cerebral exercise has been traced back to as early as the mid-eighteenth century, in written correspondence between the Swiss naturalist Charles Bonnet and the Italian scientist Michele Vincenzo Malacarne (Renner and Rosenzweig, 1987). Subsequently, Malacarne ran experiments in which eggs from the same clutches were split into two groups; one group reared in isolation, and the other given experience. Examination of the brains indicated that birds receiving the enriched experience had larger brains than birds that were reared in isolation(Renner and Rosensweig, 1987). These experiments were noted by the physiologist Samuel Thomas

von Soemmering in 1791. However, later editions of his book denied this evidence (Renner and Rosenzweig, 1987).

Thus, the first consistent evidence provided for this notion came from Darwin's (1868) examination of domestic and wild rabbits, where it was found that the cranial capacity of rabbits domesticated for many generations was proportionately smaller in relation to their body size then their wild caught counterparts. Darwin attributed this difference to the domestic rabbits being specifically bred for body size, without the attendant pressure on intellect, while the wild rabbits were being continually selected for their ability to avoid dangers and find food. Although suggestive, this evidence is only indirect, and is drawn from strains of the same animal many generations removed from a common ancestor.

1.9.2 Early Behavioral Research:

The first data to directly bear on the effect of enriched environments was behavioral and was brought forth by Hebb (1949). In the same book wherein he proposed the reverberatory cell assembly, Hebb described a small set of data, in which two litters of rat pups had been taken home and raised as pets by his young daughters. When these rats were brought back to the lab and compared to rats that had been raised in standard laboratory cages on a series of progressive maze problems, tasks developed by Hebb and Williams (1946), it was found that the "enriched" animals all performed in the top third of the total sample.

This result was formally replicated, under the supervision of Hebb, by Hymovitch (1952) and Forgays and Forgays (1952). Both of these studies confirmed that rats reared

in an enriched environment outperformed rats reared in an impoverished environment on a variant of the Hebb-Williams maze (Rabinovitch and Rosvold, 1951). These findings led to a great deal of work in many labs, all essentially replicating the basic finding of better performance on a variety of maze and discrimination tasks (Beach and Jaynes, 1954; Bingham and Griffiths, 1952; Denenberg and Morton, 1962; Denenberg, Woodcock and Rosenberg, 1968; Forgays and Read, 1962; Forgus, 1954; 1956; Meyers, 1962; Smith, 1972; Woods, 1959).

1.9.3 Early Neuroanatomical Research:

In the late 1950s David Krech, Mark Rosenzweig and Edward Bennett of the University of California at Berkeley, prompted by the early work of Hebb and his students, also began to focus their attention on the enrichment paradigm (Krech, Rosenzweig, and Bennett, 1960). The Berkeley group was interested in neural changes underlying the improved maze performance induced by the differential rearing conditions. Earlier, Krech, Rosenzweig, and Bennett (1958) had found different levels of acetylcholinesterase (AChE), the degradative enzyme of acetylcholine, in strains of rats that innately differed in their maze performance. Thus, they were now interested in whether the improved maze performance due to differential housing could also be associated with higher levels of AChE.

Immediately following weaning, Krech, Rosenzweig, and Bennett (1960) separated littermates from a number of different strains, into one of three conditions: an environmentally complex and training condition (EC), an impoverished control condition (IC), and an intermediate social condition (SC). In the EC group, rats were housed

together in a large cage containing a number of wooden toys that were selected from a larger group and changed daily. The IC group were housed individually in small cages, and the SC group were housed three at a time in intermediate sized cages. The EC group also received training on a number of maze problems, starting at 50 days of age and continuing until all the animals were sacrificed between 100-110 days of age. Results of the AChE assays indicated a reduced cortical to subcortical ratio of AChE per unit of weight in the EC group when compared to the IC group, with the SC group lying in between (Krech et al., 1960).

Since the results of their 1960 experiment were somewhat counter-intuitive, with lower AChE levels being associated with the enriched rats, a second experiment was conducted, where, in addition to taking AChE measures from more specific regions, the wet weight of cortical and subcortical sections were also recorded (Rosenzeig, Krech, Bennett and Diamond, 1962). This experiment was also performed using several strains of rats. Results from these experiments replicated the original AChE findings and indicated that the EC cortices were heavier by about 5%, although inter-strain differences were far greater than intra-strain differences induced by the enriched environment treatment.

These experiments were quickly followed by a much larger program of research that began pinpointing neuroanatomically the differences induced by complex environments. Diamond, Krech and Rosenzweig (1964) found that the heavier cortices induced by the complex environments were thicker, and consisted of fewer neurons per microscopic field than did their IC counterparts, with the greatest changes occurring in

layers II and III of the visual cortex. William Greenough of the University of Illinois in the early 1970s, also embarked upon a research program examining the neuroanatomical substrates resulting from differential housing (West and Greenough, 1972). Thus, the finding of Globus, Rosenzweig, Bennett and Diamond (1973), that denser branching at the more distal regions of the basal dendrites occurred in animals reared in complex environments was quickly confirmed by Greenough and his colleagues (Greenough and Volkmar, 1973; Greenough, Volkmar and Juraska, 1973). An extensive literature on the neuroanatomical work now exists on the effects of complex housing and various forms of maze training (Diamond, 1988; Greenough and Bailey, 1988; Renner and Rosenzweig, 1987).

1.9.4 Electrophysiological Research:

As a result of some of the early successes of the behavioral-LTP paradigm, where LTP-like, but not true LTP enhancements were found as a specific task was learned (Weiss et al., 1982; Ruthrich et al. 1982; Skelton et al., 1982), Bruce McNaughton, Carol Barnes, and their students at Colorado, combined this paradigm with that of complex environments in a series of experiments examining the relationship between LTP and learning (Sharp, McNaughton and Barnes, 1985; Sharp, Barnes and McNaughton, 1987; Silbert, Castro, Barnes and McNaughton, 1989). Thus, a third, electrophysiological, line of research involving the complex environment paradigm was initiated.

In the first study (Sharp et al., 1985), rats were implanted with a standard stimulating and recording arrangement in the perforant-path and the dentate gyrus of the

hippocampus. Following a 30 day recovery period, baseline EPs were recorded daily for a number of days. After this period, several of the rats were placed in separate rooms filled with boxes, wooden ramps and other objects, while the remaining two control animals continued to live in the standard laboratory cages. Over a period of days, both the EPSP and the pop-spike of the three rats housed in the complex environments exhibited increases as assessed by individual regressions, although the increase in the EPSP was less robust than that of the pop-spike. The EPSP and pop-spike of the control rats however, remained at baseline levels.

In the second experiment, adults of 14 months were compared to senescent animals of 32 months on the complex environment induced enhancement. Additionally, these rats were compared on the subsequent decay after removal from the enriched environment (Sharp et al., 1987). Results indicated that both the normal aged and senescent rats exhibited the synaptic enhancement of the pop-spike, but not of the EPSP, and upon removal from the complex environment, both groups showed evidence of decay, with the senescent group decaying at a faster rate than the normal aged group.

Since the EPSP data were inconsistent across these two studies and since recordings were only made every five days in the latter study, a third and final study using this paradigm was done by the Colorado group to examine the exact relation between the EPSP and pop-spike measurements during exposure to complex environments (Silbert et al., 1989). Recordings from 19 hemispheres in 11 rats were analysed as the experimental group, and recordings from 9 hemispheres in 5 rats were analysed as the control group. After five days of baseline recording, the experimental rats

were placed together in groups of 9 and 10 into .8 m^3 cages filled with junk objects that were changed or re-arranged daily, while the control rats remained housed in conditions similar to those during the baseline period. Rats housed in the complex environments exhibited increases in both the EPSP and pop-spike, which asymptoted at five days. However, over the next five days, as the rats remained housed in the complex environment, the EPSP decayed back to baseline, while the pop-spike remained at its enhanced level. From these three studies the Colorado group concluded that the animals experience a hippocampal synaptic enhancement due to the complex spatial information encountered in the enriched environment (Sharp et al., 1985; 1987).

Greenough and his students encouraged by the behavioral-LTP evidence and the initial findings of Sharp et al. (1985) also began an examination of LTP. However, in order to avoid extrahippocampal influences associated with *in vivo* preparations, Green and Greenough (1986) evaluated the enriched environment induced plasticity in hippocampal slices. At weaning, littermates were split equally into a complex environment group and an isolated control group. After approximately 30 days of differential housing, when the rats were approximately 50 days old, they were sacrificed and prepared for slice work. Orthodromic evoked potentials recorded from the granule cell layer in response to medial perforant-path stimulation across a number of intensities indicated that both the EPSP and the pop-spike were greater in the complex environment group than in the isolated control group, while E-S relations were not different between the two sets of slices.

A second experiment in this study was conducted, in which rats initially housed in the complex environment were re-housed as the isolated controls had been. Following this period the rats were prepared as before, for hippocampal slice input/output relations. Results indicated that the previously found enhancement was not present approximately 30 days after removal from the complex environment. Together, the results from these two experiments were again interpreted as resulting from the acquisition of spatial information obtained from the complex environment (Green and Greenough, 1986).

1.9.5 Recent Research:

More recently, the behavioral aspect of the complex environment paradigm has enjoyed renewed interest. However, it has now become a treatment effect interacting with other experimental manipulations, such as evaluating the complex environment's ability to ameliorate early and late brain damage, or transmitter depletion. Further, the behavioral assay of choice has now become the Morris water-maze.

Whishaw, Zabrowski, and Kolb (1984) found that postsurgical enrichment treatment following hemidecortication in adult rats improved their performance on the Morris water-maze. Whishaw, Sutherland, Kolb and Becker (1986) further found that although neonatal depletion of noradrenaline (Ne) by 6-hydroxydopamine (6-OHDA) had no effect on subsequent adult acquisition of the Morris water-maze, enriched environment experience did improve acquisition rates over non-enriched experience. Fong, Saari, Armstrong, and Shivji (1988) have also shown that enriched environment housing treatment ameliorates neonatal monosodium-glutamate administration deficits on subsequent adult water-maze acquisition. Finally, Park, Pappas, Murtha, and Ally (1991)

showed increased levels of ChAT and improved rates of maze acquisition following housing in an enriched environment. Oddly enough this last study brings the field full circle with the original AChE examination carried out by Krech et al. (1960).

1.9.6 Other Behavioral Assays:

It is surprising that research using the complex environment paradigm has not been conducted on a wider range of behaviors. The focus on mazes and learning continues in spite of reports that running-wheel exercise alone can enhance water-maze performance (Fordyce and Farrar, 1991a; 1991b), and that the learning of specific motor tasks can generate anatomical change in the sensorimotor areas controlling the limbs selectively involved in the tasks (Greenough, Larson, and Withers, 1985). Further, social housing alone has been shown to contribute intermediate anatomical (Diamond, 1988) and behavioral effects (Fisher, Turner, Pineault, Kleim, and Saari, 1991). These data suggest that factors other than the complex spatial milieu through which the rats move in the complex environment may contribute to enhanced cortical thickness and maze acquisition. These data also indicate the need for a fuller behavioral assessment of both learned and unlearned behaviors of rats reared in complex environments, in order to disentangle some of the potential contributions to the behavioral, anatomical, and electrophysiological plasticity observed in these animals.

Scattered reports of other behavioral assays do exist. Most of this literature is recent, and has largely involved the other behavioral tests as methods for assaying the amelioration of deficits caused by experimental manipulations discussed above, and not primarily to assess differences between enriched and non-enriched animals.

One group in particular has examined the amelioration of deficits caused by neonatal norepinephrine (Ne) depletion or early administration of monosodium glutamate (MSG). Located at Nipissing University and headed by Matti Saari and sometimes in collaboration with Bruce Pappas at Carleton University, this group has conducted the most extensive behavioral assessment of the effects of complex environment housing to date. With regard to mazes they have examined the Lashley III maze (Saari, Murtha, Murray, Stange, and Pappas, 1985; O'Shea, Saari, Pappas, Ings, and Stange, 1983), the Hebb-Williams maze (Pappas, Saari, Smyther, O'Shea, Murtha, Stange, and Ings, 1984), the Barnes platform maze (Fisher, Kleim, Lloyd, and Saari, 1989), and the Morris water-maze (Fisher, Turner, Pineault, Kleim, and Saari, 1991; Saari, Armstrong, Nobrega, Pappas, Coscina, 1990; Saari, Fong, Shivji, and Armstrong, 1990). The basic finding in all these studies was that the complex environment alleviated the deficits induced by either 6-OHDA or MSG. Further, the complex environment housed animals receiving vehicle injections often outperformed the individually housed animals receiving vehicle injections (Fisher et al., 1991; O'Shea et al., 1983; Pappas et al., 1984; Saari et al., 1990a; 1990b).

Saari's group has further examined social interaction using three different assays: the colony intruder test, the platform dominance test, and waterspout competition (Fisher et al., 1991; Saari et al., 1990a). The earliest version of the colony intruder test appears to have been done by Grant (1963). Normative data were generated by allowing one male rat to adapt to an observation chamber for 15 min, after which a test rat was introduced into the same chamber. The subsequent interaction was scored for a variety of

aggressive, amicable, and self-directed behaviors (Barnett, 1958; Grant and Mackintosh, 1963). File and Hyde (1978; 1979) later developed this test into a behavioral drug assay for anxiolytics, examining a number of the test parameters, such as the lighting levels, familiarity with the test chamber, prior handling experience, and age differences among the animals tested (File, 1980). A further variant of this test was used by Luciano and Lore (1975) and involved introducing the test animals, one at a time into an approximately 1^3m box, in which a number of resident rats were housed. They specifically examined the effects of individual versus social rearing of the intruder and the effects of social versus individual rearing of the rats in the resident colonies on the ensuing interactions, finding that socially reared intruders suffered less damage than individually reared intruders, and that socially reared colonies were quicker to make contact with intruders than were individually reared colonies (Luciano and Lore, 1975). Saari et al. (1986; 1990a) examined the ameliorating effects of complex environment housing on Ne depletion on the intruder in this task, introducing the test animals at 60 days of age, into a 1^3m colony cage, in which 9 resident males approximately twice the age of the test rat were housed. Comparisons between the vehicle injected groups in differential rearing conditions indicated that rats housed in the complex environments groomed and initiated social contact more often than their individually housed counterparts, providing similar findings of the earlier study by Luciano and Lore (1975). The platform dominance task appears to have been developed by Saari's group and involves placing a number of animals at a time in the Morris water-maze, in which the platform location is already known. The amount of time spent on the platform generates

a ranking system for that group, and a "round robin" system of group comparisons allows a full dominance hierarchy to be constructed. Results from this test indicated that rats involved in enriched environment treatments were ranked as more dominant than rats drawn from the other treatment conditions (Fisher et al., 1991; Saari et al., 1990a). The waterspout competition task is structured very much like the platform dominance task. Animals are water deprived and placed in small groups in a familiar test chamber with a waterspout available. The waterspout is presented in such a fashion that only one rat can have access to the water at a time. Latencies, frequencies, and order of the animals' drinking is then scored and a dominance hierarchy constructed. Results from this task indicated differences between the vehicle injected groups such that rats housed in the complex environment group rated higher on the dominance rankings than the isolated groups (Saari et al., 1990a). Aggression in isolates as compared to socially reared rats had earlier been examined by Johnson, DeSisto, and Koenig (1972) as the incidence of frog killing. Socially reared rats were less likely to kill frogs than their isolated controls. Further, Johnson et al. (1972) noted that the nature of the killing between the differently housed groups was also different, in that the socially reared rats when attacking frogs did so in a predatory manner, sometimes beginning to eat their victim before killing it. The isolates on the other hand, when initially confronted with the frog would often freeze, and/or defecate. When the attack occurred it would follow a frenzied pursuit and be consummated by a deadly bite just behind the head, afterwhich the rat would often retreat to a far corner of the arena, and remain there until the end of the test period (Johnson et al., 1972).

Fisher et al. (1991) have also examined grip strength as tested by the ability of a rat to suspend itself from a 2 cm diameter rod with its forepaws. The results indicate that overall the groups receiving enriching treatments and vehicle injections were able to suspend themselves for longer than the groups receiving no enriching treatments and vehicle injections. However, none of the individual groups were statistically different from each other on a post-hoc test of means.

In a number of these experiments the animals' body weight and the weight of the adrenal glands were also examined (Fisher et al., 1991; Nobrega, Saari, Armstrong, and Reed, 1992; Saari et al., 1990a; 1990b). Again, although the focus of these experiments was primarily on the ameliorating effects of complex environments on debilitating treatments, comparisons among the vehicle-receiving groups can still be made. The consistent findings among these studies were that rats housed in the complex environment were lighter in body weight and weight of the adrenal glands. These results suggest that the rats housed in complex environments were less affected by stress and are potentially more active, or eat less, than their isolated counterparts. Weight differences and food consumption was directly examined earlier by Fiala Snow and Greenough (1977). Food consumption and weight were monitored over a 48 hour period once a week for four weeks. Findings indicated that the complex housed rats weighed less and consumed less food then their isolated counterparts (Fiala et al., 1977). The Berkeley group had also concluded that isolation stress did not play a role in the neuroanatomical effects of enriched environments, since no differences were found in adrenal weights between enriched and isolated rats (Bennet, Diamond, Krech, Rosenzweig, 1964). However,

Geller, Yuwiler and Zolman (1965) found that when terminal body weight was covaried out of the analysis, the difference between the weight of the adrenal glands was significant with the complex environment housed animals having adrenal glands that were lighter than individually housed controls. Rats reared in a complex environment also show less stomach ulceration in response to immobilization stress (Rockman, Borowski, and Glavin, 1986; Rockman, Hall, Markert, and Glavin, 1988).

Open-field activity has been looked at directly by Saari's group, with mixed results. In the studies in which the Saari's group examined deficits induced by postnatal administration of MSG they found that the saline treated, enriched animals exhibited greater open-field activity than the individually housed saline controls (Fisher et al., 1991; Saari et al., 1990b), whereas an earlier study examining the effects of 6-OHDa administration they found that the enriched control group showed less activity than the individually housed control group (Nobrega et al., 1992). Findings by other groups in the literature are also mixed, with Manosevitz (1970) and Manosevitz and Montemayor (1972) finding enriched housing increasing open-field activity, while others found the opposite (Denenberg and Morton, 1962a; Mohammed et al., 1986; Smith, 1972).

The remaining behavior that has been examined across different housing conditions has been neophobic responses to feeding in novel locations (Holson, 1986). Findings indicating that rats reared in complex environments were less neophobic led Holson (1986) to suggest this as the basis for the observed differences in the maze performance of enriched animals over non-enriched animals. Rockman et al. (1986; 1988) have also found that rats housed in complex environments consumed greater

quanties of alcohol in two-bottle choice tests. than individually or socially housed rats.

1.10 Rationale for Current Experiments:

In summary then, the complex environment paradigm has a substantial history in the realms of behavior, neuroanatomy, and neuroelectrophysiology. Yet surprisingly, there has been no examination of all three aspects within a single study. Additionally, the focus of the behavioral studies has consistently been the animals' ability to learn mazes. Only rarely have other behaviors been examined. Further, it is surprising that no study exists whereby the effects of differential housing on the induction of electrical LTP has been evaluated. Based on these perceived shortcomings of the complex environment literature, this thesis undertook the following as its objectives: first, to examine behavior, neuroanatomy, and electrophysiology all within the same preparations, and relate the subsequent evidence from these three realms; second, to more fully describe the behavioral repertoire and capabilities of the rat in assessing the consequences of complex environment housing; finally, to evaluate the differences, if any, in the induction and maintenance of LTP in rats housed in a complex environment and rats housed in standard laboratory cages.

1.11 Outline of Thesis Experiments:

In order to determine what the behavioral consequences of complex environment housing are, this thesis assessed a wide variety of behaviors following such housing, including strength and agility, swimming ability, social interaction, spontaneous locomotor activity, consummatory behavior and preferences, and finally spatial ability. The behavioral assessment was followed by implantation and recording procedures that

examined the baseline electrophysiology using extensive input/output relations, and examined the specific ability to undergo and sustain LTP. Upon the conclusion of the electrophysiological experiments neuroanatomical cortical changes were examined through the measurement of cortical thickness at three delineated medial-lateral locations specified on five anterior-posterior planes. Finally, data collected from these behavioral, electrophysiological and neuroanatomical assessments were examined for inter-relations among the three realms.

It was thought that this set of experiments, using the facilitation aspect of the blockade/facilitation strategy would provide evidence useful in evaluating the relationship between learning and LTP.

CHAPTER 2 - GENERAL METHODS AND MATERIALS

2.1 General Procedures:

The general procedures for this set of experiments are outlined below. This chapter includes a description of the rats and their housing conditions throughout the experiments. It also includes a brief section on the behavioral, electrophysiological, and neuroanatomical assessments performed on these rats. Additionally, details of the surgical procedures and the electrophysiological recording setup are included. Specific details of the procedures and associated results are presented in subsequent chapters organized under the three realms of behavior, electrophysiology, and neuroanatomy.

2.2 Subjects:

Male rats (n=18) from 4 litters, born within 7 days of each other were used in this series of experiments. Females were culled from the litters at approximately 7 days after birth, leaving in each of the 4 litters 6, 8, 2, and 2, males respectively. The rats were weaned at approximately 22 days of age and housed together as sibling groups. At approximately 35 days of age the rats were housed individually for a 15 day habituation period in standard suspended wire mesh cages located in a climate controlled colony room. A twelve hour light/dark cycle was maintained at all times, with the lights turned on at 8:00 am and turned off at 8:00 pm. Temperature was held constant at 20°C ± 2. Rats had continuous access to food (Agway Prolab rat chow, formula 3000) and water, except where otherwise specified. The rats involved in this set of experiments were the sole occupants of the colony room. During the latter 5 days of the 15 day adaptation

period, the rats were habituated to a number of behavioral procedures, and baseline testing of consummatory behaviors and spontaneous locomotor activity was carried out, as outlined below.

At approximately 50 days of age, each of the 4 litters were divided into 2 equal groups. One group continued to be housed individually in the standard wire mesh cages, while the other group was housed together in the complex environment.

2.3 The Complex Enviroment:

The complex environment was a large 1 m^3 cage. The base was constructed from wood, and was 27 cm high. The upper portion of the cage was constructed from 1 cm wire mesh supported by a wooden frame and rested just inside the base. The top of the environment consisted of a single sheet of Plexiglas. A hinged mesh door for easy access was built into one of the sides. The wooden frame and base were laminated with a number of coats of Polyurethane.

The base of the complex environment was filled with approximately 6 litres of bedding chips which were pushed into irregular mounds. A number of Fisher Price buildings and a second level constructed of wooden platforms and cardboard boxes were placed on top of the bedding chips. A series of wooden ramps ran from the second level to an upper platform constructed of metal bars and wire mesh. From the platform a lattice work of small toys was suspended on wires, such that they were within reach of the rats. Finally, a number of plastic and wooden blocks, and various shaped toys were distributed throughout the complex environment. All the ramps, elevated mesh platforms, mobiles, Fisher Price buildings, and other toys were re-arranged on a daily basis, with

a number of the Fisher Price buildings and other toys being rotated in and out of the complex environment on a pseudo-random schedule.

Food and water were available ad libitum, except where specified below. The food in the form of dry rat chow was either distributed widely throughout the cage or located in 4 specifically placed food hoppers. Water was accessible from three .5 litre bottles located in one corner of the complex environment.

2.4 Behavioral Assessment:

After 21 days of differential housing the rats animals were tested on assays of food and water intake, fecal matter and body weight, spontaneous locomotor activity, social interaction, tests of strength, agility, and balance, and the Morris water-maze, as a spatial learning task.

Testing was carried out over a period of days, with an average of one test per day. When behavioral testing was largely completed, following 40 days of differential housing, the rats were surgically implanted with stimulating and recording electrodes in the perforant-path and dentate-gyrus respectively. At this stage the rats were approximately 90 days of age and weighed between 420 g and 550 g at the time of surgery. The implants were completed over a 4 day period using a blind procedure. After immediate recovery from the anaesthesia rats were housed individually in the standard colony room with the other laboratory animals.

2.5 General Surgical Procedures:

Rats were injected with atropine methyl nitrate (15-20 mg/kg, i.p.) 10-20 min prior to the administration of the anaesthetic to minimize respiratory problems. Animals

were anaesthetized with sodium pentobarbital (Somnotol) (65 mg/kg, i.p.) and when necessary, small supplemental injections of .05 ml were given. Surgeries were performed using standard stereotaxic equipment and techniques. The animals were placed in the stereotaxic apparatus with bregma and lambda in the same horizontal plane.

Twisted bipolar recording and stimulating electrodes were constructed of Teflon-coated stainless steel wire, 127 μm in diameter, crimped to male gold-plated pins (Amphenol 220-P02 Rella-Tac pin). Both recording and stimulating electrodes were insulated except at the cut tips, which were staggered by approximately .5-1 mm. The reference used for all monopolar recordings was constructed from the same Teflon-coated wire as the stimulating and recording electrodes. One end of the reference was crimped to a male pin and the other end was soldered to a jeweller's screw. The ground was constructed in the same fashion as the reference, except for the use of uncoated wire instead of Teflon-coated wire.

Animals were implanted unilaterally in the left hemisphere with stimulating electrodes aimed at the perforant-path and the recording electrodes aimed at the hilus of the dentate gyrus ipsilateral to the stimulating electrodes. Stimulating electrodes were placed at approximately 8.0 mm posterior to bregma (anterior/posterior axis; AP), 4.5 mm lateral to bregma (lateral axis; Lat), and 3.5 mm ventral to the dorsal surface of the skull (dorsal/ventral axis; Dep), and recording electrodes were placed at approximately 4.0 mm posterior to bregma (AP), 2.4 mm lateral to bregma (Lat), and 3.5 mm ventral to the dorsal surface of the skull (Dep). Final positioning of the depth of the stimulating and recording electrodes was determined by recording monopolar evoked potentials from

the recording electrodes in response to single diphasic pulses delivered bipolarly to the stimulating electrode during surgery, thus optimizing the placement of both pairs of electrodes. The ground lead was fastened to one of the frontal skull plates, with the uninsulated wire wrapped around three other jeweller's screws fastened to the skull, one on either side in the temporal skull plate and one in the other frontal bones, contralateral to the ground lead. The reference lead was fastened to the interparietal bone centred behind lambda, and located above the cerebellum.

Once the final positioning of the electrodes had been determined, they were cemented into place with dental acrylic and the male pins were assembled into a plastic McIntyre miniature connector plug (STC-89P1-220), which was then further anchored to the skull and jeweller's screws using dental acrylic. After the surgery was complete, rats were placed under a warming lamp until they had sufficiently recovered from the anaesthetic to be returned to the colony room.

2.6 Post Surgical Habituation to the Complex Environment:

Once all the rats had been implanted and had recovered from the anaesthetic they were returned to their differential housing conditions, on a daily basis. That is, the complex rats were placed in the complex environment in the morning and returned to their "night" cages in the standard colony room in the evening. The days spent individually housed during the post-operative period, appeared to affect the rats. Serious fighting occurred when they were placed together again in the complex environment. As a result, a number of steps were taken to ensure that the rats did not do serious harm to each other, or dislocate the stimulating and recording electrode placements that had been

implanted. First, the rats were housed in the complex environment only during the day, in the quiet portion of their light/dark cycle. Second, the rats were habituated together on an open tray filled with bedding chips, prior to being released into the complex environment. Third, a number of the large plastic structures with edges and lips, where headcaps could become stuck or caught were removed. Finally, the complex environment was re-arranged less often, with the hope that in a more stable environment it would not be necessary to re-establish the dominance hierarchy every time the animals were reinstated.

Upon full recovery and a period of readaption to the complex environment, the behavior of the rats stabilized, and baseline electrophysiological testing began. The rats at this point were approximately 120 days of age.

2.7 Equipment Configuration and Software for Recording EPs in the Dentate-gyrus:

A variety of recording chambers were used, depending upon the experiment being conducted. For the most part, the chambers were small 30.5^3 cm clear Plexiglas boxes, partially filled with bedding chips.

A series of shielded leads made of light flexible cables were crimped to female gold plated pins (Amphenol 220-S02 Rella-Tac Socket). The female pins were assembled into a plastic McIntyre miniature socket (STC-89S1-220), and then fixed to the socket with dental acrylic. During recording, rats were connected through the McIntyre assembly, which was sealed together with a ring nut (STC-89N1-220) slipped over the McIntyre socket and threaded onto the plug fixed in the rat's headcap.

The other ends of the shielded leads were connected to a mounted commutator (BRS/LVE CAY 960-12). A heavier shielded cable ran from the other end of the commutator to a Grass Model 7 Polygraph fitted with 7P511 wide band preamplifiers. The filter settings generally remained constant at .1 Hz for the low frequency half amplitude cut-off, and 3 KHz for the high frequency half amplitude cut-off. The 60 Hz notch filter was never used during recording. A further set of light shielded cables connected the J6 output channels of the driver-amplifiers to several channels on the analog to digital inputs (12 bit true differential) of a Lab Master analog/digital (A/D) system free running at a pickup rate of 20 KHz. All the electrophysiological signals of interest occurred with a frequency of less than half the pick up rate, and therefore an accurate capture of the signal was ensured. The mother board of the A/D system was inserted in an IBM 386-AT clone (25 MHz).

Stimulation was generated by an S8800 Grass stimulator coupled with a pair of stimulus-isolation/constant-current SIU6 units. The output leads of these units were attached to the cabling system described above and led to the rat. Test pulses consisted of diphasic square waves, positive phase leading, of .1 ms duration per phase. Intensity of the test pulses ranged from less than 100μa to 1000μa.

A software package developed in this lab and written in Asyst[1], managed the

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[1] Asyst is an APL-like computer language specifically developed for scientific applications and A/D data transfer (Asyst Software Technologies, Inc., ver 3.0). The software package used throughout this thesis was initially modelled after a similar package developed by Dr. R. Racine. The package employed here was developed over an initial period of a year, with subsequent modifications implemented at later times. The package was largely developed and implemented by F.H. Boon, with some assistance by the author.

triggering, collection, and analysis of the hippocampal evoked potentials (EPs). All EPs were collected at a frequency of 20 KHz (1 data bin every 50μs). Accompanying file utilities permitted the raw and averaged sweeps, and their analyses to be converted to American Standard Code II (ASCII) files, for display and further statistical analyses. EPs could be analysed individually or as averages, with 11 potential measures being derived from each sweep or average sweep.

2.8 The Dentate-gyrus EP Measures Employed:

As outlined and discussed in Appendix A the 11 different measures could be classified into variable clusters concerning the EPSP slope, the pop-spike, and latency of EP event measures (i.e. onset and offset of the pop-spike). The electrophysiological recording experiments presented in appendix A, led to the selection of only 4 of the 11 EP measures described and presented in Appendix A.

The double-ended roll-off EPSP measure was selected from the EPSP slope measures. This measure was derived by demarcating the onset of the EPSP slope and the offset of the EPSP slope (the offset of EPSP slope also marks the onset of the pop-spike, when present). The onset of the EPSP slope was defined as the first sampling interval that exceeds an increase of .02 mV, from the initial placement of a manual cursor, controlled from within the software package. Once the onset and offset were demarcated, the measure was rolled forward 15% of the time interval starting from the onset, and was rolled back 15% of the time interval starting at the offset. Subsequently, the rise over run for this interval was calculated. This EPSP slope measure was found to fall between the other two measures that were tested in terms of sensitivity/reliability. Regardless of the

EPSP operational definition employed it should be noted that the EPSP recorded at the granular/hilar level is subject to commissural and interneuronal influences, and thus do not represent a true perforant-path EPSP (Buszaki and Eidelberg, 1982). However, EPs are commonly recorded at this level for the durability and stability of the chronic recordings.

The peak-to-peak amplitude and area of the pop-spike were selected from the pop-spike measures. The former measure was simply the difference in amplitude betweeen the onset of the pop-spike and the peak of the pop-spike, while the latter measure was derived by calculating the area beneath the tangent line drawn from the onset to the offset of the pop-spike. These two measures were chosen since a number of the animals exhibited pop-spikes evoked from both the medial and lateral perforant-paths. Thus, the area measure was used to analyse the total area of the pop-spikes regardless of whether they involved components that were elicited by one or both of the perforant-paths. Alternately, only the pop-spikes evoked by the medial perforant-path stimulation were analysed by the peak-to-peak pop-spike amplitude measure. As such, multiple aspects of the granular cell discharge were captured during these analyses.

Finally, the onset of the pop-spike was selected from the latency of event measures. This was done on the basis of past research indicating a sensitivity to various LTP and LTP-like phenomena (Bliss and Lomo, 1973; Douglas and Goddard, 1975; Green et al., 1990). A fuller discussion of all 11 measures can be found in Appendix A.

2.9 Electrophysiological Assessment of EPs in the Dentate-Gyrus:

Baseline recording procedures consisted of AEPs generated by 500μa intensity test

pulses, collected at surgery and upon full recovery. Baseline recording continued with formal I/O curves. Several weeks following the baseline electrophysiological testing, the rats underwent LTP procedures, at which point they were approximately 160 days of age. Electrophysiological measures were recorded at a number of intervals subsequent to the LTP procedures, with the longest occurring 7 days following LTP induction. After one final month of daily differential housing the rats were sacrificed at approximately 185 days of age, and prepared for histological procedures.

2.10 Anatomical Assessment of Neocortical and Hippocampal Thickness:

Histological analyses were done following Stewart and Kolb (1988). Cortical thickness was measured at three points on each hemisphere from 5 different coronal planes identified by specific neuroanatomical landmarks. This assessment was carried out by Dr. Byran Kolb at the University of Lethbridge, Alberta. The experimenters were blind as to the identity of the animals. A further thickness analysis was performed upon the dentate gyrus and CA1 of the hippocampal formation at a single coronal plane adjacent to the recording electrode placement.

CHAPTER 3 - EFFECTS OF A COMPLEX ENVIRONMENT ON BEHAVIOR

3.1 Outline of the Behavioral Assessment:

After 3 weeks of differential housing with half of the littermates housed in the complex environment and the other half housed individually in standard laboratory cages, all rats were tested on a wide array of behaviors. The behaviors examined included measurements of food and water intake, an assessment of spontaneous locomotor activity, a social interaction experiment, tests of strength, agility, balance, and swimming ability, and finally an assessment of spatial learning using the Morris water-maze.

Testing was carried out over a period of days, with an average of one behavioral assay being run per day. Details of the individual behavioral assessments are described below, followed by the results and discussion of the behavioral assessment.

3.2.1 Procedures for Examining Food and Water Intake, Fecal Matter and Weight, and Assessment of the Spontaneous Locomotor Activity:

Data for the spontaneous locomotor activity assessment were collected over three phases, while data on food and water intake, fecal matter, and weight were collected for only two of those phases. The first phase of this experiment was conducted three days prior to the rats being separated into their respective housing conditions. The second phase continued on from the first, and was conducted for three days following the rats separation into their respective housing conditions. The third phase was conducted for an equal length of time at the beginning of the behavioral assessment starting on Day 21 after separation. Prior to running the first and third phases, the rats were habituated to

all the procedures for two days. Activity data for analysis were collected on the last two days of all three phases, while the food and water intake data, fecal matter and weight data were collected for the full three days of the first and third phases.

For the activity assessment rats were brought into the testing room in three groups of six, with each group of six composed of three rats from the complex environment housing condition and three rats from the individually housed condition. Each group was run at a fixed time every day, with the first group starting at 12:15pm, followed by the second group at 1:30pm, and the third group at 2:45pm. Data for the food and water intake, fecal matter and weight were collected between approximately 5:00 pm and 6:00 pm.

The food was measured by placing a weighed quantity of lab chow in each animal's cage (45 grams) and recording the amount left uneaten and recovered from beneath the cage. Water consumption was measured using graduated cylinders with ballbearing spouts. The weight of the dried fecal matter was recorded. This was done by placing paper towels beneath the suspended wire mesh cages to collect the fecal matter, which was then removed and allowed to dry for 48 hours before weighing. Finally, the weight of the rats were recorded. During the third phase procedures for the rats housed in the complex environment were different, with the bedding changed daily and sifted for the dried fecal matter and residual lab chow. Further differences for the rats housed in the complex environment consisted of the water being delivered via three communal water bottles and the food delivered via four communal food hoppers. To compensate for the social setting, and the potential loss of food through being buried in the bedding

chips, an additional amount of lab chow (+50 g) was provided to the rats housed in the complex environment. Thus, during the third phase only communal data were obtainable from the nine rats housed in the complex environment.

The Omnitech Digiscan Animal Activity Monitor (model no. RXYZCM[16]) is a 40 X 40 X 30.5 cm open-field with a grid of infrared beams mounted horizontally every 2.54 cm, and a 2nd or vertical tier of beams mounted 13.5 cm above the floor [Omnitech Electronics, Inc., Columbus, OH].

When in operation the pattern of beam interruptions is recorded and analyzed by an Omnitech Analysis unit (model no. DCM-8) and then passed on to a microcomputer where it is interpretated and stored on disk. The particular system employed here consisted of 6 RXYZCM[16] monitors, a DCM-8 analysis unit and an IBM (286AT) clone. For the assessment of spontaneous locomotor activity used in these experiments six consecutive 5 minute activity samples were collected from each rat per session.

A number of locomotor activity variables can be directly obtained or computationally derived from the obtained variables. Nine variables were chosen a priori based on earlier pilot work and other experiments performed with this apparatus in this laboratory.

Total distance travelled (TD): The distance travelled by the animal during the sample is computed by following the pattern of interruptions on the 16 X 16 grid of infrared beams and computing the subsequent distance vectors.

Average distance travelled per movement (ADM): This variable is derived from the previously computed Total distance and Number of movements. This computation is

performed by the Digiscan system.

Ave. ·ge speed per movement (AS): This variable is derived from the previously computed Total distance and Time in movement. This computation is performed by the Digiscan system.

Number of horizontal movements (NM): Each time a break in horizontal activity occurs for a period of greater than 1 second, this variable is incremented by 1. This indicates the number of separate horizontal movements executed by the animal in a given sample period. Individual movements are separated from each other by a rest period of at least one second.

Time spent in horizontal movement (MT): As long as the animal is moving, this variable is incremented. If the animal is stationary for more than 1 second, this parameter is no longer incremented. Thus, it corresponds to the amount of time the animal was in motion during a given sample period.

Time spent per horizontal movement (TM): This variable is derived from the previously collected Number of Horizontal Movements and Time spent in Movement using the formula [MT/NM] = TM. This variable was computed using supplementary software written by the author.

Number of vertical movements (VM): Each time the animal rears up and breaks the second tier of infrared beams, this variable is incremented by 1. The animal must go below the level of the vertical sensors for at least 1 second before the next rearing can be registered.

Time spent in vertical movement (VT): When the animal activates the vertical ·er of

sensors by rearing this variable starts incrementing and continues to increment until the animal goes below the level of the vertical sensors.

Time spent per vertical movement (TV): This variable is derived from the previously collected Number of vertical movements and Time spent in vertical movement using the formula [VT/VM] = TV. This computation was performed by supplementary software written by the author.

3.2.2 Procedures for the Strength and Agility Tasks:

These three tasks consisted of the balance beam, the hanging duration, and the spring scale strength task. However, portions of the simple swim and climb task, discussed below, were also included in the overall discussion of the strength and agility tasks. For all these tasks rats were tested in groups of six, with each group of six consisting of three rats from the complex environment housing condition and three individually housed littermates.

The balance beam task was run on Day 26. The apparatus consisted of two "safe" boxes placed at either end of the balance beam, which was erected 1 metre above a pile of bedding chips. The boxes were made of Plexiglas with dimensions of 30 cm x 30 cm x 30 cm, and were darkened on all sides. The boxes were spaced 1 metre apart, connected by the balance beam. A habituation beam was initially used to allow the rats to become accustomed to the test procedure. The habituation beam was 36 mm x 36 mm x 1.2 metre, with 10 cm of each end projecting into the boxes. The test beam was concealed beneath the habituation beam and was 11 mm x 45 mm x 1 metre, with the 11 mm edge of the beam facing up. Suspended approximately 45 cm above the beam was

a 150 watt light and reflecting shade.

Each animal went through the full habituation procedure and then was returned to its appropriate holding pen. The habituation procedure consisted of placing the rat in each "safe" box for a full minute. The animal was then placed at the centre of the habituation beam and then coaxed by gentle pushing at the base of the tail along the beam and into one of the boxes, where it was allowed to remain for 15 seconds. This was then repeated for the other box. The rat was again placed at the centre of the habituation beam, but then allowed to choose which "safe" box it entered. This procedure was repeated 4 or 5 times until the rat without hesitation, walked or ran down the beam to enter one of the "safe" boxes.

The larger habituation beam was removed after all rats had successfully undergone the habituation procedure, revealing the test beam, narrow edge facing upwards. For the first trial the rats were placed in each "safe" box for 30 seconds prior to being placed at the centre of the balance beam. On subsequent trials the rats were placed in each box for only 15 seconds. The rats were then placed parallel with the beam at the centre, facing the box from which they had last been removed. A stopwatch measured the time from being placed on the balance beam to the time that both hind feet entered one of the "safe" boxes. At the end of each trial the rats were allowed to remain in the chosen "safe" box for 15 seconds, before being returned to the appropriate holding pen. If an animal fell off the beam it was given two more opportunities to successfully complete the trial. If the animal fell three times during a single trial it was placed back in the holding pen and given a maximum time of 60 seconds. The rats ran a single trial

at a time. The box that a rat faced when placed on the balance beam was randomized across animals and alternated over trials. A total of 5 test trials were performed by each rat, with an inter-trial interval of approximately 10 minutes. All test trials were recorded on videotape and later scored for the following behaviors performed on the balance beam: 180^0 turns, rears, pauses, spontaneous crossings, and falls.

The hanging duration task was run on the afternoon of Day 29. The apparatus consisted of a small trapeze made of coathanger wire, 12 cm wide at the base, that hung 17 cm down from a horizontal bar, which was mounted 97 cm above a pile of bedding chips. Thus, there was an 80 cm distance from the bottom of the trapeze to the bedding chips.

Rats were placed on the trapeze such that they initially clung with their forelimbs flexed, and their heads held above the bottom of the trapeze. The time between initial placement on the trapeze to falling onto the bedding chips was recorded with a stop watch. Each rat received 3 trials with an inter-trial interval between 4 to 6 mimutes. Prior to the first trial the weight of each animal was recorded.

The spring scale strength task was run on the evening of Day 35. A 16 x 13 cm wire grid (1 cm mesh) fixed at the end of a 23 cm long rigid wire was attached to a plastic spring scale (Science Kit Inc. 2Kg). The spring scale was modified to accommodate a collar that would slide and mark the load after the spring had been released. This apparatus was placed horizontally on a table top and held down manually by one of the experimenters, with the grid overhanging the table edge.

At the start of a trial the rat was placed on the wire grid, while being grasped by

the body and the base of the tail by another experimenter. Once the rat had gripped the grid with all four feet it was gently, but firmly, pulled back by the base of the tail until it let go or began to step backwards, whereupon the experimenter would relax the tension on the base of the tail. The inserted collar indic.. ed the maximum force that the rat had tolerated before letting go, which would then be recorded by the experimenter holding down the apparatus.

Each rat was given 3 trials with an inter trial interval of approximately 3 min. Following the third trial the weight of each animal was also recorded. The experimenter recording the data was naive as to the identity of the individual rats.

3.2.3 Procedures for the Morris Water-maze and the Simple Swim and Climb Task:

This experiment was initiated on the day 27. The Morris water-maze was 91.5 cm in diameter and 44.5 cm deep from rim to floor, with an inner lip running around the circumference 15 cm below the rim. The water was brought up to a level 6.5 cm below the lip. The maze was painted black and the water was made opaque by a covering of bedding chips, which were replaced after each trial. The submerged platform was a 9 cm square made of Plexiglas with a layer of wire mesh attached to the top surface. The platform was mounted on a Plexiglas stand, which could be anchored to the floor of the maze by 2 small vertical pegs, ensuring that the platform was always in a set location. The platform was submerged below the surface approximately .5 cm, such that the chips would float freely over it. The water temperature was approximately 20-22°C. During the actual running of the water-maze, neither of the experimenters was visible to the rats, nor were there any obvious auditory cues available to the animals.

The water-maze task was run over a period of two days, with the first day consisting of a pre-acquisition probe trial, and 4 blocks of 2 acquisition trials each. The second day consisted solely of a post-training probe trial run 24 hours after the first pre-acquisition probe trial. All procedures in the water-maze were videotaped by an overhead camera.

Rats were run in groups of six with three rats from the complex environment housing condition and three rats from the individual housing condition. Each rat was run on the pre-acquistion probe trial first, and then blocks of 2 trials each until the group as a whole had completed the 4 blocks. During an acquisition block a rat was removed from its holding pen and placed in a small plastic chamber next to the maze. The rat was then picked up and released facing the inside wall of the maze from one of four compass release points. Once the rat had found the submerged platform it was allowed to remain there for a period of 15 seconds, whereupon it was placed back in the chamber next to the maze until the next trial of the block approximately 1 minute later. If the animal did not succeed in finding the platform in the allotted 60 sec search time it was removed from the water and placed on the platform for the 15 seconds. Probe trials were run similarly, except that the submerged platform was removed and the rats remained swimming in the maze for the full minute.

Acquisition trials were scored for escape to platform latencies, measured from the time of release to the time of attaining the platform. Probe trials were scored from the video tapes as time spent in each of 4 quadrants of the partitioned maze. After examining the video tapes a number of other measures were introduced and additionally scored. On

the pre-acquisition probe trials, the time spent swimming thigmotactically around the edge of the maze as a proportion of the 60 seconds, and the time from the trial onset to the time that an animal crossed into a circular area centered in the maze and .7 of the maze's diameter were recorded. Whether or not animals exhibited exophthalmus (protrusion of the eyeballs) indicating activation of the sympathetic system was judged and noted by the experimenters on the first day during training. During the post-training probe trial a majority of the animals switched strategies from searching for the platform to trying to scramble onto the lip and out over the rim. One of the rats housed in the complex environment managed to escape the maze using this strategy. As a result, post-training probe trials were scored for 5 mutually exclusive categories: time spent swimming in the 4 different quadrants and the time spent facing the inside edge of the maze scrabbling at the lip of the maze. Thus, the post training quadrant analysis was composed of quadrant time as a proportion of the summed time spent actively searching the maze for the platform. Additionally, the time to the first platform location crossing was scored, as well as the time from the trial onset to the first bout of scrambling at the lip. When the animals had completed the task on the second day their weight was obtained.

The simple swim and climb task was run on day 36. An unpainted aluminum rectangular tank 142.25 cm long, 98 cm wide, and 61 cm deep was filled 33 cm deep with 22°C temperature water. Over one end of the tank a rectangle of wire mesh (43 cm wide) was placed, such that it reached from the top of the tank to the inside bottom. The rim of this rectangle was edged with bright yellow tape blunting all the sharp ends. Rats

were released at the opposite end of the tank facing the mesh, whereupon they would swim to the mesh and climb out. Upon attaining the top of the mesh the rats were allowed 15 sec rest and explore, at which point the next trial would begin. Rats were given two blocks of 5 trials each, with the blocks separated by approximately two hours. Each rat would run through a full block before being returned to its holding pen.

The time taken from release to touching the mesh with a forepaw was taken as the swim time. Careful notes were also taken about the directness of the path chosen by the animal. During the second block of trials a climb time consisting of the interval from the forepaw touching the mesh to the first hindlimb attaining the top of the wire mesh was further recorded. During the first block of trials the release point and wire mesh were centered in the width of the tank. During the second block of trials the release point and wire mesh were placed adjacent to one of the long sides. This was done to assist the rats in swimming in a straight line, since a number of the rats displayed consistently thigmotactic paths in attaining the wire mesh throughout the first block of trials. The shortest time accomplished with a straight path was used to calculate the swimming speed of each rat, which was simply the length of the tank over the shortest time. Of the 5 rats that did not have sufficiently straight swimming paths, one was dropped from the analysis, while the remaining 4 rats had the distance swum estimated. The fastest climb time without pauses was taken as a combined measure of strength and agility.

3.2.4 Procedures for the Social Interaction Experiment:

This experiment was run over the course of two days starting on Day 33. The apparatus consisted of a Plexiglas chamber 30 cm X 30 cm X 30 cm, which was open

at the top, and filled with a 2 cm layer of bedding chips. The box was lighted on each of two sides by 43 cm long fluorescent tubes (Westinghouse 15w warm white). The whole apparatus was then wrapped in a heavy black cloth except for the open top, thus lighting the box without allowing visual access to the surrounding room. Small amplified speakers (Sanyo model MSP-20) relaying white noise were placed on either side of the chamber. All interactions that took place in the chamber were recorded by an overhead video camera and VCR.

Rats to be tested were brought in from their respective housing conditions one at a time and allowed to habituate to the chamber for 20 minutes. At the end of the 20 minute habituation period a small (224g) male stimulus rat, which had earlier been placed in a similar holding chamber was introduced into the test chamber with the rat being assessed. The next 4 minutes of interactions were recorded by the video camera. The same stimulus rat was used throughout the experiment and had previously run through an identical protocol the day before with 10 other rats of similar weight to the rats being tested.

3.2.5 Procedures for Neophobic Responses to Novel Tastes:

These two tests were carried out after the rats had been implanted with electrodes, and electrophysiological recording had begun. The animals were tested on Day 84 for their reaction to almonds, and on Day 87 for their reaction to a .3 molar sucrose solution.

For both tests the rats remained housed in their night cages, in order to prevent exploratory bouts of any other test environment, which may have been novel. Prior to

this test, anecdotal observations suggested that the rats in the complex environment would readily accept new foods. Based on these observations it was hypothesized that the animals housed in the complex environment would be less neophobic to novel tastes than their individually housed littermate controls. The almonds and the sucrose solution used in these tests were novel to the animals in both housing conditions.

Two shelled almonds were placed in each rats' cage over a period of 90 seconds starting at 1:00 pm. After a period of 15 minutes the remainder of the almonds were recovered from the cages and placed in coded weigh boats on a tray and weighed. The recovery procedure took approximately 90 seconds and was performed in the same order as the delivery procedure.

For the two bottle discrimination test all rats had their water bottles removed on the evening of the 86th day. The following day, starting at noon, the animals received a two bottle taste preference test. Two graduated cylinders with ball bearing spouts, which had been used during the collection of the data on water intake, were presented to the rats in their cages for an hour. One of the graduated cylinders contained water while the other contained a .3 molar sucrose solution dyed blue with food coloring, for easy identification. At the end of the hour the graduated cylinders were removed and the amount of liquid that the rats had consumed was recorded. Left and right placement of the sucrose solution and the water in the cages was varied across the rats. The number of graduated cylinders available limited the number of rats that could be tested simultaneously to 10. Thus, the test was run in two groups consisting of 10 and 8 animals respectively, with the two housing conditions equally represented in both groups.

The amount of sucrose solution consumed as a proportion of the total amount of liquid consumed was calculated as the dependent measure. At the conclusion of the experiment it was discovered that one of the sucrose containing graduated cylinders would not dispense any liquid. Consequently, the data collected from the two animals that had received this graduated cylinder were removed from the analysis. Both of these rats were drawn from the complex environment housing condition.

3.3.1 Results of the Food and Water Intake, Fecal Matter and Weight, and Spontaneous Locomotor Activity Analyses:

Results from pilot work suggested that once placed in the complex environment the rats would lower their spontaneous locomotor activity when tested in the activity monitors. The means for all the data collected can be seen in Figures 3 through 5.

The nine activity variables were summed or averaged across the six samples and the two days, of each phase, such that each rat had a single score for each of the three phases, for each of the nine activity variables. These data were then analysed with housing condition as a between groups factor and phase as the repeated within subjects factor. Of primary interest was the group by phase interaction, which would test whether the rats in the complex housing condition lowered spontaneous locomotor activity scores after being placed in the complex environment. Results revealed that there were differences between the housing conditions over the three phases [$F_{(18,50)}=6.63$; $p<.0005$]. The univariate group by phase interactions were also significant for all of the nine variables (Table I). A between subjects analysis was then performed on each phase separately. Results from the first phase indicated no group differences at either the

Figure 3 Results from the assessment of spontaneous locomotor activity, Movement characteristics variable cluster. Figure depicts the means and standard error of the means of the activity measures recorded during the six 5 minute samples, on day 1 and day 2, of each of the three phases. Phase 1, or the baseline data, occurred immediately prior to separation into the differential housing condition. Phase 2 occurred immediately following separation into the differential housing conditions. Phase 3 occurred approximately three weeks following separation into the differential housing conditions. Closed circles represent the complex environment housed rats, while the open circles represent the individually housed littermates. Movement characteristics variable cluster included: Total Distance Travelled (TD: top panel), Average Distance travelled per movement (ADM; middle panel), and Average Speed per movement (AS; bottom panel).

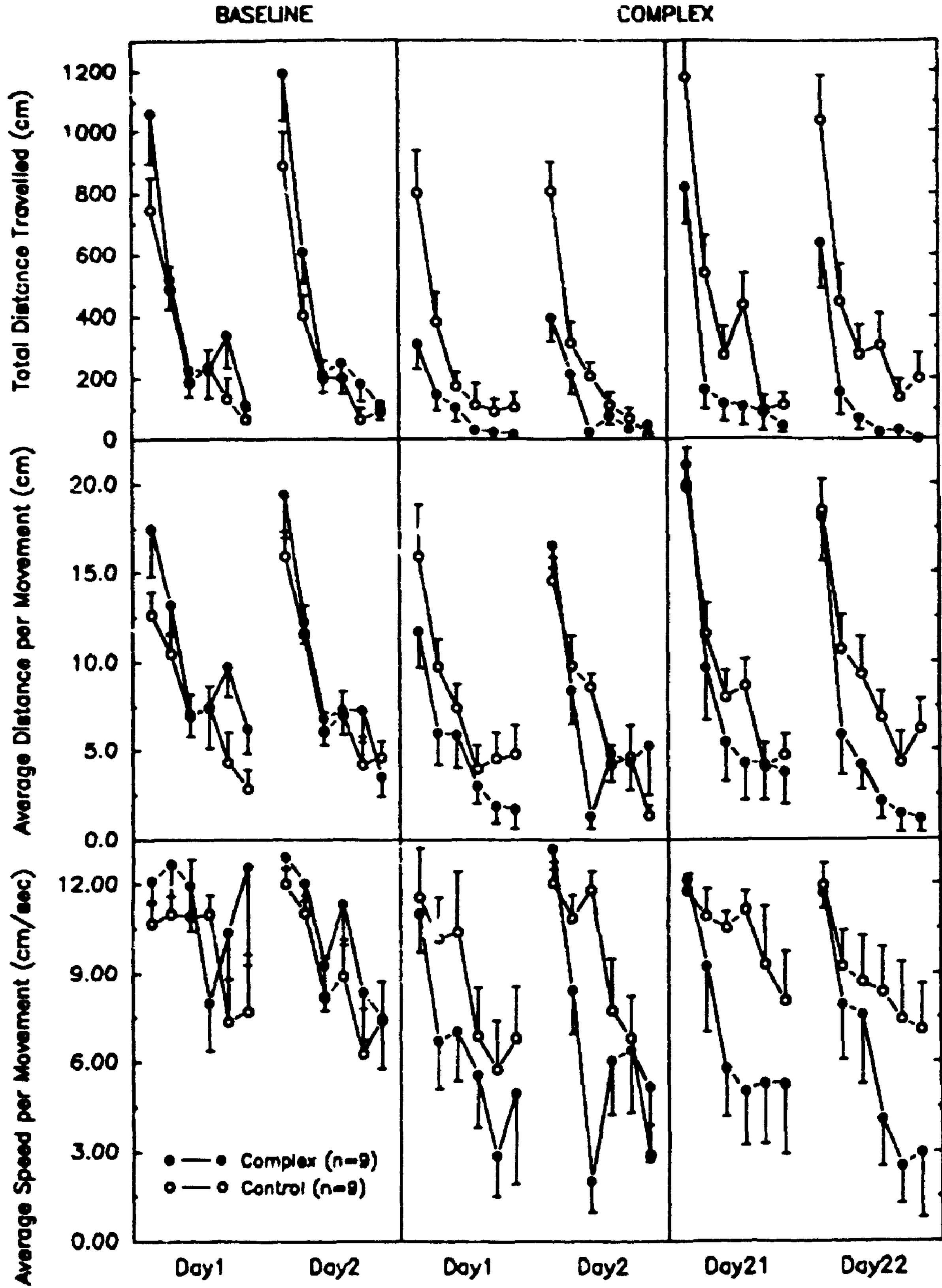

BASELINE
COMPLEX
Total Distance Travelled (cm)
1200
1000
800
600
400
200
0
Average Distance per Movement (cm)
20.0
15.0
10.0
5.0
0.0
Average Speed per Movement (cm/sec)
12.00
9.00
6.00
3.00
0.00
Complex (n=9)
Control (n=9)
Day1
Day2
Day1
Day2
Day21
Day22

Figure 4 Results from the assessment of spontaneous locomotor activity, Horizontal variable cluster. Figure depicts the means and standard error of the means of the activity measures recorded during the six 5 minute samples, on day 1 and day 2, of each of the three phases. Phase 1, or the baseline data, occurred immediately prior to separation into the differential housing condition. Phase 2 occurred immediately following separation into the differential housing conditions. Phase 3 occurred approximately three weeks following separation into the differential housing conditions. Closed circles represent the complex environment housed rats, while the open circles represent the individually housed littermates. Movement characteristics variable cluster included: Number of Horizontal movements (NM; top panel), Time Spent in Horizontal movement (MT; middle panel), and Time per Horizontal movement (TM; bottom panel).

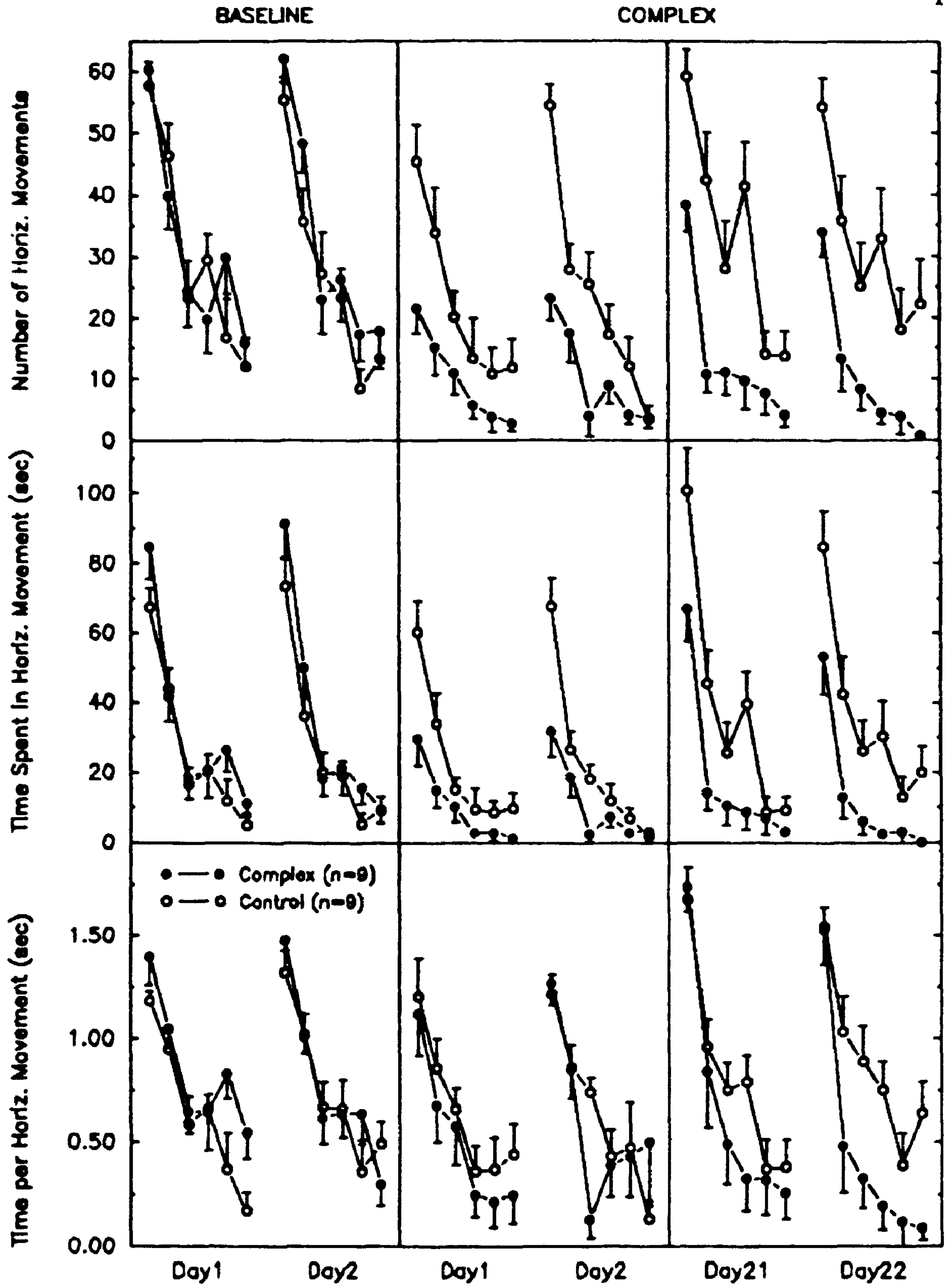

BASELINE
COMPLEX
Number of Horiz. Movements
Time Spent In Horiz. Movement (sec)
Time per Horiz. Movement (sec)
Complex (n=9)
Control (n=9)
Day1
Day2
Day1
Day2
Day21
Day22

Figure 5 Results from the assessment of spontaneous locomotor activity, Vertical variable cluster. Figure depicts the means and standard error of the means of the activity measures recorded during the six 5 minute samples, on day 1 and day 2, of each of the three phases. Phase 1, or the baseline data, occurred immediately prior to separation into the differential housing condition. Phase 2 occurred immediately following separation into the differential housing conditions. Phase 3 occurred approximately three weeks following separation into the differential housing conditions. Closed circles represent the complex environment housed rats, while the open circles represent the individually housed littermates. Movement characteristics variable cluster included: Number of Vertical movements (VM; top panel), Time Spent in Vertical movement (VT; middle panel), and Time per Vertical movement (TV; bottom panel).

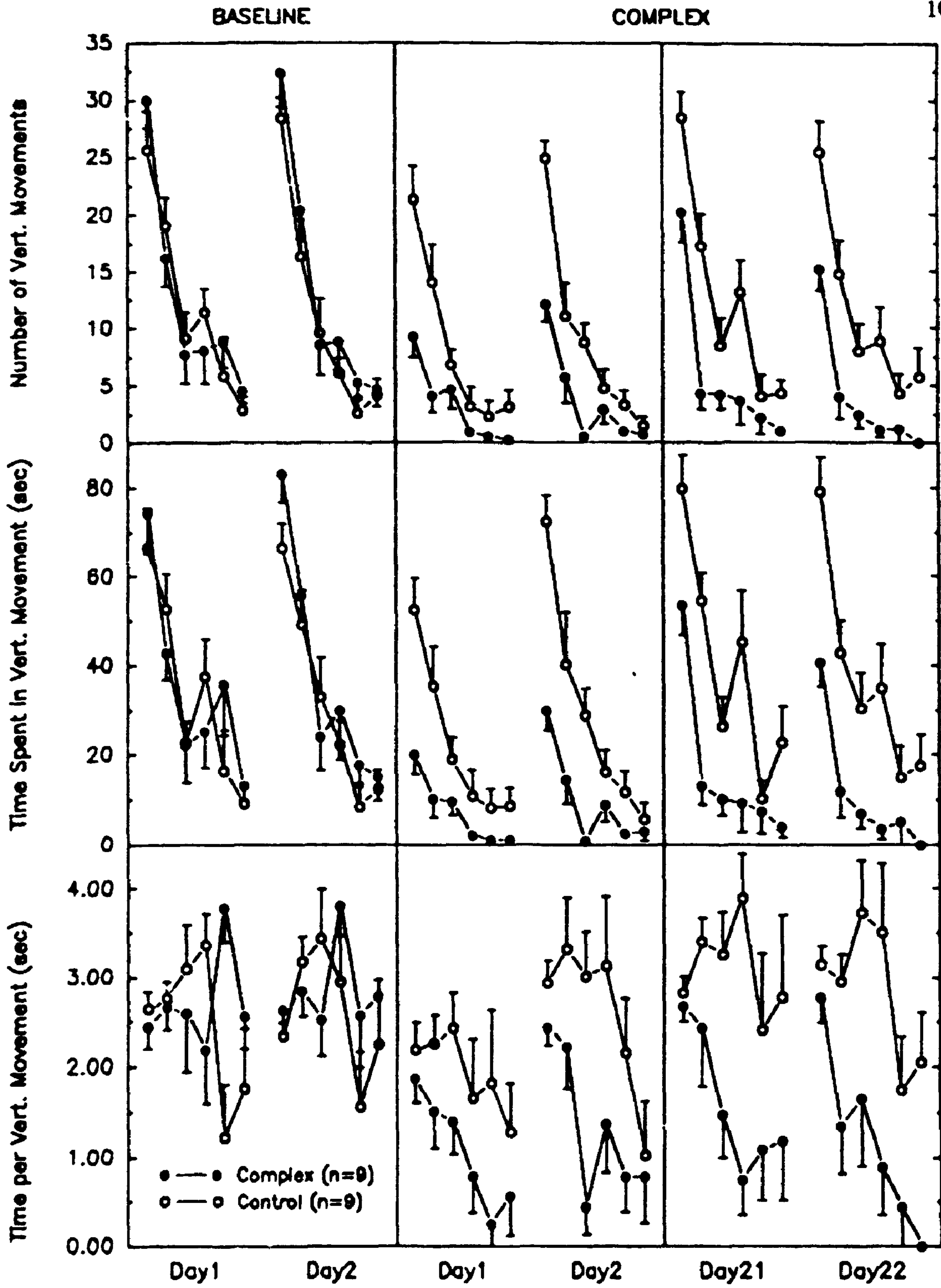

BASELINE
COMPLEX
Number of Vert. Movements
Time Spent in Vert. Movement (sec)
Time per Vert. Movement (sec)
Complex (n=9)
Control (n=9)
Day1
Day2
Day1
Day2
Day21
Day22

Table I Spontaneous locomotor activity monitor data analyses Group by Phase Interactions. Overall Multivariate and univariate F-ratios for the Group by Phase Interactions for the data collapsed across Sample and Days.

Variable	F-ratio	Significance level
Multivariate Interaction		
	$F_{(18,50)} = 6.63$;	$p < .0005$
Univariate Interactions		
TD	$F_{(2,32)} = 12.55$;	$p < .0005$
ADM	$F_{(2,32)} = 4.94$;	$p = .013$
AS	$F_{(2,32)} = 6.67$;	$p = .004$
NM	$F_{(2,32)} = 22.91$;	$p < .0005$
MT	$F_{(2,32)} = 12.39$;	$p < .0005$
TM	$F_{(2,32)} = 6.20$;	$p = .005$
VM	$F_{(2,32)} = 13.90$;	$p < .0005$
VT	$F_{(2,32)} = 14.99$;	$p < .0005$
TV	$F_{(2,32)} = 16.36$;	$p < .0005$

multivariate or univariate level [$F_{(9,8)}=1.21$; $p=.399$]. Results from the second phase indicated group differences at the multivariate level [$F_{(9,8)}=5.51$; $p=.012$] and group differences for a number of the univariate analyses at the univariate level. In particular, differences were observed for the TD, MT, VT, and TV activity variables (see Table II). Results from the third phase also revealed group differences at the multivariate level [$F_{(9,8)}=6.076$; $p=.009$] and at the univariate level for all activity variables except ADM (see Table II).

The food and water intake, fecal matter and weight data from the first phase were analysed separately from the third phase. Data from the first phase were analysed by a mixed design with housing condition as a between subjects factor and the three days of data collection as a within subjects repeated measures factor. Only the multivariate, main effect of housing was of interest, with results indicating that there were no differences between the two groups before being separated into their respective housing conditions [$F_{(3,14)}=.53$; $p=.668$].

Similar analyses for the third phase could not be performed upon the data, since the food, fecal matter and water measurements collected from the complex environment could not be attributed to individual rats. Therefore these data were analysed, following Fiala et al. (1977), using a series of nonparametric Wilcoxon matched-pairs signed ranks tests, in which individual measurements from the littermate controls were contrasted against the mean of the complex environment housed rats. Results, using a one-tailed probability distribution for evaluation, indicated that the complex environment housed animals ate less [$Z_{(27)}=-2.03$; $p=.021$], drank less [$Z_{(27)}=-4.54$; $p<.0005$], and appeared

Table II **Spontaneous locomotor activity monitor data analyses Group Main Effects. Overall Multivariate and univariate F-ratios for the Main Effect for each of the three phases for the data collapsed across Sample and Days.**

Variable	F-ratio	Significance level
Multivariate Effect for 1st Phase	$F_{(9,8)} = 1.21$;	$p=.399$
Univariate Effects		
TD	$F_{(1,16)} = 2.83$;	$p=.112$
ADM	$F_{(1,16)} = 2.58$;	$p=.127$
AS	$F_{(1,16)} = 3.24$;	$p=.090$
NM	$F_{(1,16)} = .76$;	$p=.394$
MT	$F_{(1,16)} = 1.15$;	$p=.299$
TM	$F_{(1,16)} = 1.77$;	$p=.201$
VM	$F_{(1,16)} = .55$;	$p=.466$
VT	$F_{(1,16)} = .05$;	$p=.815$
TV	$F_{(1,16)} = 1.49$;	$p=.239$
Multivariate Effect for 2nd Phase	$F_{(9,8)} = 5.51$;	$p=.012$
Univariate Effects		
TD	$F_{(1,16)} = 11.86$;	$p=.003$
ADM	$F_{(1,16)} = 1.99$;	$p=.177$
AS	$F_{(1,16)} = 2.98$;	$p=.103$
NM	$F_{(1,16)} = 1.94$;	$p=.182$
MT	$F_{(1,16)} = 9.48$;	$p=.007$
TM	$F_{(1,16)} = .78$;	$p=.388$
VM	$F_{(1,16)} = 1.29$;	$p=.271$
VT	$F_{(1,16)} = 35.76$;	$p<.0005$
TV	$F_{(1,16)} = 15.87$;	$p=.001$
Multivariate Effect for 3rd Phase	$F_{(9,8)} = 6.06$;	$p=.009$
Univariate Effects		
TD	$F_{(1,16)} = 7.30$;	$p=.016$
ADM	$F_{(1,16)} = 2.39$;	$p=.142$
AS	$F_{(1,16)} = 5.33$;	$p=.035$
NM	$F_{(1,16)} = 16.08$;	$p=.001$
MT	$F_{(1,16)} = 8.70$;	$p=.009$
TM	$F_{(1,16)} = 4.58$;	$p=.048$
VM	$F_{(1,16)} = 11.64$;	$p=.004$
VT	$F_{(1,16)} = 24.28$;	$p<.0005$
TV	$F_{(1,16)} = 22.18$;	$p<.0005$

to defecate less than their individually housed littermates, although this last difference only approached significance [$Z_{(27)}=-1.49$; $p=.068$].

Weight was properly analysed across both phases, using a mixed design with housing condition as the between subjects factor and phase and day being the two within repeated measures factors. Only the housing condition by phase interaction was of interest in this analysis. Results revealed a difference that approached, but failed to achieve, significance [$F_{(1,16)}=3.67$; $p=.073$]. Thus, although it appeared that the rats housed in the complex environment gained less weight than their individually housed littermates after being separated into their respective housing conditions, this difference did not reach statistical significance.

3.3.2 Results of the Strength and Agility Analyses:

The means of the escape latencies are presented in Table III. The balance beam escape latencies were analysed by a mixed design with housing condition as the between subjects factor and the five trials as the repeated within subjects design. Results indicated that the rats housed in the complex environment escaped to the holding boxes with shorter latencies than their individually housed littermates [$F_{(1,16)}=8.74$; $p=.009$]. One of the individually housed controls consistently fell from the beam 3 times on every trial, and consequently was scored at the maximum time for all five trials. To ensure that this rat did not induce an artifactual time that would skew the results, the data were re-analysed with this rat's data excluded. Removal of this rat's data increased the significance of the difference between groups by reducing the variance of the individually housed controls [$F_{(1,15)}=17.40$; $p=.001$]. No other factors or interactions were

Table III Analyses of Balance beam data escape latencies and the behaviors scored from video tapes. For the escape latencies the means are presented along with the s.e.m. in brackets, while for the scored behaviors the total number of animals per group that exhibited the scored behaviors are presented and the number of times that the behaviors were exhibited in brackets. The behaviors include falls during a trial, spontaneous crossings of the balance beam from one safe box to the other, spontaneous rears on the balance beam, spontaneous 180° turns on the balance beam and the number of pauses on the balance beam. The escape latency data were analysed without the data from the control animal that fell consistently. Only three of the five scored behaviors had adequate data for statistical analyses. The nonparametric Mann-Whitney U - Wilcoxon rank sum W test corrected for ties statistic was used for these comparisons. The resulting Z statistic was evaluated using a one-tailed distribution.

Behaviors		Controls	Complex
Escape Latencies			
Trial 1		25.19 (7.81)	9.22 (1.95)
Trial 2		20.88 (5.95)	8.44 (1.79)
Trial 3		18.56 (4.89)	9.89 (1.57)
Trial 4		18.75 (2.22)	9.83 (1.80)
Trial 5		15.38 (2.17)	9.00 (2.17)
$F_{(1,15)} = 17.40$; $p = .001$			
Falls		5/9 (21)	0/9
Spontaneous Crossings		1/9 (1)	3/9 (5)
Spontaneous Rears		0/9	2/9 (2)
Spontaneous 180° Turns		2/9 (3)	8/9 (12)
Pauses on the Beam		2/9 (2)	7/9 (10)
Falls	$Z_{(18)} = -2.52$;	$p = .006$	
180° Turns	$Z_{(18)} = -2.50$;	$p = .006$	
Pauses	$Z_{(18)} = -2.46$;	$p = .006$	

significant, indicating that neither group alone or together improved their performance across the five trials.

Of the five behaviors scored from the videotapes, only three were deemed to have sufficient occurrences to be tested statistically. The number of falls, spontaneous 180° turns, and pauses on the beam were analysed by a Mann-Whitney U - Wilcoxon rank sum W test corrected for ties. Results indicated that the littermate controls fell more often than the complex environment housed rats, while the complex animals paused more often, and executed more 180° turns on the balance beam. These three behaviors and the number of spontaneous crossings and rears are displayed in Table III, along with the statistical results, where appropriate.

Prior to the hanging duration task it was predicted that the rats housed in the complex environment would be stronger and more agile and therefore be able to suspend themselves longer than their individually housed littermate controls. It was also thought that the weight of the animals could be a potential confound in this test, such that the heavier animals would be disadvantaged in comparison to the lighter animals.

Thus, the data were analysed by a mixed design, with housing condition as the between subjects factor, the three trials as the repeated measure and weight as a covariate. Results of this analysis indicated that the animals housed in a complex environment were able to hang onto the trapeze for a longer duration than their individually housed littermates, regardless of their respective weights [$F_{(1,15)}=5.67$; $p=.031$]. In this analysis, weight as a covariate did not account for a significant proportion of the hanging duration across all three trials [$t_{(16)}=-1.54$; $p=.143$].

Subsequently, the analysis was re-run without the covariate, indicating that removal of weight as a covariate only improved the distinction between housing conditions [$F_{(1,16)}$=7.75; p=.013]. The mean latencies for the hanging duration, and the mean force exerted on the spring scale strength task are presented in Table IV.

Prior to the Spring Scale task it was predicted that the rats housed in the complex environment would be stronger and therefore be able to exert more force against the scale than their individually housed littermate controls. Weight of the rats was also thought to be a potential confound for this test. However, correlations between weight of the rats and their performance on the three separate trials, indicated none to be significant, with the strongest correlation accounting for less than 4% of the variance.

The force in grams weight across the three test trials were then analysed by a mixed design with housing condition as the between subjects factor and the three test trials as the repeated measures. Results indicated that the rats housed in a complex environment were able to exert more pull against the spring scale than their individually housed littermates [$F_{(1,16)}$=6.27; p=.024]. No other effects were significant.

3.3.3 Results From the Morris Water-maze and Simple Swim and Climb Task Analyses:

To test for acquisition of the task, the escape latencies on all eight trials were analysed in a mixed design, with block and trial as the repeated measures, and housing condition as a between subjects factor.

Analyses indicated that the complex environment housed rats outperformed their individually housed littermates overall, as assessed by the Group main effect

Table IV Analyses of the Hanging duration and Spring Scale Strength Tasks. The means and s.e.m. (in brackets) are presented for the hanging duration in s. and the force on the spring scale in g. Univariate F-ratios represent group main effects for the repeated measure of trial.

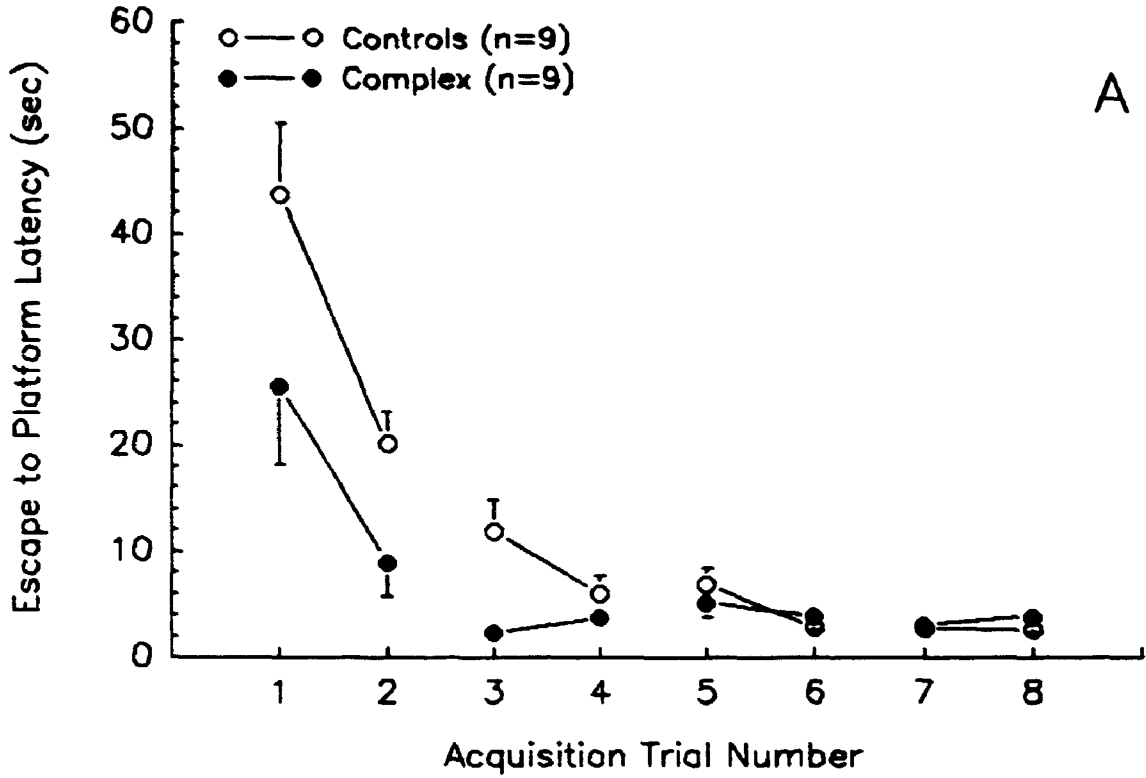
Behaviors	Controls	Complex
Hanging Duration		
Trial 1	6.94 (1.67)	10.83 (2.90)
Trial 2	5.94 (1.22)	22.83 (7.89)
Trial 3	8.22 (2.87)	19.28 (4.08)
$F_{(1,16)}=7.75$; $p=.013$		
Spring Scale Strength		
Trial 1	1491.11 (75.73)	1722.22 (103.25)
Trial 2	1433.33 (73.26)	1662.22 (82.02)
Trial 3	1355.56 (61.89)	1522.22 (87.71)
$F_{(1,16)}=6.26$; $p=.024$		

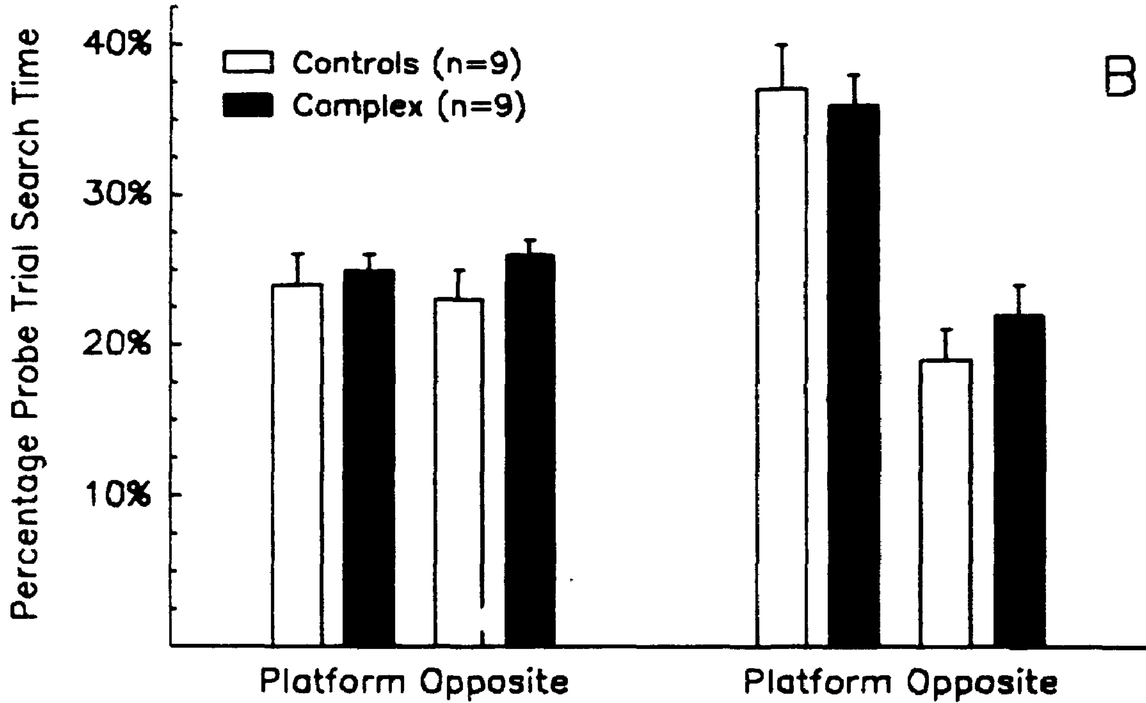
[$F_{(1,16)}$=4.61; p=.047]. However, the Group X Block interaction, indicated that the difference between the groups changed across the 4 blocks [$F_{(3,48)}$=3.44; p=.024]. Subsequently, the escape latencies of the first 2 blocks were summed together as were the escape latencies of the last 2 blocks. These data were then analysed by two univariate analyses of variance resulting in a significant difference between the groups for the first 2 blocks [$F_{(1,16)}$=5.21; p=.038], but no difference between the groups for the last 2 blocks [$F_{(1,16)}$=0.08; p=.777]. Thus, by the end of the acquisition phase rats from both housing conditions had learned the task equally well. These data and results can be observed in Figure 6a.

To test retention, the pre-acquisition and post-training probe trials were analysed by a mixed design, with pre- and post-training probe trials as a repeated measure, quadrant as a 4 level within subject factor, and housing condition as a between subjects factor. All the dependent variables were calculated as proportions of the total search time. The Time X Quadrant interaction indicated that the animals spent significantly more active search time in the platform quadrant after training than before acquisition and less time in any other quadrant [$F_{(3,48)}$=12.83; p<.0005]. The Group X Time X Quadrant interaction however, indicated no differences between the two groups in their ability to retain the maze [$F_{(3,48)}$=0.07; p=.975]. Thus, rats from both housing conditions retained the location of the hidden platform equally well. Both these results can be seen in Figure 6b.

The additional variables collected from the pre-acquisition and post-training probe trials were analysed by individual univariate analyses and on occasion subsequent

Figure 6 Results of the water-maze experiment; acquisition and retention. **A** depicts the means and standard error of the means of the escape to hidden platform latencies across the 8 trials, run in four blocks. Closed circles represent the complex environment housed rats, while the open circles represent the individually housed littermates. **B** depicts the means and standard error of the means of the pre-acquistion (left) and post-acquisition (right) probe trials, for the quadrant that contained the the platform (platform) and the quadrant that was opposite to the hidden platform (opposite). Closed bars represent the complex environment housed rats, while the open bars represent the individually housed littermates.

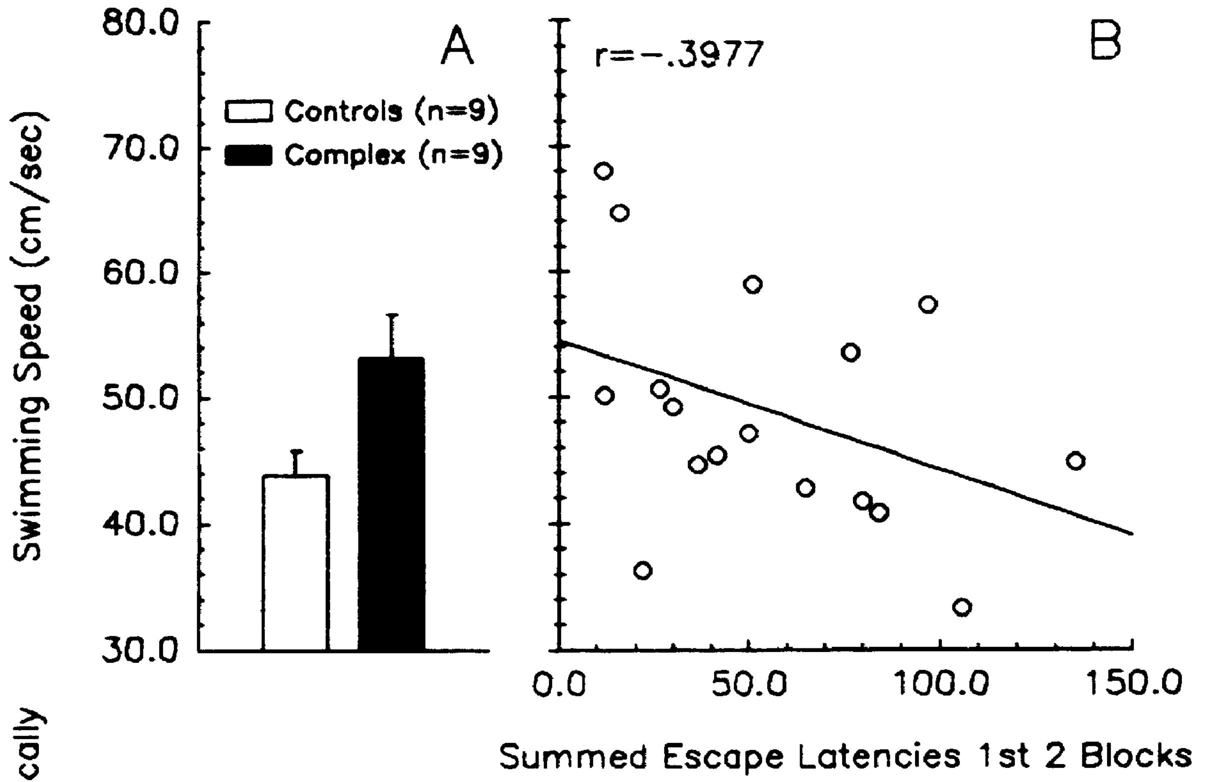

Controls (n=9)
Complex (n=9)
A
Escape to Platform Latency (sec)
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
Acquisition Trial Number


Controls (n=9)
Complex (n=9)
B
Percentage Probe Trial Search Time
40%
30%
20%
10%
Platform Opposite
Platform Opposite
Pre-acquisition vs. Post-training Probe Trial Quadrant Analysis

correlations. Time to cross into the centre area during the pre-acquisition probe trial revealed no significant difference between the groups [$F_{(1,16)}=1.07$; $p=.317$]. There was also no difference between the groups in the post-training probe trial measure of time to cross the platform location [$F_{(1,16)}=0.50$; $p=.491$], nor was there a difference between the groups in the amount of time they spent scrambling at the lip of the maze [$F_{(1,16)}=0.30$; $p=.589$]. However, the individually housed controls spent more time, as a proportion of the 60 seconds, swimming thigmotactically during the pre-acquisition probe trial than the animals housed in the complex environment [$F_{(1,16)}=5.43$; $p=.033$]. A subsequent correlation between this measure and the summed escape latencies for the 1st two blocks revealed no predictability at all [$r_{(16)}=.11$; $p>.05$]. These latter two results can be observed in Figure 7c-d. A further correlational analysis between the weight of the animals and the same acquisition measure [$r_{(16)}=.37$; $p=ns$] and retention measure of proportion of search time spent in the platform quadrant [$r_{(16)}=-.24$; $p=ns$] also showed no relation. The former of these two correlations can be observed in Figure 8a.

Of the individually housed controls 7/9 exhibited exophthalmus during maze training, while only 1/9 of the animals housed in the complex environment exhibited this phenomenon. As a consequence the presence or absence of exophthalmus was coded as a between subjects factor and the initial acquisition data were re-analysed on this factor, regardless of housing condition. Results indicated no significant difference between animals that exhibited exophthalmus and those that did not, on any of the analyses performed.

Figure 7 Results of the water-maze experiment relations of swimming speed and thigmotactic swimming to water-maze acquisition. **A** depicts the means and standard error of the means of the swimming speed calculated from the simple swim and climb task. Closed bars represent the complex environment housed rats, while the open bars represent the individually housed littermates. **B** depicts the scattergram and regression of the individual rats' swimming speed with the escape latencies summed over the 4 trials of the first 2 blocks. **C** depicts the means and standard error of the means of the percentage of time during the pre-acquisition probe trial spent swimming thigmotactically. Closed bars represent the complex environment housed rats, while the open bars represent the individually housed littermates. **D** depicts the scattergram and regression of the individual rats' percentage time swimming thigmotactically with the escape latencies summed over the 4 trials of the first 2 blocks.


80.0
70.0
60.0
50.0
40.0
30.0
Swimming Speed (cm/sec)
A
Controls (n=9)
Complex (n=9)
B
r=-.3977
0.0
50.0
100.0
150.0
Summed Escape Latencies 1st 2 Blocks

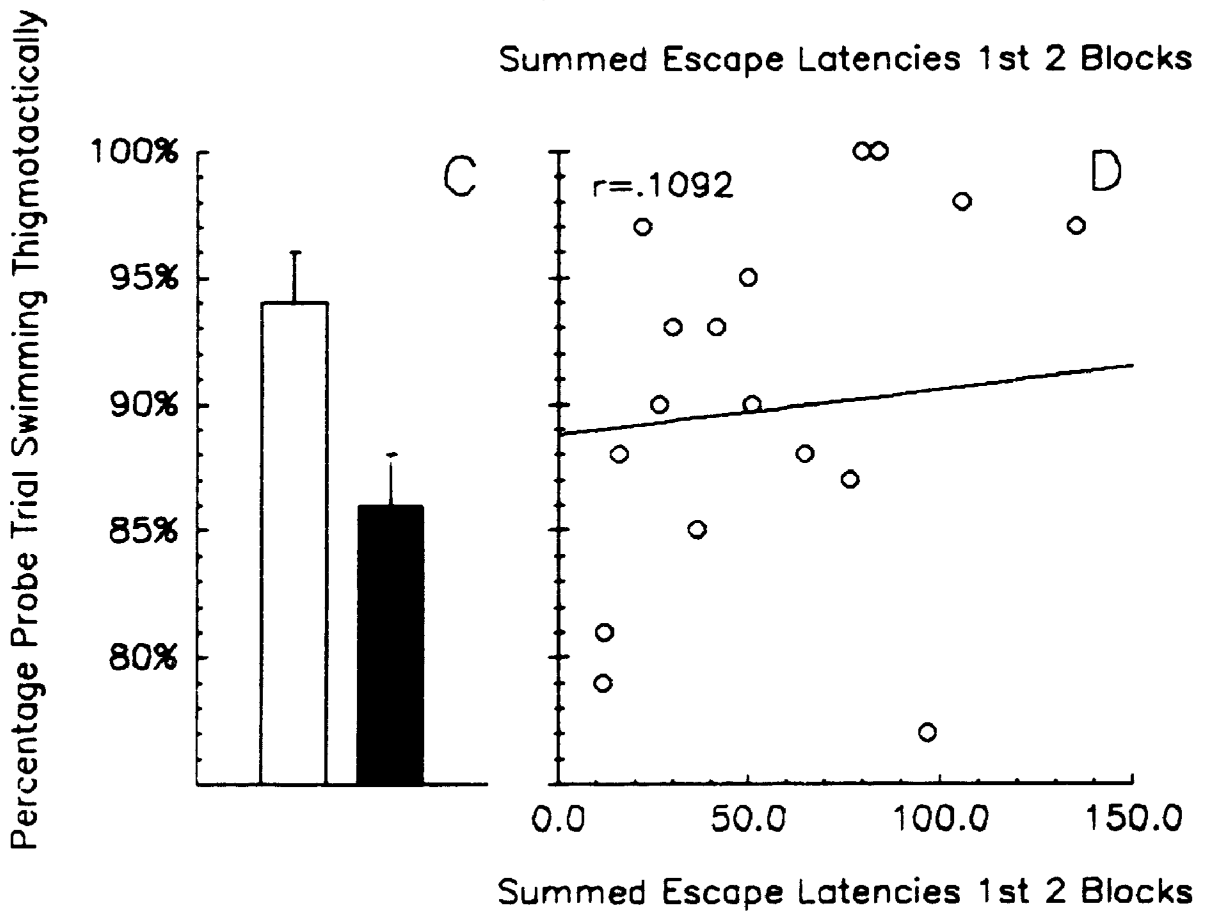
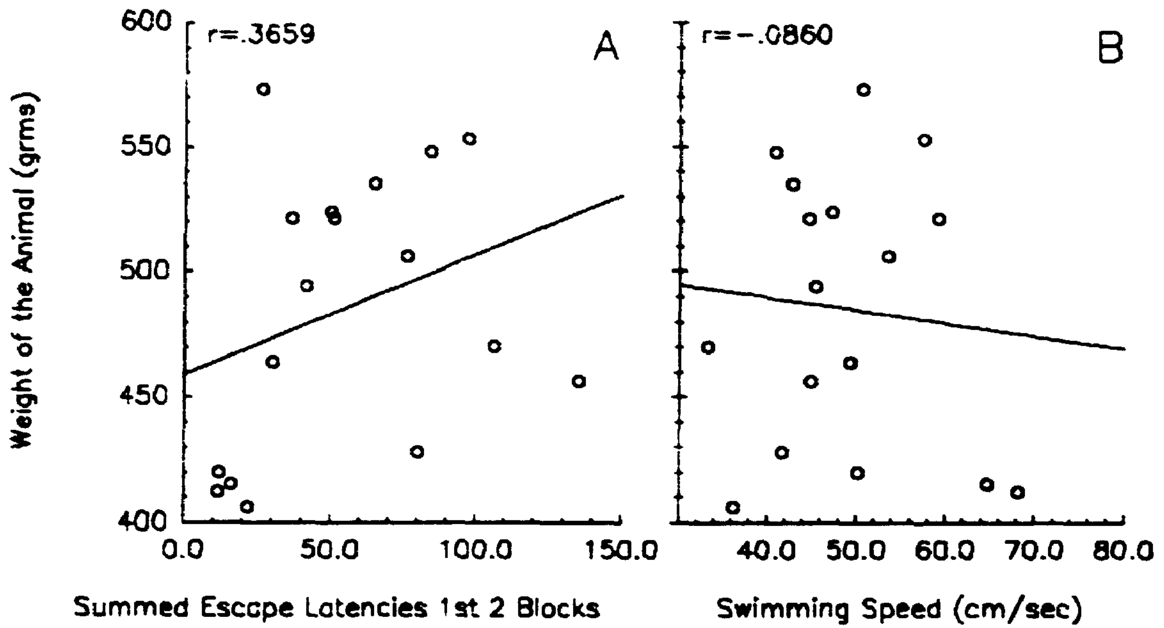
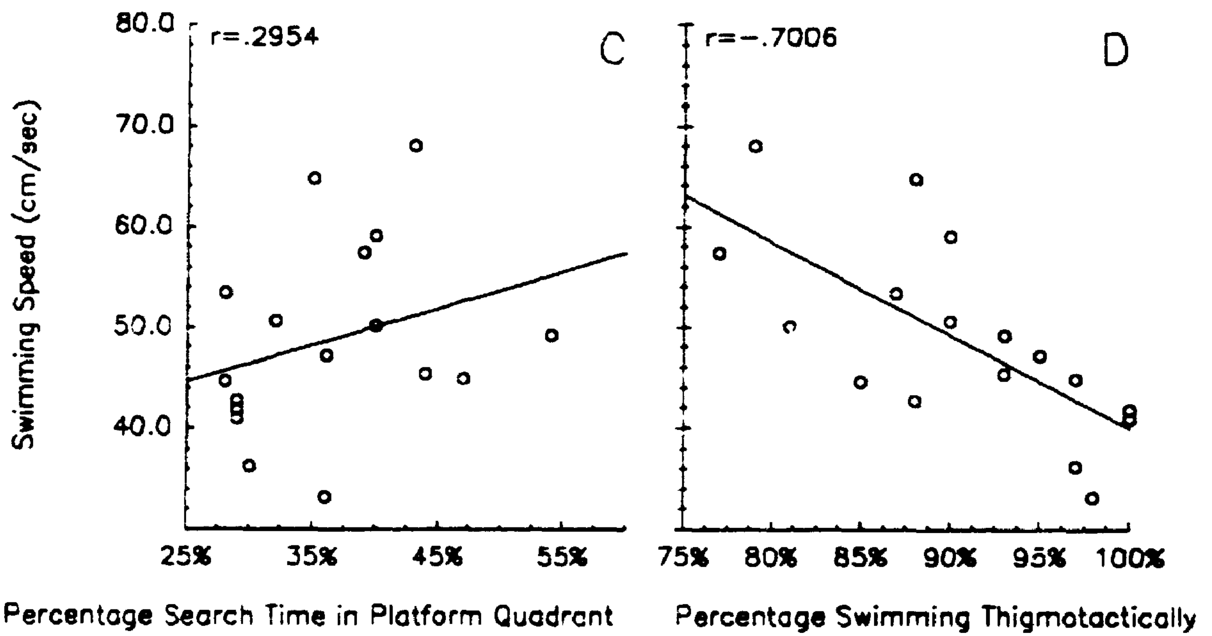

Percentage Probe Trial Swimming Thigmotactically
100%
95%
90%
85%
80%
C
D
r=.1092
0.0
50.0
100.0
150.0
Summed Escape Latencies 1st 2 Blocks

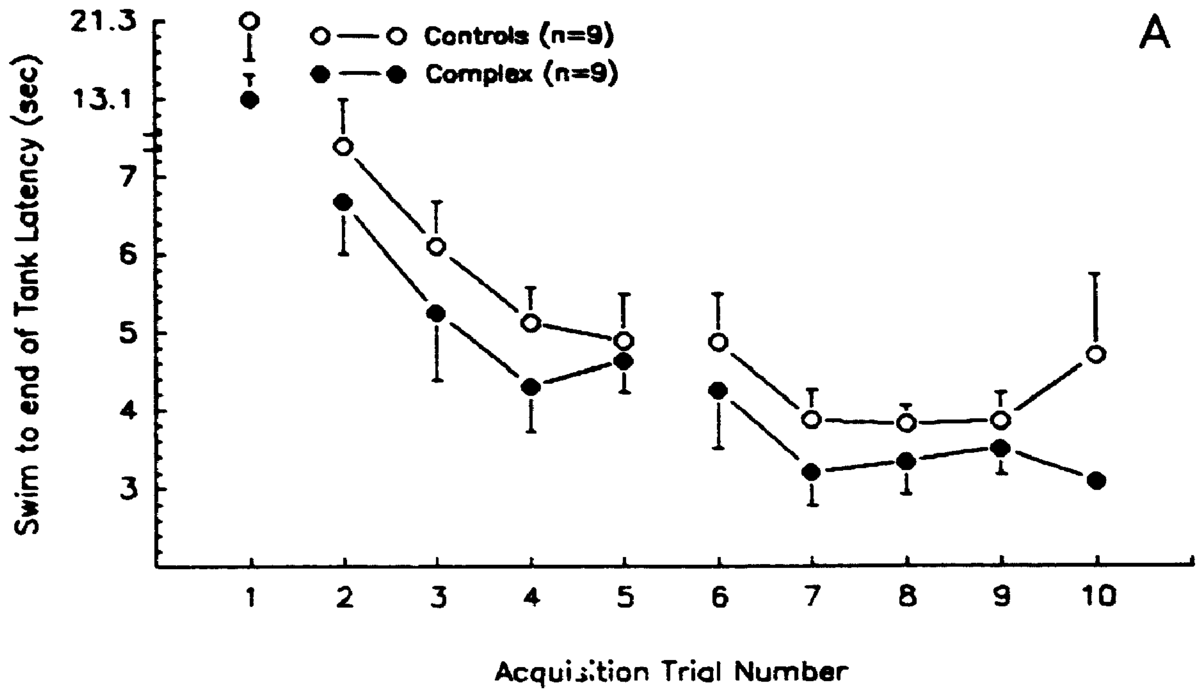
Figure 8 Results of the water-maze experiment; various relations among the recorded variables. **A** depicts the scattergram and regression of the individual rats' weight with the escape latencies summed over the 4 trials of the first 2 blocks. **B** depicts the scattergram and regression of the individual rats' weight with the their swimming speed **C** depicts the scattergram and regression of the individual rats' swimming speed with the percentage of time spent searching for the platform during the probe trial in the quadrant, where the platform had been located. **D** depicts the scattergram and regression of the individual rats' swimming speed with the percentage of time spent swimming thigmotactically during the pre-acquisition probe trial.

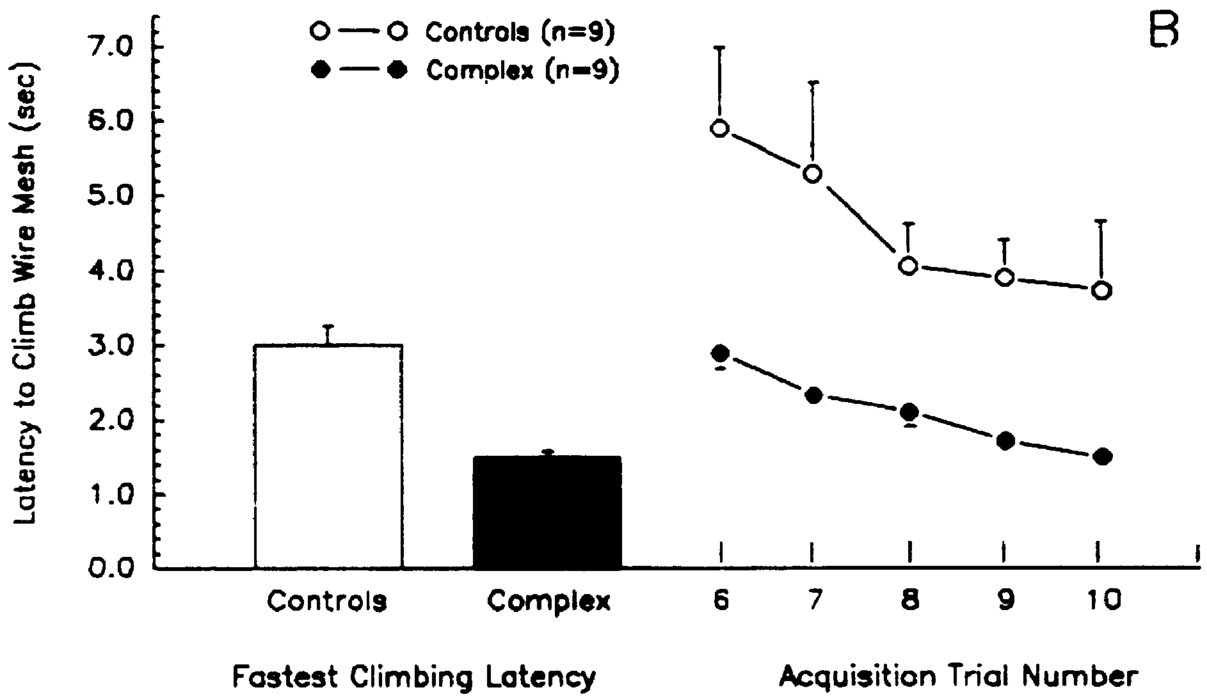

r=.3659
A
r=−.0860
B
Weight of the Animal (grms)
600
550
500
450
400
0.0
50.0
100.0
150.0
40.0
50.0
60.0
70.0
80.0
Summed Escape Latencies 1st 2 Blocks
Swimming Speed (cm/sec)


r=.2954
C
r=−.7006
D
Swimming Speed (cm/sec)
80.0
70.0
60.0
50.0
40.0
25%
35%
45%
55%
75%
80%
85%
90%
95%
100%
Percentage Search Time in Platform Quadrant
Percentage Swimming Thigmotactically

The simple swim portion of the simple swim and climb task were analysed by a mixed design with the 2 blocks of 5 trials as repeated measures and housing condition as a between subjects factor. Analyses indicated that the rats housed in the complex environment did marginally better than their individually housed littermates [$F_{(1,16)}=4.25$; $p=.056$]. Rats in both groups also improved over the 5 trials in both blocks [$F_{(4,64)}=25.21$; $p<.0005$], although no significant improvement was shown over the last 4 trials of the second block [$F_{(3,48)}=1.27$; $p=.297$]. These data and results can be observed in Figure 9a. It is also worth noting that of the 5 animals that had adjustments made to the distance swum, 4 were individually housed controls, indicating that proportionally more of the complex environment housed rats acheived more direct swimming paths in this task than did their individually housed littermates. The climbing data were analysed in a single design with the 5 trials as a repeated measure. Results indicated that the rats housed in the complex environment were faster than their individually housed controls [$F_{(1,16)}=15.63$; $p=.001$], that both groups improved over the 5 trials [$F_{(4,64)}=3.79$; $p=.008$], and that there was no difference in improvement between the groups [$F_{(4,64)}=.40$; $p=.806$]. Analysis of the fastest climb time also indicated that the animals housed in the complex environment performed the climb faster than the individually housed controls [$F_{(1,16)}=25.99$; $p<.0005$]. These data can be seen in Figure 9b. The analysis of the speed of the animals indicated that the rats housed in the complex environment swam at a significantly faster speed than the individually housed controls [$F_{(1,16)}=5.36$; $p=.035$]. This result can be observed in Figure 7a. Swim speed was then related to the weight of the rats to see if the larger animals were slower swimmers. The

Figure 9 Results of the simple swim and climb task. **A** depicts the means and standard error of the means of the escape to hidden platform latencies across the 10 trials, run in two blocks. Closed circles represent the complex environment housed rats, while the open circles represent the individually housed littermates. **B** Results from the climb portion of the simple swim and climb task. The left half represents the means and standard errors of the means of the fastest climb latency. Closed bars represent the complex environment housed rats, while the open bars represent the individually housed littermate controls. The right half depicts the means and standard errors of the means of the climb times across the recorded five trials of the second block. Closed circles represent the complex environment housed rats, while the open circles represent the individually housed littermate controls.


A
Controls (n=9)
Complex (n=9)
Swim to end of Tank Latency (sec)
21.3
13.1
7
6
5
4
3
1
2
3
4
5
6
7
8
9
10
Acquisition Trial Number


B
Controls (n=9)
Complex (n=9)
Latency to Climb Wire Mesh (sec)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Controls
Complex
6
7
8
9
10
Fastest Climbing Latency
Acquisition Trial Number

results of this analysis showed no relation between swim speed and weight [$r_{(16)}=-.09$; p=ns], as shown in Figure 8b. These data were then combined with the acquisition data from the Morris water-maze and re-analysed to see if the faster swimming rats had better acquisition curves. The correlations in the resulting matrix were all nonsignificant [$r_{(max)}=-.39$; p=ns].

Although previous analyses showed that none of the pre-acquisition probe trial measures were predictive of acquisition or retention, swimming speed was further correlated with these measures. The results of this anlysis indicated that swimming speed was significantly related to the amount of time spent swimming thigmotactically around the maze, such that the faster the swim speed the less time spent thigmotactically [$r_{(16)}=-.70$; $p<.001$], as shown in Figure 8d. Further as can be seen in Figure 8b swimming speed had no relation to the weight of the animal

3.3.4 Results of the Social Interaction Experiment:

The interactions were initially viewed on a monitor while they were being recorded and a list of observed behaviors was made. Afterwards this list of behaviors was grouped into four categories: sniffing, dominance, aggression, and self directed behaviors.

The first category contained five different sniffing behaviors. Typically, these were regions of the rat being examined and were as follows: the head, the dorsal surface, the ventral region, the anogenital region, and approaching the tail. The head as a region is self-explanatory. The dorsal surface included the area from the shoulders to the base of the tail. The ventral region was defined as the region on the ventral surface,

demarcated by the front and back limbs. The anogenital region was defined as the area immediately surrounding the genital region, the testes, and just beneath the base of the tail around the anus. The last behavior of approaching the tail was not so much an active sniffing behavior, but an attempt to engage in an active sniffing behavior. The rat executing this behavior would typically approach the other rat from behind and appear to be attempting to gain access to the anogenital region, while the rat being examined would be moving away. This was behaviorally distinct from sniffing the anogenital region, since once a sniffing bout was engaged in, if it were not mutual, the rat being examined would typically become immobile, especially if it was the submissive rat in the interaction. However, either animal in the interactive bout could execute these behaviors, and often they were mutually performed between the test rat and the stimulus rat.

The second or dominance category consisted of the following four behaviors: dorsal placement, dorsal pounce, ventral nose push, and climb over or walk over (Barnett, 1958). The dorsal placement consisted of placing the forepaws on the dorsal surface of the submissive rat with the forepaws commonly being placed directly behind the shoulder blades. The dorsal pounce was very similar in nature, except that it occurred with more speed and force. This behavior frequently occurred immediately after the end of an interactive bout following the dominant rat's disengagement from the submissive rat, or at the end of a darting and hopping sequence, often observed in rough and tumble play among juveniles. Darting and hopping were not scored as behaviors themselves due to the speed with which they occurred. However, rats that exhibited darting and hopping were noted. The dorsal pounce was also partially defined by the response of the

submissive rat, typically having the effect of immobilizing the rat, and in one case producing a submissive roll over (see below). The ventral nose push consisted of partially placing the head beneath th submissive rat, and then tossing the head in a dorsal movement, or pushing forward and upwards with the whole body. A ventral nose push always resulted in the submissive rat being tossed, pushed sideways, or actively moved in some fashion, and thereby differentiated this behavior from sniffing the ventral region (see above). A climb or walk over was defined as the dominant rat walking or climbing over the submissive rat. This behavior could be initiated from a non-engaged position or an engaged position, and always included stepping on the rat being climbed over. Again, either rat in the interactive bout could perform these behaviors, but they were largely characteristic of the test rat, and not of the stimulus rat.

The third or aggression category included the following three behaviors: boxing stance, biting, and hindlimb kick. Only a single "true" boxing sequence occurred, but a number of times the initial phase of this sequence occurred. Usually, both the test and stimulus rat mutually executed this behavior, although 4 individual incidents did occur. A boxing stance involved the rearing up of one rat in close proximity, and facing the other rat. Additionally, the head was held with the nose pointing upwards close to a vertical orientation as opposed to a horizontal orientation, and the forepaws were commonly held with the palms facing outwards. The biting that occurred never broke the skin, and more often involved clamping the jaws on a fold of skin and tugging slowly. The response to this biting was either to freeze or execute a submissive roll over (see below). The hindlimb kick involved directing the flank towards the rat being kicked and

raising the hindlimb and either pushing or actually kicking the rat. Either rat, in an interactive bout could perform these behaviors, but, except for the boxing stance, they were almost exclusive to the test rat. During one interactive bout, the stimulus rat did execute a hindlimb kick. However, in this incident there was no initial phase of directing the flank or holding the hindlimb in an upraised position, which was common to this behavior.

The fourth category of self directed behaviors consisted of rearing, grooming, and scratching. Rearing occurred either in close proximity to the other rat or away from it. Rearing also occurred either in the centre or at the sides of the testing chamber. Grooming usually involved only the beginning behaviors in the sequence and rarely progressed beyond face ellipses performed with the forepaws. For this reason grooming bouts were also brief, and in most cases less than a second in duration. Scratching was always performed by a hind limb and would typically be followed by the licking and/or chewing of the toes of the foot that did the scratching. Both rats typically executed some of these behaviors during the 4 minute test period. However, the test rat had a greater frequency of these behaviors than did the stimulus rat. Further, these behaviors sometimes occurred while the rat was engaged with the other in an interactive bout, and would have the effect of disengaging the animal from that particular bout. More often than not if this were the case the disengaging rat would be the dominant one.

Two other behaviors unique to the submissive rat in the interactive bout were also scored. These behaviors were "crawling under" and the "submissive roll over". Crawling under consisted of walking or crawling beneath the dominant rat and then lying

immobile. This behavior often occurred while the dominant rat was rearing, where it could be clearly seen that the submissive rat would lie prone crosswise beneath the dominant rat. This behavior is considered here to be the converse to climbing or walking over performed by the dominant rat. Crawling under usually occurred spontaneously, and not in response to a behavior initiated by the dominant rat. The submissive roll over on the other hand was always performed in response to a dominant or aggression behavior executed by the dominant rat in the interactive bout. The submissive roll over involved the submissive rat rolling over and exposing its ventral surface and then briefly freezing. If the dominant rat responded to the freezing by also freezing or terminating the aggressive behavior then the submissive rat would try to disengage from that interactive bout. If the dominant rat did not discontinue its aggressive behavior the submissive rat would place its hindfeet against the body of the dominant rat and appear to attempt to push off or "hold at bay" the dominant rat, and then re-initiate the brief freezing period. Both these behaviors were exclusive to the stimulus rat and were not observed in any of the test rats.

The video tapes were scored offline for the occurrence of these behaviors. Both the test rats and the stimulus rat were scored for the first four behavioral categories, while only the stimulus rat was scored for the last two behaviors of crawling under and submissive roll over.

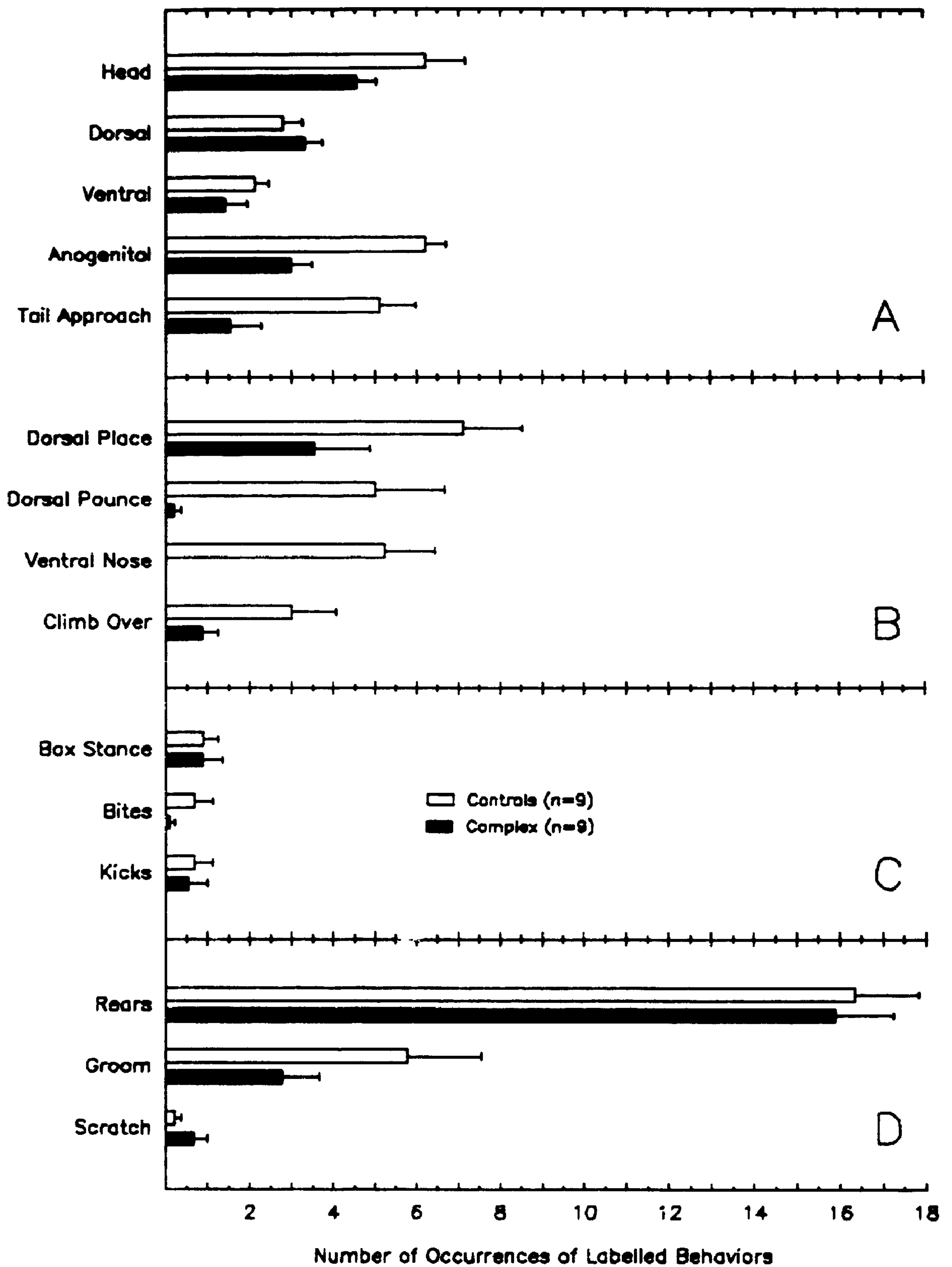
Darting and hopping behavior was observed in 9/9 of the individually housed animals and observed in 1/9 of the rats housed in the complex environment. As discussed above it was not unusual to observe the darting and hopping behavior in the rats housed

in the complex environment immediately after the environment had been re-arranged and the rats re-introduced.

The mean occurrences for 15 behaviors across the four categories that the test rats were scored on during the test period are presented as a profile in Figure 10. A between subjects multivariate analysis of variance was run on all these behaviors, with housing condition as the between subjects factor. This analysis revealed that the individually housed animals executed a greater number of overall behaviors than their complex housed counterparts [$F_{(15,2)}$=65.79; p=.015]. The 15 behaviors were then grouped into the appropriate four categories and four more similar analyses were run at this level to indicate which of the categories in which the two housing groups differed from each other. The results of this analysis indicated that the complex environment housed rats differed from the individually housed rats in the sniffing category [$F_{(5,12)}$=7.89; p=.002], and the dominance category [$F_{(4,13)}$=4.54; p=.016], but not in the aggression category [$F_{(3,14)}$=.51; p=.683] or the self directed behaviors category [$F_{(3,14)}$=2.18; p=.137]. These and the univariate F-ratios for the 15 separate behaviors can be found in Table V, where the further breakdown only confirms the results from the intermediate analysis.

A similar analysis was performed for the same 15 behaviors as scored for the stimulus rat, with the between subjects factor being the housing condition of the test rat to which the stimulus rat was reacting. The results from this analysis indicated that there were no differences in the number of behaviors executed by the stimulus rat regardless of the housing condition of the test rat with which it was interacting [$F_{(15,2)}$=.63; p=.764]. Univariate analyses indicated that none of the behaviors executed by the

Figure 10 Results from the Social Interaction experiment, data from the test rats. Figure depicts the means and standard error of the mean counts of the recorded behaviors during the four minute interaction period. **A** represents the sniffing cluster, where the test rat sniffed the stimulus rat across the various regions: head, dorsal, ventral, and anogenital, as well as the tail approach behavior. **B** represents the dominance behaviors: dorsal placement, dorsal pounce, ventral nose under, and climb over. **C** represents the aggressive behaviors: boxing stances, bites, and hindlimb kicks. **D** represents the self-directed behaviors of rearing, displacement grooming, and scratching. Closed bars represent the complex environment housed rats, while the open bars represent the individually housed littermates.



2
4
6
8
10
12
14
16
18
Head
Dorsal
Ventral
Anogenital
Tail Approach
A
Dorsal Place
Dorsal Pounce
Ventral Nose
Climb Over
B
Box Stance
Bites
Kicks
Controls (n=9)
Complex (n=9)
C
Rears
Groom
Scratch
D
Number of Occurrences of Labelled Behaviors

Table V Social interaction data analyses for the Test rats. Multivariate and univariate F-ratios for the between subjects MANOVA design performed on the four clusters of 15 behaviors scored for the test rats. All F-ratios represent the main effect of housing condition.

Variable	F-ratio	Significance level
Omnibus Effect	$F_{(15,2)} = 65.78$;	$p = .015$
Sniffing Cluster	$F_{(5,12)} = 7.88$;	$p = .002$
Head	$F_{(1,16)} = 2.50$;	$p = .133$
Dorsal	$F_{(1,16)} = .80$;	$p = .382$
Ventral	$F_{(1,16)} = 1.18$;	$p = .293$
Anogenital	$F_{(1,16)} = 22.27$;	$p < .0005$
Tail Approach	$F_{(1,16)} = 9.99$;	$p = .006$
Dominance Cluster	$F_{(4,13)} = 4.54$;	$p = .016$
Dorsal Place	$F_{(1,16)} = 3.38$;	$p = .085$
Dorsal Pounce	$F_{(1,16)} = 8.07$;	$p = .012$
Ventral Nose	$F_{(1,16)} = 18.25$;	$p = .001$
Climb Over	$F_{(1,16)} = 3.45$;	$p = .082$
Aggression Cluster	$F_{(3,14)} = .50$;	$p = .683$
Box Stance	$F_{(1,16)} = 2.56$;	$p = .100$
Bites	$F_{(1,16)} = 1.49$;	$p = .240$
Kicks	$F_{(1,16)} = .03$;	$p = .861$
Individual Cluster	$F_{(3,14)} = 2.17$;	$p = .137$
Rears	$F_{(1,16)} = .04$;	$p = .846$
Groom	$F_{(1,16)} = 2.32$;	$p = .147$
Scratch	$F_{(1,16)} = 1.49$;	$p = .240$

stimulus rat were differentially affected by interacting with either test rats housed in the complex environment or housed individually.

The two behaviors exclusive to the stimulus rat of crawling under and the submissive roll over were analysed together by a between subjects analysis, with the results indicating that the observed differences were nonsignificant [$F_{(2,15)}$ =2.17; p=.149]. However, post-hoc inspection of the two univariate analyses revealed that although there was no significant difference for crawling under [$F_{(1,16)}$=.86; p=.369], the difference for the submissive roll-over approached significance [$F_{(1,16)}$=4.11; p=.060]. A further correlation between these two behaviors found them to be unrelated [$r_{(16)}$=-.19; p=ns].

3.3.5 Results of the Neophobia Analyses:

The analysis performed on the weight of the remaining almonds indicated that the complex environment housed rats had consumed more of the almonds [$F_{(1,16)}$=12.84; p=.002]. Additionally, 4 of the 9 complex environment housed rats completely consumed the almonds placed in their cages, while none of the individually housed animals did. Conversely, 1 of the 9 individually housed rats did not consume any of the almonds placed in its cage, while all of the animals housed in the complex environment consumed some portion of the almonds.

The total amount of liquid consumed by the rats in both groups indicated no differences [$F_{(1,16)}$=.01; p=.928][2]. However, analysis of the .3 molar sucrose solution

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[2] The two animals, who received the faulty graduated cylinder containing sucrose were included in this analysis, since they were still able to consume liquid from the graduated cylinder containing water. An analysis with these data removed, indicated no change in the results.

as a proportion of the total liquid consumed, indicated that the rats housed in the complex environment consumed significantly more of the sucrose solution than did their individually housed littermates [$F_{([illegible])}=8.51$; $p=.011$].

3.4.1 Discussion of the Spontaneous Locomotor Activity Assessment and the Examination of Food and Water Intake, Fecal Matter and Weight:

Results from the spontaneous locomotor activity assessment were clear and fairly straight forward. There was a pattern of declining activity over the six activity samples for every session, on all of the nine variables. No differences were observed between the groups, when all the rats were similarly housed in individual cages. However within the first 24 hour period following placement in the complex environment, differences between the housing conditions were apparent on a number of the activity variables and by the third phase of testing differences were exhibited on all variables except average distance travelled per movement. Thus, the results presented here indicate that the complex environment housing had an almost immediate effect of lowering the spontaneous locomotor activity of rats, and that continued housing of this sort strengthened and generalized these differences. These results also match data derived from the two earlier pilot studies run in this laboratory, particularly the immediate differences following the 24 hrs of differential housing. Although a number of studies have examined the effects of complex environment housing on open-field activity, none have been as comprehensive in their measures, nor as long in the individual recording sessions, nor have been able to show the immediacy of the housing effects, as has been done here.

The relevant literature on open-field activity as it relates to complex environment housing, briefly discussed in Chapter 1 is mixed. As such, a number of groups have found that complex environment housing increases open-field activity (Fisher et al., 1991; Manosevitz, 1970; Manosevitz and Montemayor, 1972; Saari et al., 1990b), while a number of groups have found the opposite effect (Denenberg and Morton, 1962a; Mohammed et al., 1986; Nobrega, et al., 1992; Smith, 1972). Surprisingly, even the results of a single group's data are split on the obtained effects, with the Nipissing group showing both increases (Fisher et al., 1991; Saari et al., 1990b) and decreases (Nobrega et al., 1992) in open- field activity as a consequence of complex environment housing.

It is possible that differences in the size, procedure, and method of scoring the open-field and its activity may account for these discrepencies in results. Both Fisher et al. (1991) and Saari et al. (1990b), who found that complex environment housing increased spontaneous activity used a relatively large open-field, divided into 9 squares (90 X 90 cm; 30 cm squares) and observed activity over a brief period of time (3 one min samples). Nobrega et al. (1992) on the other hand, used an automated open-field that was smaller in size, but with the same number of divisions demarcated by infrared beams (49 X 49 cm, 15 cm squares), and recorded activity over a longer period of time per sample and overall (9 five min samples). All open-field assessments run by the Nipissing group consisted of a single session.

Mohammed et al. (1986) similarly, used an automated open-field based on the breaking of infrared beams, recording activity continuously for 30 min on two consecutive days, obtaining results showing less activity in the complex environment

housed rats. The activity monitors in this experiment were relatively small (40 X 25 cm). The earlier work of Denenberg and Morton (1962a) observed rats in the open-field for 3 min each day for 6 consecutive days, after the rats entered the field through a small side compartment. The open-field itself was large (114 X 114 cm), and broken down into 25 divisions, approximately 23 cm to a side. They too found that the complex environment housed rats entered fewer squares than their individually housed counterparts. Finally, of those who found that the complex environent housed animals exhibited less activity, Smith's (1972) version of the open-field was circular, approximately 80 cm in diameter, and divided into 19 equally sized areas, demarcated by 3 concentric circles divided by lines radiating from the centre. Rats were tested for 5 min a day for 3 consecutive days in this open-field.

Finding results opposite to those of Mohammed et al. (1986), Dennenberg and Morton (1962a), and Smith (1972) were Manosevitz and his colleagues, studying the effect of rearing conditions on activity in various strains of mice, used an open-field that was small and square (50 X 50 cm), divided into 25 equal square areas. However, this open-field further had barriers projecting into the field at regular alternating intervals such that the space was broken up into zigzagging alley-ways (Manosevitz, 1970; Manosevitz and Montemayor, 1972). Mice were observed on 5 consecutive days for 2 min each, with the number of squares entered counted as the activity of the animal.

In order to include the results from this thesis in this comparison, it should be noted that each session was a half hour in duration, and that prior to each of the three phases the rats had been habituated to the full procedure for a minimum of two days.

Also the size of the activity monitors were on the small side, when compared to the other studies (40 X 40 cm), and operated automatically using infrared beams to monitor activity.

Thus, a number of factors may have influenced the different results obtained, either acting singly or in parallel. The potential influencing candiates are the shape and size of the open- field, the duration of the sampling period, whether there was prior habituation or multiple days involved in the procedure, and whether acitivity was automatically recorded or scored by a human observer present during the open-field assessment. However, none of these factors alone clearly separates those studies that found activity increased and those studies that found activity decreased as a result of complex environment housing. Generally though, the experiments that found increased activity were automated, were run over days, collected data for longer periods per session, and used smaller arenas. As such, Mohammed et al. (1986), Nobrega et al. (1992), and the results presented here, automatically collected data in an open-field less than 50 X 50 cm, for a period equal to or greater than half an hour. Both Denenberg and Morton (1962a) and Smith (1972) however, used human observers, open-fields greater than 50 X 50 cm, and observation periods of 5 min or less, but observed open-field activity over a period of days, as did Mohammed et al., (1986) and the results presented here. On the other hand Fisher et al. (1991), Saari et al. (1990b), Manosevitz (1970), and Manozevitz and Montemayor (1972), all used human observers, collecting data for periods of 3 min or less. Further, the Nipissing group in these studies used a large open-field (90 X 90 cm), and ran only a single session. Although Manosevitz and his

colleagues, did use open-fields that were 50 X 50 cm, and ran the experiment over a number of days, they did employ an irregularly shaped field with barriers protruding into the open area, and they were examining mice (Manosevitz, 1970; Manosevitz and Montemayor, 1972).

Thus, the findings presented here of lowered activity levels in rats housed in the complex environment, match those of other studies employing similar activity systems and procedures. It is interesting to note again that the differential housing effects were almost immediate upon being placed in the complex environment, a finding that is novel.

Prior to being placed in the different housing conditions, rats in these series of experiments consumed similar amounts of food and water, defecated an equal amount, and weighed similar amounts.

Differences between the two housing groups during the third phase indicated lower overall food and water intake, less defecation and less weight for the rats housed in the complex environment, although the rats' weight and fecal matter only exhibited trends towards statistical differences.

Weight data collected from two earlier pilot studies have provided similar results, in that the complex environment housed rats had a lighter weight, but that these differences were non-significant. Both these studies were similar to the one reported here. The Berkeley group consistently found weight differences in their experiments (Bennett, Diamond, Krech, and Rosenzweig, 1964). However, they typically averaged the weights across a number of experiments, as in Globus et al. (1973). Consequently, the data from the two previous pilot studies were combined with the data from the

present study and reanalysed. This analysis resulted in a significant effect of housing condition, such that rats housed in the complex environment weighed less than their littermate controls [$F_{(1,25)}$=4.26; p=.049].

As to why rats housed in a complex environment consumed less than did their individually housed littermates, probably had to do with the opportunities for exercise provided by the different housing conditions.

Thus, the results presented here of a lower body weight, and lower food and water intake are in agreement with that of previous research (Bennett, Diamond, Kreck, and Rosenzweig, 1964; Fiala et al., 1977; Globus et al., 1973). In particular, Fiala et al. (1977) examined the amount of food and water consumed during a 48 hr interval, once weekly, over a period of 4 weeks, with the methods and analyses and results identical to those employed here. Thus, the analyses on intake and weight confirm previous findings.

3.4.2 Discussion of Strength and Agility Tasks:

As predicted the rats housed in the complex environment outperformed their individually housed littermates on all tasks of strength and agility. These tasks included the hanging duration, the spring scale strength task, the balance beam, and additionally portions of the simple swim and climb task. Of these tasks only the hanging duration has been examined previously in the complex environment literature (Fisher et al., 1991), and thus effects of complex environment housing upon the balance beam, the spring scale strength task, and the simple swim and climb task are novel.

That the rats housed in the complex environment outperformed their individually housed littermates on these tasks should not be surprising considering the opportunities available to them in the complex environment to engage in the range of behaviors that could enhance both strength and agility. The relations between some of the behaviors observed in the complex environment and the formally tested strength tasks are obvious, such as climbing the walls of the cage and the climb portion of the simple swim and climb task or walking along the horizontal bars that supported the highest level platform in the complex environment and the balance beam task. A number of rats on different occasions were additionally observed to slip off these same horizontal bars and suspend themselves until they either dropped or were able to regain their balance, behaviors that were very similar to those necessary for the hanging duration task.

Thus, the rats housed in the complex environment in some cases had actual practice in the specific behaviors necessary to perform some of the strength and agility tasks. However, specific practice in the task cannot account for the performance of the complex environment housed rats on the swim portion of the simple swim and climb task, since by the time these tasks were run both groups had equal swimming experience, but not in the apparatus used during this task. Further, none of the rats had any experience with the procedure or demands of the spring scale strength task. Therefore, direct practice cannot account for all the observed effects, although some of the effects may have generalized. It should also be noted here that although the individually housed littermates did weigh more than their complex environment housed counterparts, the weight of the animals which was specifically tested for, and recorded at the time of each

task, did not correlate with their performance on any of these tasks. Another possible explanation for the differences observed on the unpracticed tasks is that the individually housed rats performed more poorly than their complex environment housed littermates due to greater levels of corticosteriods. Unfortunately there is no direct evidence from these tasks that is relevant to this issue. However, others have found that individually housed rats have larger adrenal glands than rats housed in complex environemnts (Fisher et al., 1991; Geller et al., 1965; Nobrega et al., 1992; Saari et al., 1990a; 1990b) and show more ulceration of the stomach in response to immobilization (Rockman et al., 1986; 1988). The adrenals were not weighed at the end of this set of experiments. Indirect evidence arises out of the water-maze task where 7/9 of the individually housed rats exhibited exophthalmus, whereas only 1/9 of the complex environment housed rats did. Similarly, during the pre-acquisition probe trial of the same task, the individually housed rats swam thigmotactically for a longer period than did their complex environment housed counterparts. However, when the data from the water-maze were analysed according to the animals that did and that did not exhibit exophthalmus, no differences were found. Further, duration of thigmotactic swimming did not correlate with water-maze acquisition, although such correlations do exist among rats treated with NMDA antagonists (Cain et al., 1993). The relevance of the rats' performance on the Morris water-maze to the strength tasks discussed here however, is questionable, and therefore a convincing argument that the observed strength and agility differences were a result of differing corticosteroid levels cannot be made. One final and obvious explanation, is that the complex environment housed rats were simply in better physical

condition then their individually housed littermates. By having a lowered food and water consumption, by weighing less, and by having greater opportunity to engage in a larger variety of behaviors that would increase their exercise levels the rats housed in the complex environment were possibly healthier or more fit than their individually housed littermates. In support of this Fiala et al. (1977) note that at the end of a number of these experiments the individually housed rats exhibited greater levels of adipose tissue than the complex environment housed rats. Such observations were also made here, when the rats were perfused at the end of the experiments.

Thus, the complex environment housed rats may have utilized the greater opportunity available to them for a more active existence, which would improve health and prolong life. Only one report in the literature appears to have tested the strength of complex environment housed rats in comparison to individually housed ones. As mentioned in the introduction Fisher et al. (1991) in a test similar to that of the hanging duration used here, found that complex environment housed rats could suspend themselves longer from a 2 cm diameter bar than could the individually housed rats. No other reports in the literature appear to have measured strength or agility differences between complex environment housed and individually housed rats.

3.4.3 Discussion of the Social Interaction Experiment:

Results from this experiment revealed that the individually housed rats overall executed far more of the scored behaviors than did their complex environment housed counterparts. Of the four behavioral categories that the behaviors were grouped into, differences were found between the first and second categories of sniffing and

dominance, but not between the third and fourth categories of aggression or self directed behaviors. Specifically, the individually housed rats responded to the stimulus rat with a greater number of anogenital sniffing bouts, tail approaches, dorsal pounces, ventral nose pushes, and climb overs than did their complex environment housed counterparts. Also within the second behavioral category the behavior of dorsal placement exhibited a trend towards significance, again with the individually housed rats showing a greater frequency of these behaviors. In return, the stimulus rat responded to the behavior of the individually housed rats with a greater number of submissive roll overs, but in all other behaviors, the stimulus rat reacted similarly to both groups of rats, regardless of the test rats' housing condition. Thus, even though the much smaller stimulus rat's behavior was the same for both groups of test rats, the individually housed rats responded with a greater number of anogenital sniffing bouts, and displayed more behaviors that attempted to exert dominance over the smaller rat, than did their complex environment housed counterparts, to which the smaller stimulus rat responded in turn with a greater number of submissive roll overs. None of the test situations actually evolved into a formal fight, and the number of boxing stances, bites, and kicks that occurred was extremely low for both groups. It should also be remembered that the stimulus rat had been previously exposed to this test situation with 10 other rats, similar in size to those used in this experiment.

The test situation was intentionally designed to induce a social interaction of a potentially aggressive nature, and in the case of such an encounter to allow the test rat to dominate over the stimulus rat, as set out by Barnett (1958; 1963). First, the test rat

was placed in the test chamber before the stimulus rat and given adequate time to explore the chamber, thereby making it the resident male. Second, the stimulus rat was considerably smaller and introduced 20 m following placement of the test rat in the test chamber, making it the strange intruder.

Grant and Mackintosh (1963) examined social interactions in a number of species and Barnett (1958; 1963) described social interactions among wild caught rats (Rattus norvegicus). As such, many of the behaviors previously described, were identified in the present experiments.

The colony intruder task, in which a single test animal is introduced into a colony of resident male rats, has been used to examine the effects of social or isolate rearing on both the intruder and the resident colony (Luciano and Lore, 1975). Results indicated that the social history of the intruder and colony interacted with each other, such that socially reared <u>intruders</u> were far less likely to be attacked and injured during the encounters than the individually reared intruders. Socially reared <u>colonies</u> were far quicker in initiating contact with the intruder than the individually reared colonies. Thus, the social - social matchings resulted in the most amicable interactions, while mixed pairings resulted in less amicable matchings (Luciano and Lore, 1975).

Johnson et al. (1972) studied interspecific aggression, by rearing rats with frogs in various exposure conditions and then during adulthood allowed them to interact in a test arena. They found that the rats minimally exposed to frogs, attacked them after a very long latency, characterized by frenzied activity afterwhich the rats would retreat and not go near the dead frog, whereas the maximally exposed rats were often observed to

capture the frogs quickly and start feeding on them, before dispatching them completely (Johnson et al., 1972).

Saari et al. (1990) examined social interaction between complex environment and individually housed rats, using the colony intruder test, the platform dominance task, and the waterspout dominance task. Results from the colony intruder task indicated that the complex environment housed group groomed, and initiated social contact more than their individually housed littermates, a result that agrees with the earlier findings of Luciano and Lore (1975). Results from both dominance ranking tasks indicated that the complex environment housed rats were higher on the constructed hierarchies than their individually housed littermates (Saari et al., 1990; Fisher et al., 1991).

On the surface these findings appear contrary to those found in the social interaction experiment run here, in that the individually housed rats exhibited a greater frequency of behaviors in the dominance category. However, in the colony intruder task designed by the Nipissing group the test rat was subordinate since the nine resident males were twice the age of the test rat. Additionally, during the dominance task each rat was paired with a littermate from one of the different housing conditions, matched for age and weight. The social interaction experiment that was run here was specifically configured to allow the test rat to be dominant over the stimulus rat. Thus, these experiments are not necessarily directly comparable.

However, it may be argued that in the colony intruder test run by the Nippissing group the rats housed in the complex environment acted more socially, and therefore more appropriately than their individually housed littermates. Further, the more dominant

chosen ceiling that exacerbated the difference between the housing groups; in the case of the second trial, the complex environment housed rats performed close to asymptotic levels, which precluded their showing greater learning than their individually housed littermates.

These difficulties in a straight-forward interpretation of the initial results indicate that either a different task or a different protocol could have been employed. This is partially supported by the data, in that by the last four acquisition trials all the rats are near asymptotic performance. However, that rats reared in a complex environment outperform their individually housed littermates early in the acquisition of the Morris water-maze has been found by others (Fisher et al., 1991; Saari et al., 1990a; 1990b). These studies were run by the Nipissing group, using a four trial per day protocol. Analyses and figures used did not allow a breakdown of the data beyond a single day's worth of data, but all indicated that the rats housed in the complex environments outperformed their individually housed littermates on the first day. Performance on subsequent days showed that this advantage lessened. Thus, the specific pattern found here on the water-maze matches that found by others. That rats reared in complex environments are more flexible in their problem solving behavior has been specifically argued by Juraska (1990), who found that rats reared in a complex environment used a nonspatial strategy to solve a large 17-arm radial maze (Juraska, Hendersen, and Muller, 1984). She contrasts this nonspatial strategy with the spatial strategy employed by complex environment reared rats in the work of Hebb's students, who showed that the enriched animals were more disturbed by maze rotation than their individually housed

the stimulus rat was less than half the average weight of the test rats. Further, the rats housed in the complex environment were able to discriminate more quickly that the stimulus rat was not a mating opportunity. Therefore, the rats housed in the complex environment were overall more sensitive and reacted more appropriately to the social cues than their individually housed littermates. Finally, although effects of complex environments on social interaction has previously been examined, none of the examinations were as thorough nor designed to allow the test rats the dominant position in the interaction.

3.4.4 Discussion of Neophobic Responses:

Both tests of neophobic responses to novel tastes revealed that the rats housed in the complex environment were less neophobic than their individually housed littermates. Thus, the rats housed in the complex environment consumed more of the almonds and drank proportionately more of the 0.3 molar sucrose solution. It should be noted that the complex environment housed rats however, consumed as much liquid as their individually housed controls during the two b[illegible]e test. Also worth noting is that the differences observed on these tests run counter to the overall pattern of lowered weight and consumption of the standard rat chow and tap water in rats in a complex environment.

Neophobic responses to novel food tastes, probably enhance survival by avoiding the ingestion of fatal levels of poisons (Barnett, 1963; Rozin, 1977). This response is especially important in rodents which cannot vomit in order to reduce the amount of the toxic substance ingested. Thus, on the surface, the results presented here appear to contradict the overall pattern of rats housed in complex environments as being

"enriched", since it is an implicit assumption that an enriched animal would have enhanced survivability. It is possible however, that there may be an optimal level of neophobia, and that too great a neophobic response would result in a much restricted diet, which in turn could lead to malnutrition. Unfortunately, evidence to support this notion is not forthcoming from these experiments.

However, Holson (1986) found that rats raised individually were more reluctant to consume the food at the end goalbox of a Lashley III maze, than their complex environment housed littermates. As a result the rats reared in the complex environment outperformed their littermate control groups. Therefore Holson (1986) suggested that the rats housed in the complex environment were less neophobic to feeding in a novel location. Holson (1986) further suggested that the increased neophobia on part of the individually housed rats may account for their poor performance on most other maze tasks. This latter argument of course falls down in light of more recent work with the Morris water-maze.

Other studies have shown that rats raised in a complex environment given a two bottle preference test will consume more of an alcohol solution, than do individually housed littermates (Rockman, Borowski, and Glavin, 1986; Rockman et al., 1988). Additionally, the complex environment housed animals showed decreased ulceration of the stomach, in response to immobilization, which was further reduced by the alcohol consumption, although signs of intoxication and withdrawal were never observed (Rockman et al., 1986; 1988).

Thus, the reduced neophobic responses measured here were corroborated by studies showing that complex environment reared rats exhibit less neophobia to feeding in novel situations, and drinking novel tasting solutions.

3.4.5 Discussion of Morris Water-maze and Simple Swim and Climb Tasks:

Rats housed in a complex environment acquire the Morris water-maze, and the simple swim to end of tank task more quickly then individually housed controls. Both groups however, by the end of training have acquired the Morris water maze equally well and similarly retain this task over a 24 hour period equally well. By the end of the simple swim and climb task, the rats housed in the complex environment still outperformed their individually housed controls, in both swimming and in climbing the wire mesh placed at the end of the tank. Rats housed in a complex environment also displayed less exophthalmus, and spent less time swimming thigmotactically around the Morris water maze during the pre-acquisition probe trial than did the individually housed controls. However, neither of these factors had any predictive or discriminative ability with respect to the acquisition or retention of the water-maze. Further, the rats housed in the complex environment swam faster than individually housed controls. Yet, this too did not relate to any acquisition or retention measure in the Morris water-maze. Swimming speed also did not relate to the weight of the animal. Swimming speed did however relate to the amount of time spent swimming thigmotactically, such that the faster swimmers spent less time during the pre-acquisition probe trial swimming around the perimeter of the Morris water-maze. This may indicate that a set distance of a region is explored to a certain level before other areas are explored. Animals that swim faster will cover this set

amount of territory in less time than animals that do not and can subsequently move on sooner to other regions of the water-maze.

Thus, the rats housed in the complex environment learned the Morris water-maze faster than their individually housed littermates, and this acquisition did not relate to the swimming speed, levels of sympathetic activation at the time of acquisition, pre-acquisition swimming patterns, or weight of the individual animals. Once the task was learned equally well however, subsequent retention measures collected 24 hours later did not exhibit differences between rats housed in the complex environment and individual housing. Although better spatial learning does appear to be a consequence of complex environment housing it is still puzzling that the animals housed in the complex environment outperform the individually housed controls on the very first trial.

One potential explanation may be that the rats housed in the complex environment were more flexible in their behavior, as evidenced by a reduction in thigmotactic swimming during the pre- acquisition probe trial. This may enable the complex housed rats to seek out other portions of the water-maze sooner than their individually housed littermates and therefore encounter the hidden platform sooner than their littermates. This idea is also supported by data from the first acquisition trial in which 2/9 of the complex reared rats versus 6/9 of the individually reared littermates were unsuccessful in locating the platform and had to be placed on it at the end of the 60 s. Thus, a majority of the individually housed controls were at the maximum swim values or at ceiling, while only a small minority of the complex environment rats were at this level.

A consequence of this result is that the individually housed littermates exhibited a greater difference from trial 1 to trial 2, and thus, it could be suggested that the individually housed rats learned more about the location of the hidden platform from the first trial to the next than the complex environment housed rats. Yet, had the maximum escape time allowed been two minutes, as in some studies, this difference would probably have increased, given the tendency of the individually housed controls to use a thigmotactic escape strategy. Conversely, had the maximum escape time been limited to 30 s, as in some studies, then the difference between trial one to trial two would have been dramatically decreased. Thus, it can be argued that the difference observed on the first trial between the housing groups was somewhat arbitrarily determined by the maximum allowable escape time.

Additionally, a close examination of the raw data from the second trial indicates that 5/9 of the complex environment housed versus 2/9 of the individually housed littermates had an escape time that was less than the maximum escape time of the final four acquisition trials, when the rats were assumed to asymptote in their performance. Thus, one could argue that the complex environment rats reached asymptotic or floor levels of performance much sooner than their individually housed littermates, and therefore were prevented from exhibiting greater learning, as evidenced by the difference between trials one and two. A similar argument can be made for the difference between trial two and trial three, in which 8/9 of the complex environment housed rats reached asymptotic performance while only 3/9 of the individually housed littermates did. Thus, in the case of the first trial, the individually housed controls performed at an arbitrarily

chosen ceiling that exacerbated the difference between the housing groups; in the case of the second trial, the complex environment housed rats performed close to asymptotic levels, which precluded their showing greater learning than their individually housed littermates.

These difficulties in a straight-forward interpretation of the initial results indicate that either a different task or a different protocol could have been employed. This is partially supported by the data, in that by the last four acquisition trials all the rats are near asymptotic performance. However, that rats reared in a complex environment outperform their individually housed littermates early in the acquisition of the Morris water-maze has been found by others (Fisher et al., 1991; Saari et al., 1990a; 1990b). These studies were run by the Nipissing group, using a four trial per day protocol. Analyses and figures used did not allow a breakdown of the data beyond a single day's worth of data, but all indicated that the rats housed in the complex environments outperformed their individually housed littermates on the first day. Performance on subsequent days showed that this advantage lessened. Thus, the specific pattern found here on the water-maze matches that found by others. That rats reared in complex environments are more flexible in their problem solving behavior has been specifically argued by Juraska (1990), who found that rats reared in a complex environment used a nonspatial strategy to solve a large 17-arm radial maze (Juraska, Hendersen, and Muller, 1984). She contrasts this nonspatial strategy with the spatial strategy employed by complex environment reared rats in the work of Hebb's students, who showed that the enriched animals were more disturbed by maze rotation than their individually housed

littermates (Forgays and Forgays, 1952; Hymovitch, 1952). Thus, Juraska (1990) concludes that rats reared in a complex environment use a wider variety of cues to solve maze problems than their individually housed controls.

Exophthalmus exhibited no relation to the water-maze acquisition, and rats from both housing conditions were handled approximately an equal amount during behavioral testing. However, without the the weights of the adrenal glands the corticosteroid issue cannot be fully resolved, since in studies where the adrenal glands have been weighed it has been found that rats housed in a complex environment have reduced weights in comparison to individually housed littermates (Fisher et al., 1991; Geller, et al., 1965; Nobrega et al., 1992; Saari et al., 1990a; 1990b). Geller et al. (1965), in particular examined not only the weight of a number of organs but also sampled adrenal and serum levels of corticosteroids at the time of sacrifice. They found no differences between individually or complex environment housed rats in the levels of corticosteroids, but did find differences in adrenal weight, even after differences in body weight were accounted for through an analysis of covariance. They interpretated these findings as indicating that chronic corticosteroid activation may have occurred early during the individual housing condition and that it had habituated after prolonged exposure (Geller et al., 1965). In a review paper Bennet et al. (1964) had earlier argued that their data, derived over a number of experiments, indicated no effect of housing condition on adrenal weight. However, in a 1972 article they clarified this finding as no difference, when the adrenal glands were taken as a percentage of bodyweight, with the data from the absolute weights not being reported (Rosenzweig, Bennett, and Diamond, 1972). This method of

accounting for differences in bodyweight between the housing conditions is cruder than the analysis of covariance used by Geller et al. (1965). Further, Fiala et al. (1977) specifically noted that the individually reared animals exhibited an excess of adipose tissue, but no difference in skeletal size, suggesting that bodyweight differences were selectively due to fat, and was not an overall size difference. However, to further test the effects of endocrine gland hormones Rosenzweig et al. (1972) hypophysectomized rats at weaning age and subsequently reared them in complex or individual housing conditions. Although hypophysectomy retarded both brain and body growth this treatment did not prevent the effect of the complex environment upon cortical thickness, the effects being as strong in hypophysectomized rats as in unoperated controls. Also the hypophysectomy affected body growth by 60%, whereas it affected brain growth by only 10%. Thus, Rosenzweig et al. (1972) argued that the effects of complex environment housing upon the brain were not due to hormones released by endocrine glands, such as the adrenals. However, the hypophysectomy did affect brain development by an average of 10%. Additionally, Sloviter and colleagues have shown that adrenalectomy in adult rats results in the loss of hippocampal granule cells, which could be prevented by the chronic administration of corticosterone (Sloviter, Valiquette, Abrams, Ronk, Sollas, Paul, and Neubort, 1989). Further, McEwen and his students have found that repeated corticosterone injections over 12 weeks caused pyramidal cell loss in the hippocampus (Sapolosky, Krey, and McEwen, 1986) while injections over 3 weeks selectively reduced the apical dendrites of CA3 pyramidal cells (Wooley, Gould, and McEwen, 1990). Finally, even if corticosteroid levels do not interact with complex environment housing

to affect cortical thickness, this does not mean that corticosteroid levels do not affect behavioral acquisition of the water-maze. As a result, the argument that differing corticosteroid levels may account for the enhanced acquisition of the Morris water-maze cannot be fully ruled out.

Regardless of the explanation, the results that the rats housed in the complex environment outperform their individually housed littermates on a task of spatial ability is consistent with older literature finding similar effects on a variety of maze tasks (Bingham and Griffiths, 1952; Denenberg and Morton, 1962b; Denenberg et al., 1968; Forgays and Forgays, 1952; Forgus, 1954; Hymovitch, 1952; Mohammed et al., 1986; O'Shea et al., 1983; Pappas et al., 1987; Smith, 1972; West and Greenough, 1972), and more recently, and specifically in the Morris water-maze (Fisher et al., 1991; Park et al., 1991; Saari et al., 1990a; 1990b). Finally, although the finding that complex environment housing enhances water-maze acquisition is not new, the indepth analyses of maze acquisition and additional behavioral control conditions have not been performed before.

CHAPTER 4 - EFFECTS OF A COMPLEX ENVIRONMENT ON EVOKED POTENTIALS IN THE DENTATE-GYRUS

4.1 Outline of Electrophysiological Assessment of Dentate-gyrus EPs:

When behavioral testing was largely completed, the rats were surgically implanted with stimulating and recording electrodes in the perforant-path and dentate-gyrus, respectively.

Upon full recovery and readaption to the complex environment, baseline electrophysiological testing began. These procedures consisted of collecting AEPs during surgery and during the first recording session, and were completed with the recording of formal I/O curves. Several weeks following baseline electrophysiological testing, the rats underwent LTP procedures. Decay of the LTP was recorded at a number of intervals subsequent to its induction, with the longest occurring 7 days afterwards.

Details of these procedures are presented below, along with the results and discussions of the electrophysiological assessment. Complete verification of the electrode placements could not be done due to the neuroanatomical assessment used. The data on electrode placement are presented in appendix B.

4.2.1 Procedures for Electrophysiological Recordings During Surgery and Following Recovery:

During a four day period, starting 40 days after the animals had been separated into their respective housing conditions all rats in the experiment were surgically implanted with unilateral recording and stimulating electrodes in the dentate gyrus and

perforant-path, as described in Chapter 2. Final positioning of the electrodes was optimized by electrophysiological recording, as was standard throughout these procedures. An equal number of individually housed rats and complex environment housed rats were implanted each day. During implantation the surgeon was naive to the identity of the rats, on all other electrophysiological procedures the experimenter knew the identity of the rats. Immediately prior to the electrodes being cemented in place, once final positioning had been achieved, an average of 10 evoked potentials was recorded using a constant intensity of 500 μa across all rats.

After all the animals had recovered for a few days under these conditions the complex housed rats were re-introduced into the environment.

The animals were allowed to fully recover from the surgery and re-adapt to the complex environment for about three weeks. After this time the rats were tested for placements and another average of 10 evoked potentials was recorded, again using a constant intensity of 500 μa for all animals, and recorded during behavioral immobility. These recordings were taken 70 days after being initially separated into the different housing conditions.

These electrophysiological data were then analysed for differences between the two housing conditions and for similarities from the time of recording at final positioning under anaesthetic to the time of recording following full recovery.

4.2.2 Procedures for the Electrophysiological Recording of Formal I/O Curves:

Formal I/O curve recording began once the rats housed in the complex environment no longer fought among themselves in a manner that would inflict physical

damage. This was carried out over a period of 5 days and was initiated within a few days of the re-adaption recordings taken at 500 μa, which had occurred on day 70.

As outlined in Chapter 2 rats were habituated to the recording chamber for half an hour prior to the I/O curves being collected. The rats were watched to ensure that they did not drift off into sleep, as defined by the rat lying down with its head resting on its forepaws and eyes closed. Constant current biphasic pulses were used, as described in Chapter 2, and all recording took place during behavioral immobility. As a result the test pulse frequency was often less than .10 Hz, but it was never greater than this value.

A modal number of 8 different intensities, ranging from 8 to 12 intensities per I/O curve, were used for recording. The stimulation intensity at which a pop-spike, could first be observed (but not necessarily consistently observed), was noted. The lowest intensity that the first average was recorded at was within 50 μa of this threshold and was demarcated by the consistent appearance of the pop-spike. The maximum intensity for all I/O curves was 1000 μa, since results from Appendix A indicated that pop-spike measures recorded 500 μa and 1000 μa above this intensity showed little change.

These average EP data were then analysed for the 4 selected EP measures at each intensity and analysed statistically as raw data and as percentages of the maximum values recorded at the 1000 μa stimulation intensity.

4.2.3 Procedures for the Electrophysiological Induction and Reording of LTP:

Since the I/O curve recording procedure occurred over a number of days, and since preliminary data from Appendix A indicated that low frequency potentiation could be induced by I/O recording, the rats were again allowed to re-adapt to the different

housing conditions for a substantial period of time. During this time period the last behavioral tests of neophobic responses to novel tastes were also completed. LTP induction began approximately 110 days after the initial separation into the differential housing conditions, with all rats being induced over a 4 day period, after which the decay was traced for another 7 days. Based on the I/O curves low, medium and high stimulation intensities were selected for each animal, and attempts were made to match pairs of rats from each housing condition on these intensities, and to further match them loosely based on the shape, and size of the EPs, as has been done earlier in this laboratory (Hargreaves et al., 1990). These pairs of animals, drawn from the different housing conditions, were then run together throughout the LTP procedure, such that one animal from each housing condition was run in any given recording session.

All the test pulses given during the abbreviated I/O recording were delivered while the animal was immobile. After an abbreviated baseline I/O curve had been recorded, LTP was induced using one of the standard set of parameters employed in this laboratory (Cain et al., 1993; Hargreaves et al., 1990). Briefly, a series of 20 tetanizing trains were applied to the perforant-path of each rat. Trains consisted of diphasic square wave pulses given at a constant intensity of 800 μa, with each pulse having identical parameters to those of the test pulses. Each train had a duration of 20 ms, with an intra-train frequency of 400 Hz. Trains were applied during periods of immobility, with approximately 30 to 45 s between each train. While test pulses were delivered bipolarly to the stimulating electrode tips, the LTP trains were delivered monopolarly against the reference lead implanted in the skull. Finally, a 21st high frequency train was delivered

bipolarly to the stimulating electrodes. This last train of high frequency pulses was delivered to permit a comparison of the effects of monopolar trains and bipolar trains on the behavior of the rats at the time of delivery. This was of interest in another series of experiments, and thus will not be commented upon further here.

Immediately following LTP induction abbreviated I/O curves were recorded, and again an hour later. The decay of the induced LTP was then traced at 3 hrs, 24 hrs, 72 hrs, and 7 days post LTP induction. Following LTP and each subsequent decay recording session after 3 hrs, the rats were returned to their respective housing conditions.

4.3.1 Results of Surgical and Post-surgical Electrophysiological Recordings:

For one of the complex housed animals no pop-spike was achieved, and the final placement AEP was not recorded. Prior to the full analysis of the evoked potential data recorded at the time of surgery, all variables were tested for equality of variances from the two housing condition samples. This was done by producing F-ratios of the larger sample variance over the smaller sample variance. None of these values were shown to be significant and thus the two samples from the different housing conditions were deemed to be homogenous. Subsequently, these evoked potential data were analysed for differences on the the single slope measure, the two pop-spike measures, and the single latency measure. Overall, no differences were found, either at the multivariate or univariate levels for all 4 EP measures.

In analysing the electrophysiological data recorded following the recovery and re-adaption period it was discovered that in addition to the complex housed rat that had had no pop-spike during the surgery, another one of the complex housed rats had its electrode

placement shift during the recovery period, resulting in a loss of a recordable EP. Thus, all subsequent electrophysiological analyses were performed on a maximum of 16 animals. As with the electrophysiological data collected at the time of surgery, the samples for all the variables were tested for equality of variances. The F-ratios indicated that none of the variances were statistically different from each other. Consequently, an identical analysis was run for the evoked potential data recorded after the full recovery and re-adaption period. At the multivariate level the analysis was non-significant. However, both the pop-spike measures and the latency measure were significant at the univariate level, indicating that the individually housed rats had larger pop-spikes with shorter latencies (Table VI).

4.3.2 Results of the Formal I/O Curve Recordings:

The pop-spike threshold intensity was tested by a between groups t-test using separate sample variance estimates. Results indicated that there was no difference between the two housing conditions on the stimulation intensity necessary to elicit an initial pop-spike [$t_{(6.54)} = 1.64$; $\underline{p} = .148$], with the average threshold for both groups equal to or below 75 μa.

The I/O curve data were analysed in two clusters, one involving the raw data from the average EPs, and the other cluster involving the data as percentages of the maximum values. Both clusters were examined by parallel analyses, consisting of an overall mixed between within subjects repeated measures MANOVA, and subsequent ANOVAs for each individual measure. The means and standard errors of the means for the I/O curve data are presented in Figures 11 and 12.

Table VI Analyses of evoked potential data, recorded following recovery and re-adaption procedures. Top: means and s.e.m. (brackets) of the AEP measures for the different housing conditions. Bottom: Multivariate and univariate F-ratios for the between subjects MANOVA design performed on evoked potential measures. All F-ratios represent the main effect of housing condition. The units of measure for the different variables are as follows: the double-ended roll-off EPSP slope measure is in Mv/ms, the peak-to-peak pop-spike amplitude is in mV, the pop-spike area is in arbitrary units, and the latency to onset of the pop-spike measure is in ms.

Variable	**Control**		**Complex**	
	Mean	**s.e.m**	**Mean**	**s.e.m.**
Double ended Roll-off	7.01	(3.26)	5.69	(1.20)
Peak to Peak	9.75	(1.16)	5.63	(2.39)
Area	11.38	(0.72)	7.99	(1.65)
Pop-spike Onset	4.16	(0.03)	4.59	(0.22)

Variable	**F-ratio**	**Significance level**
Omnibus Effect	$F_{(4,11)} = 1.78$,	$p = .203$
Univariate Effects		
Double ended Roll-off	$F_{[illegible]} = .66$;	$p = .431$
Peak to Peak	$F_{(1,14)} = 6.13$;	$p = .027$
Area	$F_{[illegible]} = 6.98$;	$p = .019$
Pop-spike Onset	$F_{[illegible]} = 5.04$;	$p = .041$

Figure 11 Means and standard errors of the means for the raw data from the formal I/O curve recordings of rats from the different housing conditions. The four measures analysed are shown which include the double ended roll off EPSP slope, the peak to peak pop-spike amplitude, the pop-spike area, and the latency to the onset of the pop-spike. Stimulation intensities were matched across all the rats for the 4 highest intensities. Lower intensities were not as closely matched, and therefore have simply been labelled in descending order from the highest intensity recorded. Open circles represent data from the complex environment housed rats, and closed circles represent data from the individually housed littermates.

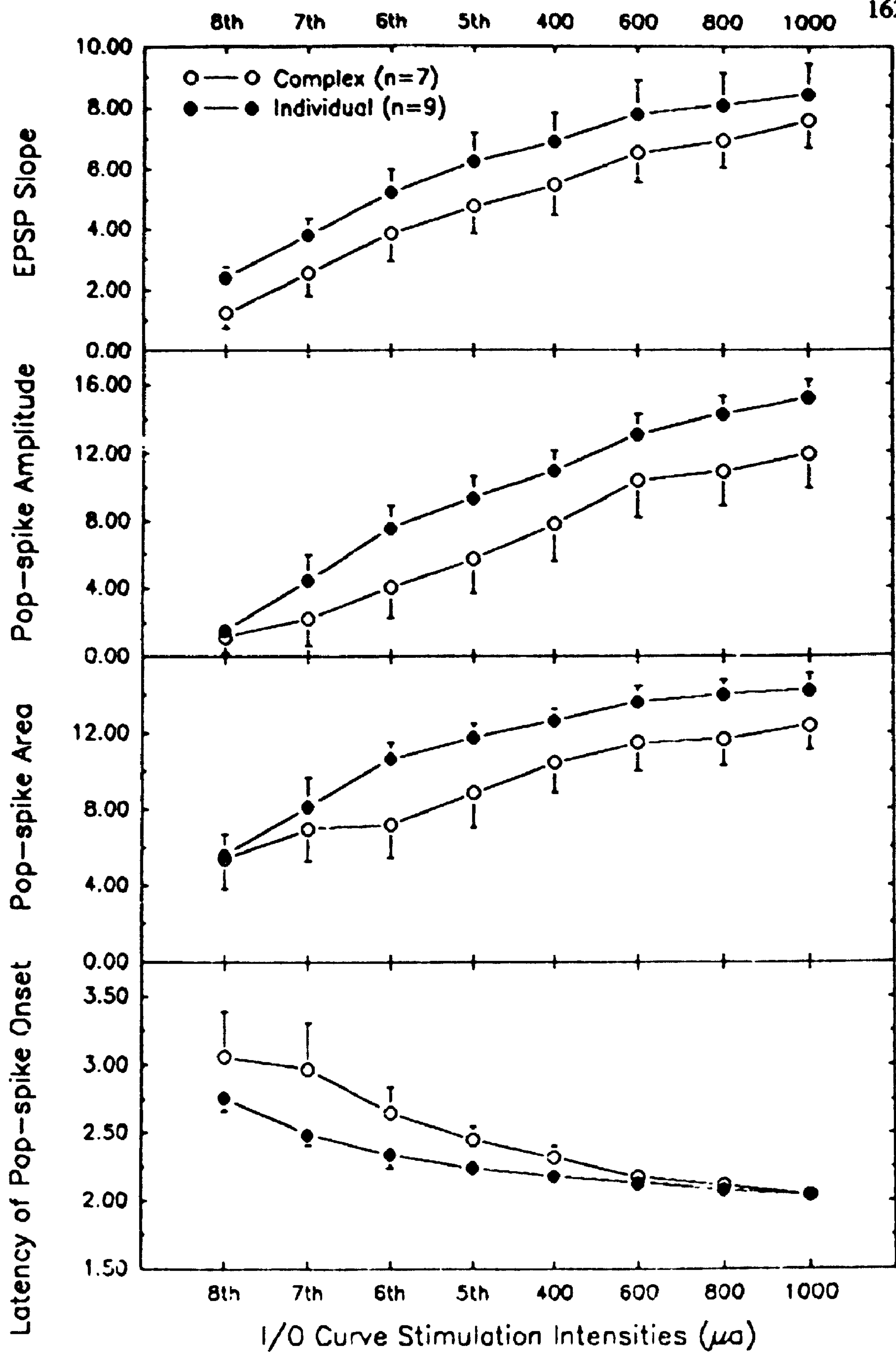
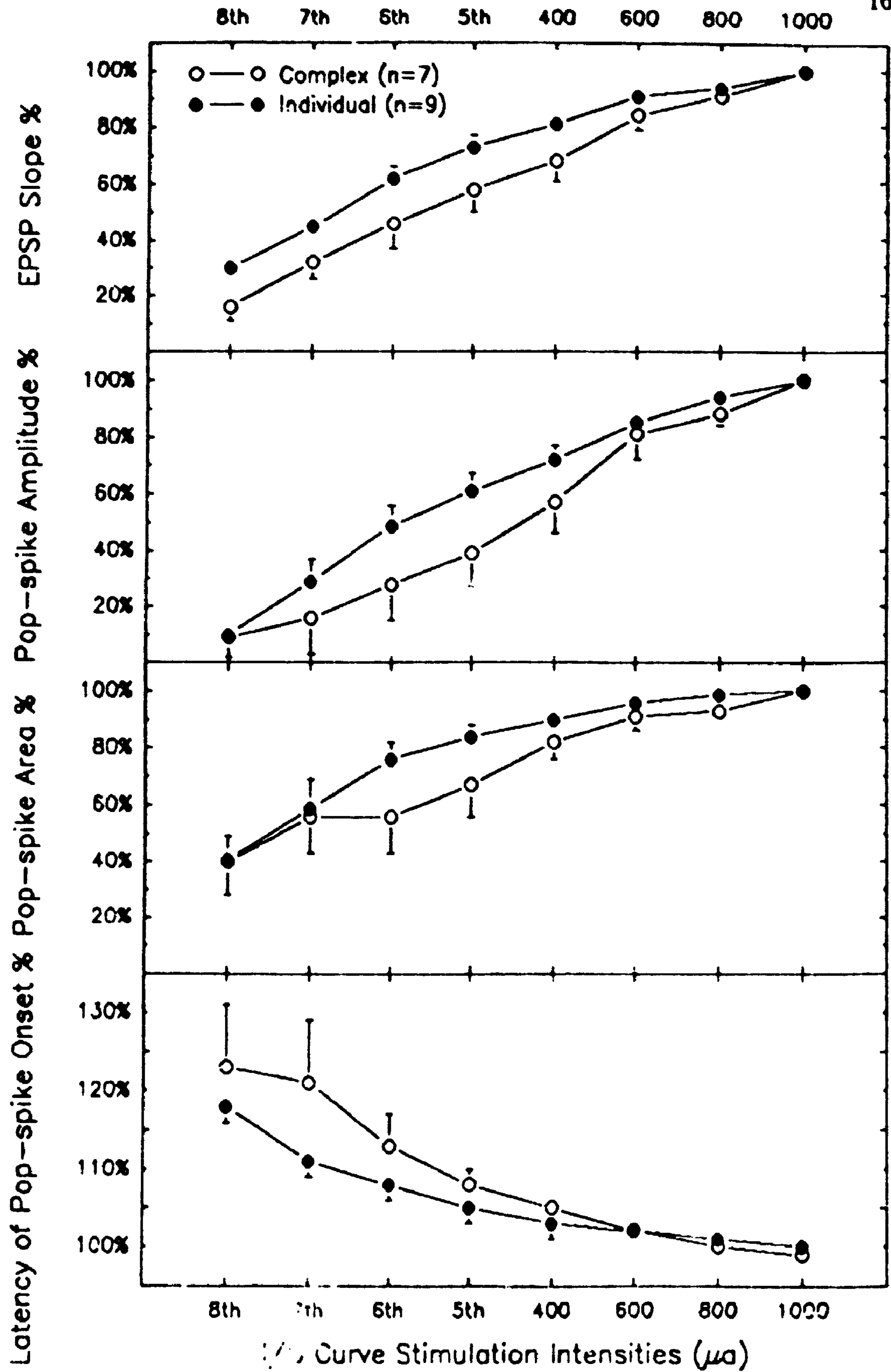

8th
7th
6th
5th
400
600
800
1000
Complex (n=7)
Individual (n=9)
EPSP Slope
10.00
8.00
6.00
4.00
2.00
0.00
Pop-spike Amplitude
16.00
12.00
8.00
4.00
0.00
Pop-spike Area
12.00
8.00
4.00
0.00
Latency of Pop-spike Onset
3.50
3.00
2.50
2.00
1.50
I/O Curve Stimulation Intensities (μa)

Figure 12 Means and standard errors of the means of the data from the formal I/O curve recordings of rats from the different housing conditions, transformed as percentages of the maximal I/O values. The four measures analysed are shown which include the double ended roll off EPSP slope, the peak to peak pop-spike amplitude, the pop-spike area, and the latency to the onset of the pop-spike. Stimulation intensities were matched across all the rats for the 4 highest intensities. Lower intensities were not as closely matched, and therefore have simply been labelled in descending order from the highest intensity recorded. Open circles represent data from the complex environment housed rats, and closed circles represent data from the individually housed littermates.


8th
7th
6th
5th
400
600
800
1000
Complex (n=7)
Individual (n=9)
EPSP Slope %
100%
80%
60%
40%
20%
Pop-spike Amplitude %
Pop-spike Area %
Latency of Pop-spike Onset %
130%
120%
110%
100%
Curve Stimulation Intensities (μa)

Analyses of the raw data indicated that there were no effects of housing condition, or housing condition by stimulation intensity interactions, either at the multivariate or univariate levels (Table VII). The within subjects main effect of stimuluation intensity was significant for all variables, at all levels, simply indicating that the EP measures changed as the stimulation intensity increased.

Analyses of the proportional data indicated no effects of housing condition, or housing condition by stimulation intensity interaction at the multivariate level, although for the interaction there was a slight trend towards significance [$F_{(28,374)}=1.39$; $p=.094$]. However, at the univariate level the proportional EPSP slope measure indicated both a significant group effect [$F_{(1,14)}=5.16$; $p=.039$] and a trend towards a group by intensity interaction [$F_{(7,98)}=1.88$; $p=.076$]. Similarly the porportional pop-spike amplitude also showed this trend [$F_{(7,98)}=1.81$; $p=.093$]. As such, the individually housed controls had higher proportional EPSP and pop-spike amplitude values, particularly at the lower stimulation intensities (Table VIII). As with the raw data the within subjects effect of stimulation intensity was significant at both the multivariate and univariate levels for all measures of the proportional data.

4.3.3 Results of the LTP Analyses:

Prior to the analysis of the LTP data, it became apparent from data collected in this laboratory (Cain et al., 1993) and another laboratory (Au, 1990; Au and Leung, 1989; Leung and Au, submitted) that the degree of observed LTP was highly dependent upon the test pulse intensities used to evaluate it. Specifically, the EP values evoked by the test pulse related proportionately to their maximally evoked values. As such, greater

Table VII Analyses of raw evoked potential data from the full I/O curves. Multivariate and univariate F-ratios for the mixed between within subjects repeated measures MANOVA design performed on evoked potential measures. F-ratio clusters represent the main effect of housing condition, stimulation intensity, and the interaction between these two effects respectively.

Variable	F-ratio	Significance level
Multivariate		
Between Groups Housing Effect	$F_{(4,11)}$ = .91;	p=.490
Univariate Effects		
Double ended Roll-off	$F_{(1,14)}$ = 1.17;	p=.297
Peak to Peak Pop-Spike	$F_{(1,14)}$ = 2.38;	p=.145
Area	$F_{(1,14)}$ = 2.09;	p=.170
Pop-spike Onset	$F_{(1,14)}$ = 2.75;	p=.120
Multivariate		
Within Subject Intensity Effects	$F_{(28,374)}$ = 33.74;	p<.0005
Univariate Effects		
Double ended Roll-off	$F_{(7,98)}$ = 72.33;	p<.0005
Peak to Peak Pop-Spike	$F_{(7,98)}$ = 59.73;	p<.0005
Area	$F_{(7,98)}$ = 25.51;	p<.0005
Pop-spike Onset	$F_{(7,98)}$ = 23.13;	p<.0005
Multivariate		
Housing by Intensity Interaction	$F_{(28,374)}$ = 1.33;	p=.125
Univariate Effects		
Double ended Roll-off	$F_{(7,98)}$ = 16;	p=.992
Peak to Peak Pop-Spike	$F_{(7,98)}$ = .89;	p=.517
Area	$F_{(7,98)}$ = .83;	p=.565
Pop-spike Onset	$F_{(7,98)}$ = 1.65;	p=.132

Table VIII Analyses of the proportional evoked potential data from the full I/O curves. Multivariate and univariate F-ratios for the mixed between within subjects repeated measures MANOVA design performed on evoked potential measures. F-ratio clusters represent the main effect of housing condition, stimulation intensity, and the interaction between these two effects respectively.

Variable	F-ratio	Significance level
Multivariate		
Between Groups Housing Effect	$F_{(4,11)} = 1.14$;	$p = .388$
Univariate Effects		
Double ended Roll-off	$F_{(1,14)} = 5.16$;	$p = .039$
Peak to Peak Pop-Spike	$F_{(1,14)} = 1.70$;	$p = .213$
Area	$F_{(1,14)} = 1.14$;	$p = .305$
Pop-spike Onset	$F_{(1,14)} = 1.30$;	$p = .273$
Multivariate		
Within Subject Intensity Effects	$F_{(28,374)} = 48.14$;	$p < .0005$
Univariate Effects		
Double ended Roll-off	$F_{(7,98)} = 150.77$;	$p < .0005$
Peak to Peak Pop-Spike	$F_{(7,98)} = 103.06$;	$p < .0005$
Area	$F_{(7,98)} = 29.84$;	$p < .0005$
Pop-spike Onset	$F_{(7,98)} = 22.91$;	$p < .0005$
Multivariate		
Housing by Intensity Interaction	$F_{(28,374)} = 1.39$;	$p = .094$
Univariate Effects		
Double ended Roll-off	$F_{(7,98)} = 1.88$;	$p = .076$
Peak to Peak Pop-Spike	$F_{(7,98)} = 1.81$;	$p = .093$
Area	$F_{(7,98)} = .88$;	$p = .522$
Pop-spike Onset	$F_{(7,98)} = 1.56$;	$p = .156$

amounts of LTP were observed at the low end of the I/O curve and lesser amounts of LTP were observed at the high end of the I/O curve. Thus, the selection of the test pulse intensity and its resulting EP values could easily distort and bias the amount of LTP reported if not chosen with great care, in relation to the intensity at which maximal values could be evoked.

Consequently, because of the small sample size in this experiment and the variability of the stimulation intensities used for the low, medium and high test pulses across rats, it was deemed critical that the EP values as proportions of the maximum values were as homogenous as possible. However, it was also deemed important that the test pulse intensities did not differ significantly between the groups. As a result prior to any analyses, baseline EP values were transformed into proportional values and matched across pairs of animals drawn from each of the housing conditions, and thus, a single test pulse intensity was selected from the baseline data for each rat. LTP data for each rat were then derived from this stimulation intensity throughout the duration of the experiment. The selected baseline values and associated intensities attempted to reduce the variance of the proportional EP measure values, and the variance of the stimulation intensity. Thus, for some animals the low intensity was selected, while for other animals the medimum intensity was selected, and for other animals still, the high intensity was selected.

The matching of the baseline EP measures as percentages of the maximal I/O values yielded a double-ended roll-off EPSP slope value approximately 47% of the maximal I/O value, a peak to peak pop-spike amplitude approximately 55% of the

maximal I/O value, a pop-spike area 74% of the maximal I/O value, and a latency to the onset of the pop-spike approximately 22% greater than the maximal I/O value. Descriptive statistics from the two different housing conditions of the raw EP baseline values and their proportions can be found in Table IX.

Since the data selection was nonstandard, analyses were performed on two data transformations 1) proportions of the selected baseline values, and 2) proportion of maximum I/O values. These transformations were performed upon all four EP measures analysed.

Prior to the analyses of the data for group differences, repeated measures analyses were performed on the EP measures and their transformations to ensure that all measures did exhibit LTP and its subsequent decay across the recording sessions of the experiment. The results of these analyses indicated that the latency of the onset of the pop-spike measure and its transformations did not exhibit significant effects of LTP (Table X). Subsequently this measure was dropped from the remainder of the analyses. All other EP measures and their transformations indicated significant changes across the recording sessions.

Also, prior to analysing the data for group differences in the ability to sustain LTP, a number of comparisons were run on the baseline data, recorded immediateley before LTP was induced, and on some of the associated parameters used to record LTP. This was done to ensure that the matching procedure employed in this experiment did not differentiate the groups on baseline rankings and stimulation parameters. These analyses were performed by simple oneway ANOVAs, and additionally by independent sample

Table IX Analyses of LTP baseline evoked potential data. Means and standard errors of the means of the baseline EP values from the selected intensities. Data are represented as raw values, and as proportions of the maximum I/O values on which the intensities were selected.

Variable	Complex		Individual	
	Mean	s.e.m.	Mean	s.e.m.
Double ended Roll-off EPSP Slope				
Raw values	3.41	0.23	3.99	0.58
Proportional values	44.79%	2.4%	48.96%	5.1%
Peak to Peak Pop-spike Amplitude				
Raw values	6.66	1.30	7.91	1.42
Proportional values	56.15%	9.30%	55.12%	7.9%
Area of the Pop-spike				
Raw values	9.23	1.39	10.24	1.24
Proportional values	73.54%	8.4%	73.80%	7.4%
Latency of the Pop-spike Onset				
Raw values	2.58	0.05	2.52	0.08
Proportional values	119.12%	2.1%	123.67%	3.5%

Table X Analyses of the LTP evoked potential data. Univariate F-ratios represent within subjects repeated measures effects, collapsed across housing condition. Analyses indicate whether the EP measures and their transformations were able to detect the induced LTP. EP data analysed for each measure and its associated three transformations, of difference from baseline, proportion of baseline and proportion of maximum value. Univariate F-ratios for the raw data and for the proportions of the maximum values were run on 7 repeated measures including baseline, while difference scores and baseline porportional scores were run on 6 repeated measures excluding the baseline values.

Variable	F-ratio	Significance level
Double ended Roll off EPSP Slope		
Proportion Transformation	$F_{(5,75)} = 5.63$;	$p<.0005$
Proportion of Maximum Value	$F_{(6,90)} = 6.81$;	$p<.0005$
Peak to Peak Pop-spike Amplitude		
Proportion Transformation	$F_{(5,75)} = 4.85$;	$p=.001$
Proportion of Maximum Value	$F_{(6,90)} = 9.29$;	$p<.0005$
Pop-spike Area		
Proportion Transformation	$F_{(5,75)} = 6.68$;	$p<.0005$
Proportion of Maximum Value	$F_{(6,90)} = 10.37$;	$p<.0005$
Latency to Pop-spike Onset		
Proportion Transformation	$F_{(5,75)} = .24$;	$p=.946$
Proportion of Maximum Value	$F_{(6,90)} = 1.01$;	$p=.422$

t-tests. Results from both sets of analyses indicated that none of the measures tested were dissimilar from each other in the two housing conditions.

Analyses were therefore carried out comparing the amount of LTP sustained by the rats in the different housing conditions. Parallel analyses were performed on all the data transformations and consisted of separate mixed between within subjects repeated measures MANOVA designs for each set of the transformed EP measures. Thus, all the proportional baseline scores were analysed together, while the dataset based on proportions of the maximal I/O values were analysed together. The group means and standard errors of the means for proportional transformations across the LTP recording sessions are presented in Figures 13 and 14.

Multivariate and univariate assessments of the main effect of housing condition indicated significant effects on the proportion of baseline data transformation favouring the complex environment housed animals with greater LTP than their individually housed littermates (Table XI). Group by time interactions were not found for these data indicating that both groups decayed at the same rate, and therefore at the end of the 7 day recording period rats housed in the complex environment still exhibited greater amounts of LTP than their individually housed littermates.

Simple main effects of housing were not sought for the proportion of maximal values data transformation, since the repeated measures included the baseline measures, on which the data were specifically matched to be equal. Consequently, the group by time interaction was of greater interest, with this result indicating a strong trend towards significance at the multivariate level, and significant univariate effects for the pop-spike

Figure 13 Means and standard errors of the means of the data from the LTP experiment, transformed as percentages over the baseline values. The three EP measures that were analysed are shown, in addition to the onset latency of the pop-spike which was not analysed. The time line for the recording sessions represents pre-LTP baseline recordings followed by immediate post-LTP recordings, followed successively by 1, 3, 24, hours and 3 and 7 days post-LTP recording sessions. Open circles represent data from the complex environment housed rats, and closed circles represent data from the individually housed littermates.

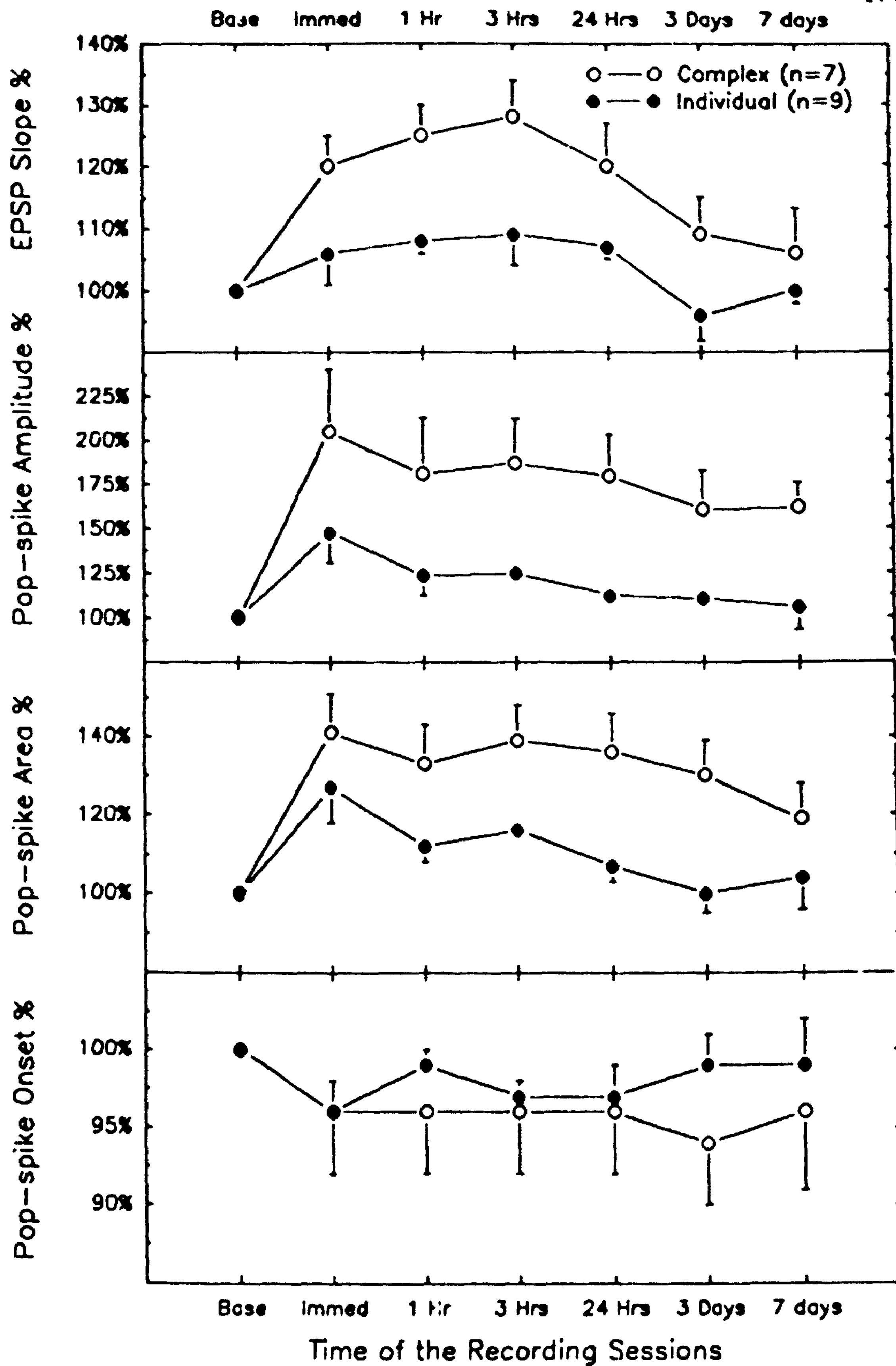

Base
Immed
1 Hr
3 Hrs
24 Hrs
3 Days
7 days
EPSP Slope %
140%
130%
120%
110%
100%
Complex (n=7)
Individual (n=9)
Pop-spike Amplitude %
225%
200%
175%
150%
125%
100%
Pop-spike Area %
140%
120%
100%
Pop-spike Onset %
100%
95%
90%
Base
Immed
1 Hr
3 Hrs
24 Hrs
3 Days
7 days
Time of the Recording Sessions

Figure 14 Means and standard errors of the means of the data from the LTP experiment, transformed as percentages of the maximal I/O values. The three EP measures that were analysed are shown, in addition to the onset latency of the pop-spike which was not analysed The time line for the recording sessions represents pre-LTP baseline recordings followed by immediate post-LTP recordings, followed successively by 1, 3, 24, hours and 3 and 7 days post-LTP recording sessions. Open circles represent data from the complex environment housed rats, and closed circles represent data from the individually housed littermates.

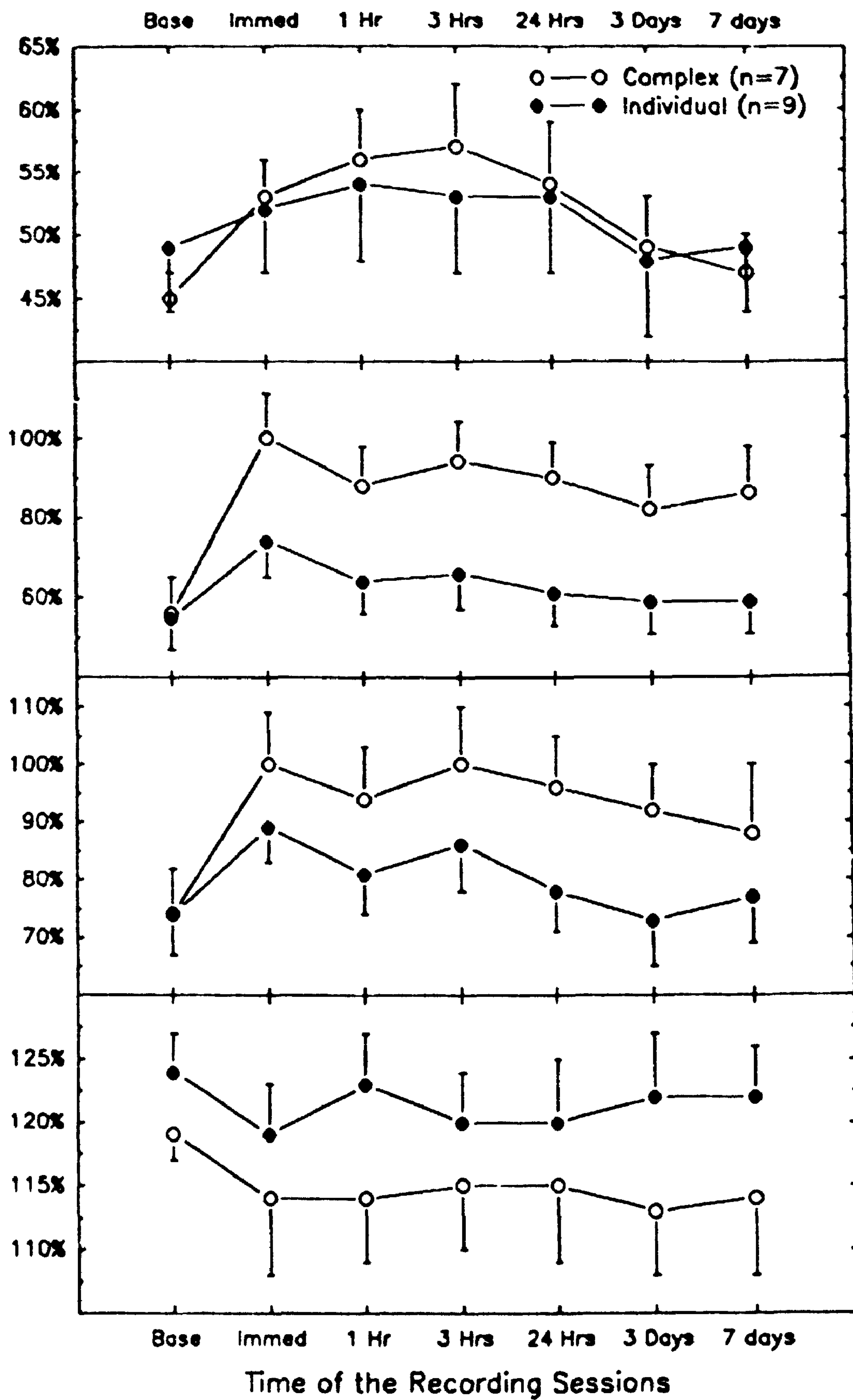

Base
Immed
1 Hr
3 Hrs
24 Hrs
3 Days
7 days
Complex (n=7)
Individual (n=9)
EPSP Slope % Max.
65%
60%
55%
50%
45%
Pop-spike Amplitude % Max.
100%
80%
60%
Pop-spike Area % Max.
110%
100%
90%
80%
70%
Pop-spike Onset % Max.
125%
120%
115%
110%
Time of the Recording Sessions

Table XI Analyses of the LTP data. Multivariate and univariate F-ratios represent between subjects main effects of housing condition collapsed across the repeated measures. The three EP measures of each data transformation were analysed together. The main effect of housing condition on the proportion of maximum values, is not of great interest, since this analysis includes the baseline values on which the data were specifically matched.

Variable	F-ratio	Significance level
Multivariate Baseline Proportion Scores	$F_{(3,12)} = 3.95$;	$p=.036$
EPSP Slope	$F_{(1,14)} = 9.68$;	$p=.008$
Pop-spike Amplitude	$F_{(1,14)} = 6.93$;	$p=.020$
Pop-spike Area	$F_{(1,14)} = 6.67$;	$p=.022$
Multivariate Maximum I/O Proportion Scores	$F_{(3,12)} = 1.53$;	$p=.258$
EPSP Slope	$F_{(1,14)} = 0.09$;	$p=.927$
Pop-spike Amplitude	$F_{(1,14)} = 3.54$;	$p=.081$
Pop-spike Area	$F_{(1,14)} = 1.14$;	$p=.303$

amplitude, and pop-spike area, but not for the EPSP slope measure (Table XII). However, posthoc tests indicated that when the baseline measures were contrasted at the one and three hour recording sessions only, both the multivariate [$F_{(6,50)}=2.79$; $p=.020$] and the univariate interactions were significant, indicating that rats housed in the complex environment exhibited greater amounts of LTP over baseline measures than did their individually housed littermate controls (Table XIII). Additional analyses of the LTP data transformed as difference scores from baseline values, also gave robust effects similar to those of the proportional baseline values.

Further, as part of the post hoc analyses, correlations were run on the baseline ratio scores, at the one hour post LTP mark, collapsed across the housing conditions. For these analyses all four EP measures were used, including the onset latency of the pop-spike. Both these correlation matrices can be observed in Table XIV. Results of the baseline proportional scores, indicated a high correlation between the pop-spike amplitude and the pop-spike area. Further, in this correlation matrix, both the pop-spike measures correlated with the EPSP slope measure, but only the area correlated with the latency measure. The EPSP slope measure did not correlate with the latency measure.

Correlations between LTP and learning were also run. In this analysis learning was assessed as the sum of the first four acquisition trials of the Morris water-maze that previously had shown significant differences between the two housing conditions. Potentiation measures examined for this analysis were the EPSP slope, pop-spike amplitude and the pop-spike area recorded immediately, 1 hr, 3 hrs, and 24 hrs after LTP induction. All three of the data transformations used to analyse the LTP data were

Table XII Analyses of the LTP data. Multivariate and univariate F-ratios represent mixed between and within subjects interaction of housing condition by time or LTP. The three EP measures of each data transformation were analysed together. The interaction on the proportion of maximum values, is of the key interest, since this analysis includes the baseline values on which the data were specifically matched.

Variable	F-ratio	Significance level
Multivariate Baseline Proportion Scores	$F_{(15,200)} = .76$;	$p = .721$
EPSP Slope	$F_{(5,70)} = .65$;	$p = .659$
Pop-spike Amplitude	$F_{(5,70)} = .15$;	$p = .979$
Pop-spike Area	$F_{(5,70)} = .91$;	$p = .483$
Multivariate Maximum I/O Proportion Scores	$F_{(18,242)} = 1.64$;	$p = .052$
EPSP Slope	$F_{(6,84)} = 1.30$;	$p = .266$
Pop-spike Amplitude	$F_{(6,84)} = 2.94$;	$p = .012$
Pop-spike Area	$F_{(6,84)} = 2.53$;	$p = .027$

Table XIII Post hoc analysis of the LTP data. Multivariate and univariate F-ratios represent mixed between and within subjects interaction of housing condition by time or LTP on the proportion of maximum values. Only the repeated values of the baseline, 1 hour, and 3 hour recording sessions are included.

Variable	F-ratio	Significance level
Multivariate Maximum I/O Proportion Scores	$F_{(6,50)} = 2.79$;	$p=.020$
EPSP Slope	$F_{(2,28)} = 4.26$;	$p=.024$
Pop-spike Amplitude	$F_{(2,28)} = 6.41$;	$p=.005$
Pop-spike Area	$F_{(2,28)} = 4.84$;	$p=.016$

Table XIV Correlational analyses of the LTP data. Intercorrelations among the EP measures at 1 hour post LTP induction. Only the transformations based on the baseline scores are included. As such, the first matrix is based on the difference scores, and the second matrix is based on the proprortional scores. All correlations were evaluated at p=.01, using a one-tailed distribution. Also the latency of the pop-spike onset was re-introduced in this an^lysis.

	Baseline Proportional Data		
	Slope	**Amplitude**	**Area**
Amplitude	.70*		
Area	.68*	.92*	
Onset	-.18	-.41	-.57*

*Indicates that the correlation is Significant at the $p < .025$ alpha level.

examined in this analysis. Results from this analysis of the 36 correlations indicated that none were significant at the $p = .05$ alpha level using a one-tailed distribution [$r_{(max)} = -.35$; $p = ns$].

4.4.1 Discussion of the Electrophysiological Data Recorded Prior to LTP Induction:

That no differences were found between the rats at the time of recording, before the electrodes were cemented in place, either in the variance of the measures within each group or the actual value of the measures themselves indicates that the surgeon would not have been able to differentiate rats from the different housing conditions on these criteria alone. Therefore, any biases which may have been possible through recognition of group membership was not a factor.

However, the null group differences observed at the time of surgery, as well as the potential group differences observed during both the post-recovery recording session and during the full I/O recordings run counter to the predicted differences and to previous literature on the matter. As such, Green and Greenough (1986) using a slice preparation, found that slices drawn from rats that had been housed in complex environments had larger EPSPs and pop-spikes across the I/O curve than slices drawn from their individually housed littermates. Similarly in a series of experiments McNaughton and Barnes and their colleagues found that both the EPSP slope and pop-spike increased gradually over a period of five days, after being placed in complex environments (Sharp et al., 1985; 1987; Silbert et al., 1989). They further found that the EPSP measure decayed back to baseline over the next five days, while the pop-spike remained elevated, for as long as the differential housing continued and for some time

after (Sharp et al., 1985; 1987).

A number of differences exist between the studies presented here and those done by McNaughton and Barnes and colleagues and by Green and Greenough (1986). First, those studies done by McNaughton and Barnes and colleagues involved within animal changes, such that in the critical complex environment housing manipulation, rats were compared to their own pre-housing baseline. The absolute values of the pop-spike amplitude, and EPSP slope measure from both groups in Sharp et al. (1985) showed considerable overlap, indicating the great variability in EPs due to slight differences in electrode placement. In support of this argument are some of the early depth profiles recorded by Lomo (1971), in which a .1 mm shift in recording electrode position could result in a 6 mv pop-spike amplitude decreasing to a 2 mv pop-spike amplitude. Similarly, depth profiles recorded by Brankack and Buzsaki (1986) indicated that a .0825 mm shift in electrode position resulted in a 10 mv pop-spike amplitude becoming a 2.5 pop-spike amplitude.

In contrast to the variability of the absolute measures was the relatively small, but uniform and reliable change occurring after placement in the complex environment. Of the studies reporting an LTP-like effect after complex environment housing, only Sharp et al. (1985) display raw measures indicating that the change in pop-spike amplitude is less than 2.5 mv. Untransformed data was not shown in either Sharp et al. (1987) or Silbert et al. (1989). The number of animals in Sharp et al. (1985) was extremely small with the total number of experimentals and controls together equalling five. The number of rats used in Sharp et al. (1987) and Silbert et al. (1989) was far more comparable to

the numbers used here. However, it still remains true that the rats in those studies were implanted prior to complex environment housing and thus the effect of the complex environment was not a between groups comparison.

A between groups comparison was made, subsequent to the complex environment manipulation, by Green and Greenough (1986). Their findings indicated that slices drawn from rats housed in the complex environment exhibited greater pop-spike amplitude and EPSP slope values across the I/O curve, than those slices drawn from their individually housed littermates. First, differences were only observed in the latter portion of the I/O curve, which was discontinued at intensities which evoked a pop-spike amplitude that was half its maximal value. The pop-spike amplitude recorded at the maximal value did not exhibit differences between the two groups and values of the EPSP slope were not shown at this intensity. Thus, in Green and Greenough (1986) the EP measures recorded at the extremely low values and at extremely high values did not exhibit differences between the groups. As such, the results from the raw data presented here, in part, appear to conform to this pattern, with no differences on the pop-spike measures shown at the low I/O curve intensities, and lesser differences exhibited by the EPSP slope and latency of the pop-spike onset at the extremely high intensities. Second, five or six slices were taken from each animal, of which the three healthiest appearing slices were selected for electrophysiological measures and the slice that gave the most robust response was used in the final analyses.

However, it should be remembered that at the multivariate level, the observed differences were statistically non-significant. It should also be noted that at the univariate

level, different results were produced by the recording sessions taken at the time of surgery, upon recovery, and during the I/O procedures. The inconsistencies observed across the recording sessions probably have to do with differences in anaesthetic condition and electrode placement. Similarly, differences between previous studies and the present analyses are probably attributable to more variability in electrode positioning and a weaker between subjects design. Therefore, the overall results should not be altogether surprising nor contradictory in their implications for previous literature. However, that differences between housing conditions were not found in this set of analyses indicates that previously observed differences are not a robust phenomenon.

4.4.2 Discussion of LTP Induction and Maintenance Data from Rats Housed in Complex Environments and Individual Housing Conditions:

In their analyses of data from the LTP saturation experiments Cain et al. (1993) reported successively smaller amounts of LTP at the low, middle and high test pulse intensities used during the abbreviated I/O curves. Further, unpublished analyses of these data in conjunction with those from the behavioral-LTP experiment in the same study, revealed similar relations within each of the low, medium and high intensity levels, on a total of 37 hemispheres from 24 rats. In these analyses significant correlations were found between baseline pop-spike measures, transformed as proportions of the maximal I/O values and LTP amounts, reported either as differences or proportions over baseline measures, such that greater amounts of LTP were found at proportionately smaller I/O values. These correlations can be found in Table XV, and accounted for up to 62% of the LTP variance.

Leung and Au (submitted) have also examined this issue in the CA1 region of the hippocampus using *in vitro* slice preparations (See also Au, 1990; Au and Leung, 1989). After recordings had stablized, full I/O curves were recorded followed by the induction of LTP using primed burst stimulation. I/O curves evaluating the PTP and LTP were then recorded at 10 s, 15 min, 30 min, and 60 min following the potentiation procedures. Results indicated greater amounts of LTP over baseline values were found at the lower end of the I/O curve than at the higher end, for both EPSP slope and pop-spike amplitude values.

In light of these findings and given the small numbers of rats in each housing condition, and the variablility of the stimulation intensities used, it w[illegible] be more important to appropriately match animals on the values from a single te[illegible] pulse intensity then to report the abbreviated I/O curves, as had been recorded throughout the LTP procedure. As such, the I/O curve data were not analysed as I/O curves, but matched and analysed as described above. Once the the test pulse intensities for each animal had been selected, the data from those intensities were transformed into two sets of scores. These transformations were proportional scores based on baseline values, and proportional scores based on the maximal I/O values, for which the baseline test pulse intensities had been selected. Proportional or percentage increases over baseline values have been traditionally used to evaluate LTP. The proportional scores of maximal I/O values were employed in light of the more recent analyses linking the amount of LTP recorded to the baseline intensity values. This latter transformation has also been used by Leung and Au (submitted). This transformation was also used to evaluate the amount

of LTP in this experiment, since it was also used as the basis for selecting the test pulse intensities. Similarly, this latter transformation was used throughout Appendix A and as one of the data transformations used to analyse the formal I/O curves in the current study.

Since the data were selected in a non-standard way, a number of statistical tests were run on the raw data used to select the test pulse intensities and generate the data transformations. Findings from these tests indicated that the selected test pulse intensities, the selected baseline values, and the maximal I/O values did not differ between complex environment and individually housed conditions The stimulation intensity used to induce the LTP was held constant for both groups at 800 μa, and thus was not individually fitted for each rat as the test pulse intensities had been. However, the LTP intensity was proportionately close to the maximum I/O curve intensity used for both groups, and the EP measures resulting from this intensity did not differ from each other. Therefore, it can be assumed that the stimulation intensity selection, data transformation process, and the LTP parameters did not bias the analyses towards finding differences.

Prior to the analyses of the LTP data for differences among the housing conditions, an overall assessment of the EP measures and their transformations ability to detect LTP was made. Findings indicated that the latency to the pop-spike onset, as recorded here, was not sensitive enough to detect changes after LTP induction, and thus this measure was dropped from the remaining analyses. Previous studies found that this measure changed as a function of LTP (Bliss and Lomo, 1973; Douglas, 1977). As such, it is odd that this measure did not exhibit LTP in the current study. However, in the LTP

studies, analog instead of digital methods were used to record EPs, and thus the resolution of the recording was greater than the 50 μs digital pickup rate used here. The amount of shift observed in the sample given by Douglas (1977) was .15 ms, a difference which may not have been detectable in the current study, given that the margin of error of the 20 KHz pickup rate would be one data bin on either side of the recorded time, which would amount to a potential error of .10 ms. Yet, results from Appendix A indicate that this measure shifts a minimum of 1 full msec across the intensity range of the I/O curve. However, little change in this measure occurred at stimulation intensities greater than 200 μa above pop-spike threshold, and the average test pulse intensity used in the current LTP evaluation was approximately 420 μa. Unfortunately, stimulation intensities were not given in Douglas (1977), and thus identical contrasts cannot easily be made. However, the pre-LTP pop-spike amplitude (peak to peak) of the sample given by this same study was a little less than 2 mv, which contrasts with average pre-LTP pop-spike amplitude of 7.36 mv used here. As a result, it is possible, due to the positioning of the pop-spike on the I/O curve that less LTP would be exhibited in this study by the latency measure than that exhibited by the latency measure in Douglas (1977). Thus, that the latency of the pop-spike onset did not exhibit good LTP in the current examination, possibly should have been expected.

Results from the LTP induction and maintenance analyses indicated that rats housed in the complex environment exhibited greater amounts of LTP than their individually housed littermates, in all cases except the proportion of maximal I/O transformation of the EPSP slope. Additionally, by the end of the 7 day period much of

the LTP exhibited by the individually housed littermates had decayed back to baseline. However, the decay rate for both groups was similar as indicated by the null interaction effects. Thus, the complex environment housed rats were still elevated by the end of the 7 day period largely due to greater amounts of LTP initially induced. These results were in the predicted direction and show that rats undergoing environmental enrichment treatments exhibit a greater capacity to sustain and maintain electrophysiologically induced plasticity than their individually housed littermates.

These data are new and have not been reported before, except by this laboratory and in abstract form (Hargreaves et al., 1992). However, in the saturation experiments discussed in Chapter 1, both the original Castro et al. (1989) study and the same laboratory's replication (Korol et al., 1993) employed complex environment housed rats in both experimental and control groups, although this was not stated in the original report (McNaughton, personal communication). As such, LTP data from complex environment housed rats have been reported, but contrasts between housing conditions were not possible since both experimental and control groups received the enrichment treatment. The amount of LTP observed in these studies as compared to that found in the current examination is also difficult to assess, since multiple LTP sessions were delivered and only the final saturation levels were reported in Korol et al. (1993). However, in the earlier Castro et al. (1989) study the daily LTP amounts were displayed. Unfortunately, the test pulse intensities used in the original study evoked baseline pop-spike amplitudes that were approximately 30% of the maximal I/O values (McNaughton, personal communcation), which were different from the approximately 55% of maximal I/O

values used here. It is also difficult to contrast and compare the current LTP data with previous data from this laboratory since LTP was induced using monopolar stimulation trains instead of the more traditional bipolar stimulation trains normally used in this laboratory.

As to why complex environment housing enhances the ability of a rat to sustain and maintain electrophysiological LTP is unknown and was not further explored in this set of experiments. It can probably be safely assumed that the biochemical mechanisms underlying LTP in the different housing conditions is the same. As such, LTP in complex environment housed animals probably involves the NMDA subreceptor, as well as a number of the phosphorylation cascades and protein syntheses discussed in Chapter 1. However, it is possible that the degree of change in any one or number of the processes underlying LTP may be different in the two housing conditions.

Given the history of neuroanatomical differences associated with enriched environments, it is also possible that structural differences between the two groups exist, which may also enhance LTP. Thus, much like the neuroanatomical differences in the molecular layer of the dentate gyrus accounting for the differences in LTP saturation rates of young versus senescent rats found by Barnes and McNaughton (1985), differences in the molecular layer may account for the differences in LTP reported here. However, as will be discussed presently in the neuroanatomical section, there is some debate as to whether or not neuroanatomical changes take place in the hippocampus, as a result of complex environment housing. Regardless of whether it is argued that structural, biochemical, or some combination of these two facets underly the differences

in LTP induction the data currently do not allow firm conclusions.

4.4.3 Discussion of the Relations Between LTP and Learning Analyses:

It was of interest to see that learning and LTP did not exhibit any relations to each other, regardless of the EP measure, data transformation, or the time at which potentiation was evaluated. Correlations between measures of LTP and learning have been examined previously by a number of laboratories. In a close replication to the present analyses Cain et al. (1993) found that a number of water maze acquisition and retention measures did not correlate with subsequent LTP measures of either the EPSP slope or pop-spike amplitude. As such, the analysis reported nere has replicated those findings. However, significant correlations between error scores on the lit platform task and subsequent LTP, after repeated high frequency sessions were found by Barnes (1979). These relationships indicated that lower error scores and distances travelled were associated with greater amounts of LTP. Jeffery and Morris (1993) similarly found positive correlations in the water-maze between the amount of saturated LTP and the amount of time spent in the region of the platform location during the post-acquisition probe trial. Thus, it may require that LTP has reached ceiling or at least near ceiling amounts before any relations are seen. However, correlations between saturated LTP and learning were also examined by Cain et al. (1993). This examination did not reveal any significant patterns of learning and retention with any of the LTP measures. As such, the ongoing evidence from this laboratory suggests that the two phenomena are unrelated.

In conclusion then, the present findings that rats housed in complex environments sustain and maintain greater levels of LTP are novel. Thus, housing in complex

environments appears to enhance both learning and LTP. However, correlations between these two phenomena continue to indicate that learning and LTP may be unrelated.

CHAPTER 5 - EFFECTS OF A COMPLEX ENVIRONMENT ON NEOCORTICAL THICKNESS

5.1 Outline of the Anatomical Assessment of Neocortical Thickness:

After one final month of daily differential housing the rats were sacrificed at approximately 185 days of age. Cortical thickness was measured at three points on each hemisphere from 5 different coronal planes identified by specific neuroanatomical landmarks. In analysing the effects of complex environment housing, data from within each litter were matched across the two housing conditions. Additionally, within each rat the pattern of cortical thickness was analysed for the standard anterior-posterior, medial-lateral, and hemispheric differences, found by others.

These analyses are detailed below, along with the anatomical procedures for generating the data, and discussions of the relevant findings.

In a separate analysis, the thickness of specific layers of the dentate gyrus and CA1 regions of the hippocampal formation were also examined and compared across housing conditions.

5.2 Procedures for the Neuroanatomical Assessment:

Following the final decay recording session of the last rat during the LTP experiment, animals were kept in their respective housing conditions for a further 20 days, after which they were sacrificed with an overdose of sodium pentobarbital and perfused through the heart with a 10% formalin solution. The brains were removed and placed in 10% formalin. Thus, sacrifice occurred approximately 140 days after the rats

were separated into the complex environment and individually housed control conditions. At this time the rats were between 185 and 190 days of age.

Histological analyses used the methods of Stewart and Kolb (1988). Briefly, at the beginning of the histological procedures the brains were removed from the formalin and placed in a 30% sucrose formalin solution. The brains were then sectioned coronally, with each slice 40 μm thick, in a cryostat set at -20°C. Every tenth section was saved and subsequently stained with cresyl violet, a Nissl body stain.

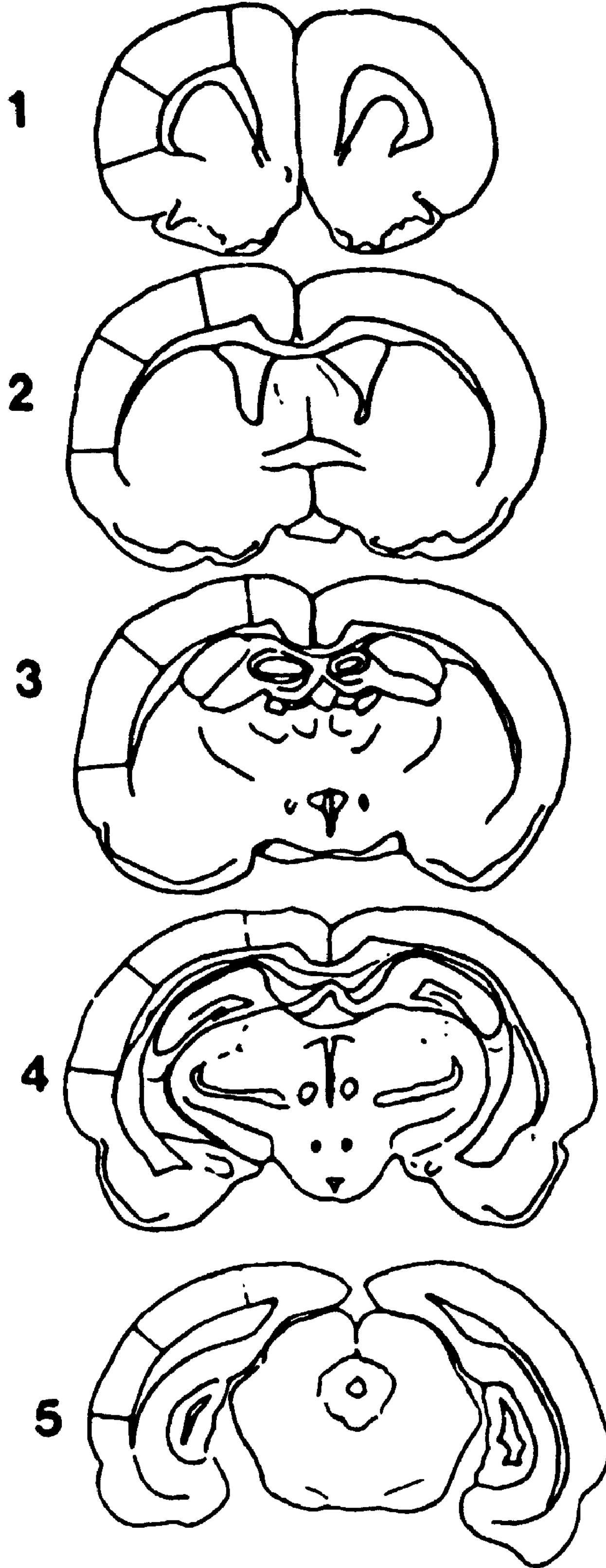
Cortical measurements were taken at medial (M), central (C), and lateral (L) positions, along 5 coronal planes, identified by the nomenclature used by Zilles (1985). The coronal planes were identified by specific landmarks and the MCL positions were identified by specific cortical divisions. Both the landmarks and the divisions used are outlined in Table XVI and illustrated in Figure 15.

The mounted slices were projected onto a horizontal surface by a Zeiss DL 2 POL petrographic projector set at a magnification of 13 X. Cortical thickness was measured by a plastic ruler placed at the 3 specificed cortical divisions on each hemisphere at the 5 specified coronal planes. The experimenters were blind as to the identity of the rats' brains. Some data were unobtainable due to histological error. When this occurred in the litter consisting of 8 siblings, the missing data were estimated as the average of the remaining rats 3 rats in that housing condition. This was done for 6 of the 540 data points. Data in the first coronal plane were also missing from other rats whose data could not be accurately estimated and subsequently this plane was dropped from the analyses.

Table XVI The neuroanatomical landmarks for measuring cortical thickness in the complex environment experiment. Landmarks are based on Zilles' (1985) nomenclature. Three medial-lateral positions on both left and right cortices were measured at 5 different coronal planes.

Plane Location		Relation to Bregma	Landmark/Division
First		+2.2 mm	Caudate-putamen
	Medial		Frontal 2 - Frontal 1
	Central		Frontal 3 - Parietal 1
	Lateral		Parietal 1 - Gustatory
Second		-0.3 mm	Anterior Commissure
	Medial		Frontal 1 - Hindlimb
	Central		Forelimb - Parietal 1
	Lateral		Parietal 1 - Parietal 2
Third		-1.8 mm	1st Hippocampal section
	Medial		Frontal 1 - Hindlimb
	Central		Hindlimb - Parietal 1
	Lateral		Parietal 2 - Gustatory
Fourth		-4.8 mm	Posterior Commissure
	Medial		Agranual Retrosplenial - Occipital 2 medial medial
	Central		Occipital 2 lateral - Parietal 1
	Lateral		Temporal 1 - Temporal 3
Fifth		-6.6 mm	Last Hippocampal section
	Medial		Occipital 2 medial lateral - Occipital 1 medial
	Central		Occipital 1 binocular - Occipital 2 lateral
	Lateral		Temporal 1 - Temporal 2

Figure 15 Schematic of the coronal planes 1 - 5, with the Medial Central, and Lateral cortical thickness measures demarcated on each section. The MCL divisions are based on the nomenclature of Zilles (1985), as delineated in Table XVI. The figure tracings are based on a figure from Kolb and Stewart (1988).


1
2
3
4
5

For the analyses of the hippocampal formation a single coronal slice was selected for each animal. Sections were selected on the basis of two criteria; 1) being adjacent to slices containing the tract of the recording electrode and 2) displaying portions of the habenular commissure. Thus, these slices were posteriorly located at approximately -4.5 mm from Bregma.

The selected slices were magnified 45 X by a Bausch and Lomb overhead microscope and projected onto a horizontal surface, where outlines of the hippocampal formation were traced. Five thickness measures were then made medial-laterally from the centre of the granule cell layer of the superior blade of the dentate gyrus, to the centre of the CA1 pyramidal cell layer of the hippocampus proper, with each measure transecting the centre of a blood vessel situated at the hippocampal fissure and oriented perpendicular to the two cell layers. Thus, thickness measures of the molecular layer of the superior blade of the dentate gyrus, and of the apical dendrites of the CA1 pyramidal cells (stratum radiatum and lacunosum moleculare) were made. Measures of the inferior blade of the dentate gyrus and the CA1 stratum oriens were not taken due to the obvious distortion of the tissue, which probably occurred during the histological procedures. The five recorded measures were then averaged to obtain a single measure for the thickness of the molecular layer and the length of the CA1 apical dendrites, of each the left and right portions of the hippocampal formation. The experimenter was blind as to the identity of the rats' slices during the tracing and measuring procedures.

5.3 Results of the Neuroanatomical Assessment:

For the analysis to determine the nature of the differences between housing

conditions, the data were averaged across hemispheres, and rats in each of the housing conditions were ranked separately within each litter by an aggregate measure of cortical thickness. The two housing conditions were then matched against each other on all cortical measures and analysed by a within subjects matched design. The anterior-posterior location of the coronal sections, and the medial-lateral position of measurements were the within subject factors, while housing condition was the matched factor.

The results from this analysis again indicated significant main effects of the anterior-posterior location of the coronal section [$F_{(3,24)}=637.53$; $p<.0005$] and the medial-lateral position of the cortical measurement [$F_{(2,16)}=74.94$; $p<.0005$]. Further, and critically, a main effect of housing condition was found, with rats housed in the complex environment having thicker cortices than their individually housed littermates [$F_{(1,8)}=5.91$; $p=.041$].

Subsequent paired t-tests performed post-hoc, revealed that four measures that showed significant differences 1) medial location, on the second coronal section, demarcated by the anterior commissure, 2) lateral location, on the third coronal section, demarcated by the first appearance of the hippocampal formation, 3+4) central and lateral locations, on the fourth coronal section, demarcated by the posterior commissure (Table XVII). Of the 4 significant differences the greatest indicated an increase of 7%, while the least indicated an increase of 3.4%. All other differences were non-significant, but it is worth noting that 10/12 of these differences favoured the rats housed in the complex environment as having thicker cortices. Further, the magnitude of the 2/12 non-significant differences going in the opposite direction were the smallest of the 12 absolute

Table XVII Analyses of anatomical data from the complex environment experiments. Paired t-tests from the post-hoc analysis of the housing condition analysis. T-tests contrast the thickness of cortices from the rats that were housed in the complex environment to the thickness of their individually housed littermates cortices. Data were averaged across hemispheres and matched within each litter for cortical thickness. Cortical thickness measured at 4 different anterior-posterior locations and 3 cortical positions. T-tests were initially evaluated using a one-tailed distribution, due to the predicton that the cortices of the rats housed in the complex environment would be thicker. Additionally, group means of cortical thickness are presented. Thickness in mm was estimated by dividing the measurements taken by the magnification factor.

Variable	Complex	Individual	$t_{(8)}$ value	Significance level
2nd Coronal Section				
Medial	2.74	2.61	2.32;	$p=.025$
Central	2.77	2.79	-.30;	$p=.386$
Lateral	2.33	2.30	.60;	$p=.282$
3rd Coronal Section				
Medial	2.33	2.22	1.23;	$p=.126$
Central	2.70	2.62	.96;	$p=$ [illegible]2
Lateral	2.47	2.38	2.10;	$p=$ 035
4th Coronal Section				
Medial	1.83	1.79	1.40;	$p=.100$
Central	2.05	1.95	3.60;	$p=.004$
Lateral	2.21	2.07	2.57;	$p=.016$
5th Coronal Section				
Medial	1.55	1.57	-.68;	$p=.258$
Central	1.87	1.83	.93;	$p=.191$
Lateral	1.66	1.60	.91;	$p=.194$

differences.

A second analysis, similar to the first was performed, except that each housing condition was ranked within each litter by bodyweight at the time of perfusion, instead of using an aggregate of cortical thickness. Results from this analysis were similar in pattern to the first, but not as robust, such that only the differences observed on the coronal plane marked by the posterior commissure were significant [$t_{(8)}=2.26$; $p=.027$, $t_{(8)}=3.31$; $p=.006$], and with the difference observed on section marked by the first appearance of the hippocampus exhibiting only a trend towards significance [$t_{(8)}=1.78$; $p=.056$]. Again however, 10/12 of the differences favoured the animals housed in the complex environment. Similarly, the opposing differences were ranked as the smallest of the 12 comparisons.

The neocortical data were also analysed for within subject patterns across all 18 rats. In this design hemisphere, anterior-posterior location of the coronal section, and medial-lateral position of measurement were all within subject factors.

Results indicated that the neocortex became thinner in the posterior coronal sections, [$F_{(3,53)}=713.44$; $p<.0005$], that the central measure was typically thicker than the lateral measure, which in turn was typically thicker than the medial measure [$F_{(2,34)}=112.99$; $p<.0005$], and that differences between the hemispheres interacted in a complex way with the position of the measure and the anterior-posterior location of the coronal section section [$F_{(6,102)}=3.44$; $p=.004$].

Subsequent paired t-tests performed post-hoc, revealed that the four measures exhibited hemispheric differences, at medial and central locations on two of the coronal

sections, demarcated respectively by the appearance of the anterior commissure and the posterior commissure. Of these differences 3/4 favoured the right hemisphere as having the thicker neocortex, with only the medial measure located at the level of the posterior commissure indicating a difference in the opposite direction.

Similar analyses were performed on the thickness data derived from the hippocampal formation, using the within litter cortical thickness and bodyweight rankings to match the measures on. Results from these analyses showed no differences between housing conditions at the multivariate level nor at the level of the post-hoc t-tests for either the dentate gyrus molecular or CA1 apical dendritic measures or both measures combined. Further, within subject analyses revealed no hemispheric differences on any of these measures.

A set of correlational analyses were performed using selected measures of neocortical thickness, LTP and learning. The neocortical thickness measures were selected on the basis of being located on coronal sections which had shown significant differences between the two housing conditions. Thus, the three measures from each of the second, third, and fourth coronal sections were chosen, for a total of nine cortical thickness measures. Only the EPSP slope and pop-spike amplitude, recorded one hour after LTP induction, and transformed as proportions over baseline values were selected as the LTP measures. The EPSP slope and pop-spike measures were selected since they measured different aspects of the EP. The pop-spike amplitude, and the pop-spike area were virtually identical in the amount of LTP that they had exhibited and thus, the pop-spike ampitude was chosen of the two. The one hour interval was chosen since the

induced LTP would be the most robust at this time, but without any of the contaminating effects of shorter duration potentiation phenomena. The proportional transformation of the data was chosen since it gave the largest range of values and exhibited the most robust LTP effects. Learning was assessed as the sum of the first four trials acquisition trials of the Morris water-maze. One set of correlations related the two LTP measures to the nine neocortical thickness measures for a total of 18 correlations, while another set of correlations related the single measure of learning to the nine neocortical thickness measures. If significant correlations were to arise from these analyses it was predicted that LTP would correlate positively with neocortical thickness and that learning would correlate negatively with neocortical thickness.

Results from the first set of correlations indicated that the EPSP slope LTP measure was significantly correlated with the medial measure on the second coronal section [$r_{(14)}=.44$; $p=.045$] and that the pop-spike amplitude LTP measure was significantly correlated with the lateral measure on the second coronal section [$r_{(14)}=.58$; $p=.009$]. Results from the second set of correlations indicated that learning was significantly correlated with the lateral measure on the fourth coronal section [$r_{(16)}=-.42$; $p=.041$]. None of the other correlations were significant.

Finally, correlations between learning, LTP, and the hippocampal formation thickness measures, similar to those above were also run. The results from these analyses indicated that none of the correaltions were significant.

5.4 Discussion of the Neuroanatomical Results:

The within animal patterns of cortical thickness observed in the data presented

here have been observed by others. The reduction in thickness seen moving from the anterior coronal sections to the more posterior sections is similar to data and sections presented by Diamond (1987; 1988), Diamond, Johnson, and Ingham, (1975), Kolb, Sutherland, Nonneman, and Whishaw (1982), Krieg (1946), Paxinos and Watson (1986), Pelligrino, Pelligrino and Cushman (1979), Stewart and Kolb (1988) and Zilles (1985). Similarly, the rankings of the three medial-lateral measures can also be generally seen in such atlases, although not at all coronal planes (Diamond, 1987; 1988; Diamond et al., 1975; Krieg, 1946; Paxinos and Watson, 1986; Pellegrino et al., 1979; Zilles, 1985).

The nature of differences between the left and right hemispheres however, appears to be less clear. However, a majority of the 12 measures analysed here favoured the right cortex over the left, with three of those measures deemed statistically significant. A fourth statistically significant difference was found favouring the left cortex over the right, but this measure matched Diamond's area 18 measure and thus, the left - right asymmetry results found here are not inconsistent with other reports derived from Long-Evans male rats (Diamond, 1985; 1987; 1988; Diamond et al., 1975).

The increased cortical thickness found here, as a result of complex environment housing has been found numerous times previously and was one of the key rationales for doing this experiment (Diamond, 1988; Renner and Rosenzweig, 1987). The pattern of greater effects of the complex environment in the occipital cortex is also consistent with the literature (Diamond, 1988; Renner and Rosenzweig, 1987). In fact, the original Berkeley group's report of enhanced thickness was measured on coronal sections located at the same planes as used here, identified by the anterior and posterior commissures,

with the greater differences occurring in the neocortex at the level of the posterior commissure (Diamond, et al., 1964). Their original finding of a difference of 6.2% in this latter region matches up well with the results found here of a 7% increase in the same location. Although deemed to be occipital cortex, the posterior landmark typically used places this region more in the area of associative cortex. The data presented here also showed the greatest difference in the central and lateral measures of this plane, an area encompassing the occipito-parieto-temporal junction of the rat neocortex, which has been deemed to be largely associative cortex (Zilles, 1985).

Rats in the original 1964 experiment were placed in the complex environment immediately following weaning at 25 days of age (Diamond et al., 1964). This differs from the experiments run here, in which the rats were differentially housed at approximately 50 days of age. This was done in order to habituate the animals to many of the experimental procedures, as well as to allow baseline data to be collected on a number of the behavioral variables. This does not appear to be a critical factor since no differences in the enrichment effects on cortical thickness were found between rats placed in the complex environment for 80 days, either immediately after weaning, or after 105 days had elapsed (Diamond, 1988). Similarly, Green, Greenough and Schlumpf (1983) have found neuroanatomical changes in rats housed in complex environments starting at 450 days of age.

However, Greenough and his colleagues have reported differences in dentate gyrus granule cell morphology between littermatte pairs split across complex environments and individually housed conditions at 23-26 days of age or at 145 days of

age (Fiala, Joyce, and Greenough, 1978). Essentially they found that rats placed in the complex environment after weaning had wider, but not taller granular cell dendritic trees and greater granular cell dendritic field branching, proximal to the cell body. Differences in the granular cell dendritic trees among the adults placed in the complex environments at 145 days of age were not found. Juraska (1984) using a similar analysis did not find the same differences, and in fact found that individually housed rats often had greater number of branchings in the region of 60 to 200 μm from the granule cell bodies. The strain of rats in these studies was not identified, but the animals were placed in the differential housing conditions at identical times, and for identical durations. A similar debate exists as to whether increases in hippocampal thickness occur as a result of complex environment housing, with one group of researchers finding positive results (Walsh, Budtz-Olsen, Penny, and Cummins, 1969), and with the Berkeley group and others finding negative results (Diamond, Ingham, Johnson, Bennett, and Rosenzeig, 1976; Jones and Smith, 1980).

Thus, in lieu of these findings it is not surprising that differences were not found here. Further, even in allowing Fiala et al.'s (1978) findings of a widened dendritic tree and denser dendritic branching proximal to the granule cell body, these results would not necessarily translate into a thicker molecular layer. In fact, the height of the granular cell dendritic trees was not different between the housing groups. Therefore anatomical differences between housing conditions in the hippocampal formation of the rats used here may have gone undetected.

Yet, evidence that the hippocampus is essential for the effect of complex

environment housing on neocortical thickening has been provided by Kolb and Sutherland (Sutherland, personal communication). In a series of experiments they unilaterally destroyed the hippocampus using chemical lesioning techniques, of rats prior to placement in complex environments. Subsequent histological analyses revealed that the neocortex contralateral to the destroyed hippocampus exhibited the standard thickening, whereas the neocortex ipslateral to the lesion did not exhibit any increase in thickness, as a result of complex environment housing. Thus, whether or not the hippocampus itself exhibits neuroanatomical changes as a result of complex environment housing, it has been shown that an intact hippocampus is necessary for related neuroanatomical changes to take place in the neocortex.

Measures of neocortical thickness, such as have been used in the experiments presented here, provide only gross estimates of neuroanatomical change, and much had been done elsewhere to elucidate the constituents responsible for the increased thickness. It is generally accepted that the increased neocortical thickness is due to an enlargement of the space occupied by individual neurons and their supporting glial and vascular celltypes (Diamond, et al., 1964). Thus, it has been found that cortical pyramidal cells of rats housed in complex environment have longer apical dendrites (Diamond, 1967), greater basal dendrite branching of the more distal dendrites, and a greater number of spine densities per unit area on the basal dendrites (Globus et al., 1973; Greenough and Volkmar, 1973; Greenough, Volkmar and Juraska, 1973; Turner and Greenough, 1985). Electron microscopy has further found that the actual synaptic junctions themselves are thicker and cover a larger surface area (Mollgard, Diamond, Bennett, Rosenzweig, and

Lindner, 1971; West and Greenough, 1972).

That these neuroanatomical changes are not due to visual input alone has been shown by Rosenzweig, Bennett, Diamond, Wu, Slagle and Saffran (1969), who blinded or dark reared rats in complex environments and found that these treatments did not prevent the cortical thickening.

However, training in the Hebb-Williams maze series alone, has been shown to alter cortical pyramidal cells by increasing the branching of the more distal apical dendrites (Greenough, Juraska, and Volkmar, 1983). Further, split brain rats trained under monocular conditions did not show these changes in the hemisphere ipsilateral to the occluded eye (Chang and Greenough, 1982). Thus, visual input is necessary in some cases for learning associated changes to take place. In this context, it would have been interesting to know how nonoccluded split-brain rats performed on the maze series in relation to the monocularly occluded split-brain rats. Training in a unilateral forepaw reaching task has also been shown to specifically affect the sensorimotor cortex contralateral to the trained limb (Greenough, Larson, and Withers, 1985). Similarly, group housing or social conditions have also been shown to result in necortical changes, albeit intermediate effects between complex environment and individual housing (Greenough and Volkmar, 1973; Rosenzweig et al., 1964; 1972; Turner and Greenough, 1985). Thus, a variety of housing and learning conditions can have varying generalized and specific effects on the cortical neuroanatomy of rats.

Finally, it is likely that a number of influences other then the complex environment manipulations carried out in this thesis affect cortical thickness. Prominent

among these influences are probably genetic factors. As such, the anatomical analyses assessing the effect of housing condition matched animals within each litter to either body weight or an aggregate of cortical thickness. This was done such that the comparisons made were between genetically similar rats.

Results from the correlational analysis indicated that 2 of the 18 LTP and neocortical thickness correlations were significant, and that 1 of the 9 learning and neocortical thickness measures were significant. Given that an alpha level of $p = .05$ was used in these analyses it is possible that the correlations reported in these analyses represent a type I error or spurious correlations. However, an argument can be made that there were two separate analyses of 18 and 9 correlations run and therefore the type I error is less than 1 correlation per analysis. Further, all three correlations were in the predicted directions. Finally, the two significant LTP correlations were located on the same coronal section. The proximity with which these correlations occurred in relation to each other suggests a non-random and therefore non-spurious pattern.

However, another statistical difficulty is that a number of the variables were selected on the basis of being able to successfully discriminate between the two housing conditions. As a consequence, some of the variables will have had a bimodal distribution of the scores, which may have added to the strength of the correlations, much like the Ramirez and Carrer (1989) study discussed in chapter 1. However, the strongest of the three correlations was between the pop-spike measure of LTP and the lateral neocortical thickness measure located on the second coronal section, the latter measure which had not shown a significant difference between the two housing conditions.

Regardless of the statistical difficulties the indicated relationships are still worth discussing. It was of interest to see that both learning and LTP exhibited relations to cortical thickness, especially given the coarseness of the anatomical measures. However, it was of greater interest to see that these two anatomical locations were quite distinct from each other. This finding further supports the earlier results from chapter 3 of no relation between LTP and learning. However, it may be noted, as discussed in chapter 1 that correlations between repeated LTP and learning have been found independently by Barnes (1979) and Jeffery and Morris (1993). Thus, near saturation levels of LTP may be correlated with learning capacity. Further, the present set of studies have only examined LTP in the perforant-path/dentate-gyrus monosynpatic pathway. LTP has been shown throughout the trisynaptic circuit, and additionally, the perforant-path also involves direct afferents to CA3 and CA1. These pathways should also be examined before a definitive statement of the unrelatedness of LTP to learning is made.

That LTP and neocortical thickness on the second coronal section exhibited a joint relatedness, should in no way be indicative of a causal link going in either direction, or even of a direct link between the anatomy located at this coronal plane and that of the hippocampus. It is more likely indicative of a third underlying factor that equally affects both the anatomy at this location and LTP. Given the relation between LTP and cortical thickness reported here, it would be of great interest to know how LTP related to hippocampal thickness, and additionally to know how well it related to neocortical thickness. However, as discussed above there is some debate as to whether or not the hippocampus anatomically exhibits the effects of complex environment housing (Diamond

et al., 1976; Fiala et al., 1978; Jones and Smith, 1980; Juraska, 1984; Walsh et al., 1969). Yet, the earlier work of Barnes, contrasting the learning and electrophysiology of senescent rats to younger adults, as discussed in Chapter 1 did suggest that differences in neuroanatomy could underly both electrophysiological and learning differences (Barnes, 1979; 1983; Barnes and McNaughton, 1985; Geinisman and Bondareff, 1976; Geinisman et al., 1992a; 1992b). Further, these studies found differences in the dentate gyrus, Although it was not the number of granule cells and mossy fibres that changed with age, but the complexity of the granule cells dendritic branching. Therefore the differences may not be expressed as overall increases in hippocampal thickness, as has been discussed earlier.

Regardless of whether changes in the hippocampal formation can be found after complex environment housing, an intact hippocampal formation does appear to be requisite for the expression of the complex environment housing effect upon neocortical thickness (Sutherland, personal communication).

To summarize the findings presented here then, the data have shown that the complex environment treatment used in this set of experiments did in fact alter the neuroanatomy by increasing the thickness of the neocortex over that of individual housing. Further, the pattern and degree of cortical thickness increases, as a result of complex environment housing as well as the overall within animal patterns of cortical thickness found in the data presented here match those found in past literature from several research groups (Diamond, 1988; Juraska, 1990; Kolb and Stewart, 1988; Renner and Rosenzweig, 1987). Finally, correlational analyses revealed relations betweeen LTP

and cortical thickness, and between learning and cortical thickness. Statistical difficulties weaken the strength of these relationships, but more importantly they further indicate a separation of learning and LTP.

CHAPTER 6 - SUMMARY AND CONCLUSIONS

The electrophysiological phenomenon known as LTP exhibits a number of features that are similar to the Hebbian synapse (Morris et al., 1989). As such, many of the explorations of LTP's underlying mechanisms have been, in part, driven by the assumption that LTP and learning hold a number of mechanisms in common. However, in contrast to the amount of LTP literature that currently exists, the number of direct attempts to associate learning and LTP have been few.

Generally, the research associating learning and LTP can be categorized into four strategies, outlined by Morris and Baker (1984). A critical and detailed review of the current literature indicates that of these four strategies, prior enhancement and prior learning have been unsuccessful in providing consistent and reliable evidence linking LTP and learning. Evidence from the correlational strategy, for the most part, has also not been forthcoming. However, both Barnes (1979) and Jeffery and Morris (1993) have found that the amount of LTP induced after multiple sessions, is related to performance on spatial tasks, such that greater amounts of LTP are associated with better performance. Therefore, it is possible that capacity or near capacity levels of LTP function as some sort of learning index. However, causal inferences cannot be made from correlational data and consequently, this strategy has lacked explanatory power.

Thus, most of the evidence linking LTP and learning mechanisms has come from the blockade/facilitation strategy. In this strategy, if an experimental manipulation hampers both learning and LTP, or conversely facilitates both learning and LTP, then

some link is said to exist between the two phenomena and the experimental manipulation. Much of the evidence from this strategy has been provided by Richard Morris and his colleagues, who have extensively examined the effects of NMDA blocade on spatial learning and LTP induction. Additionally, recent genetic manipulations have provided two sets of mice that are missing specific genes that regulate certain biochemical functions that previously have been shown to be necessary for the induction of LTP. Consequently, these mice have shown deficits in both learning and LTP induction (Grant et al., 1992; Silva et al., 1992).

Although a substantial amount of work has been done using the blockade approach, little, if any, has been done using the facilitation approach. As a result, this thesis attempted to focus on the facilitation approach using environmental enrichment as an experimental manipulation. Historically, through independent lines of research, this paradigm has shown that housing rats in complex environments leads to improved maze performance, neuroanatomical growth, and increases in hippocampal synaptic communication. However, prior to this thesis, behavioral, neuroanatomical, and electrophysiological realms had not all been examined together in the same preparation. Also, prior to this thesis the induction of LTP in animals housed in complex environments had not been examined. Finally, prior to this thesis, behavioral assessments of animals housed in complex environments had not been very extensive, with each study narrowly focused on only one or two behaviors. Thus, these were the goals set out for this thesis at the end of chapter 1.

In addition to a comprehensive examination of complex environments, this thesis

set out to examine a number of different I/O curve assessments and properties, in relation to ongoing behavior at the time of recording. Further, since I/O curves were integral to this thesis, the question of low frequency potentiation was re-examined. The results from these electrophysiological experiments, as well as from other experiments not fully reported upon, and those of others (Leung and Au, submitted) constrained the way LTP was induced and subsequently analysed in the complex environment experiments.

Results from Appendix A indicated that the different intra-class EP measures of the EPSP slope, pop-spike and latency measures were largely in agreement with each other. So too were the inter-class EP measures, but these relations tended to breakdown at the high-end of the I/O curve. Further, previous literature indicated that these relations also broke down at the low-end of the I/O curve (Wilson, 1981; Chavez-Noriega et al., 1989). Also, as had been previously found, differences in the ongoing behavior at the time of recording influenced the amplitude of some of the EP measures. As such, only a few measures were selected to assess LTP induction in the complex environment experiments, and all EPs recorded were behaviorally "clamped" to immobility. Further results from Appendix A indicated that I/O curve recording could potentiate the EP measures, albeit for a relatively short time. As a result there was a large separation of time between the running of the full I/O curve in the complex environment experiments and the assessment of LTP. Further, the procedures for recording LTP used abbreviated I/O curves instead of the extended I/O curves used to assess the baseline connectivity. Although abbreviated I/O curves were used to record the LTP in the complex environment experiments, these data were analysed at a single matched intensity. This

alternate analysis was performed as a result of unpublished, as well as published analyses of LTP data, indicating that the amount of LTP recorded, greatly depended upon the stimulation intensity used to assess the LTP (Cain et al., 1993; Leung and Au, submitted).

The critical focus of this thesis were the effects of the complex environment manipulation on the behavior, electrophysiology, and neuroanatomy of the rat, and the implications of these results for associating learning and LTP. Behavioral results indicated that the rats housed in the complex environment were different from their individually housed littermates on virtually all behaviors tested, showing improved performance on all tasks of ability and learning, and exhibiting less neophobic responses, spontaneous locomotor activity and dominance asserting behaviors during social interactions with a smaller conspecific. Differences in learning the water maze could not be attributed to weight, or swimming ability alone, or to sympathetic activation. These results were in agreement with much of the past literature that had examined the effects of complex environment housing on individual behavioral assays. However, a number of new tasks were employed, and the extensive analysis of the water-maze and control conditions presented in this thesis had not been published previously.

The baseline electrophysiological data showed no statistical differences between the two groups, but trends favouring the individually housed rats were evident. However, when these potential differences were taken into account and the animals were specifically matched for recording and LTP inducing parameters, the complex environment housed rats exhibited greater amounts of LTP, which were still present

seven days following induction. The trends observed in the baseline data ran counter to what had previously been found. However, numerous procedural differences existed between the various studies that may have accounted for the differences in findings. The LTP results were novel, and as a result there were no precedents with which the data could be compared.

Cortical thickness measures, when matched within each litter, indicated significant differences in several regions, all favouring the complex environment housed rats with thicker cortices than their individually housed littermates. The pattern of differences was similar to that found by others, as were many of the within animal patterns of cortical thickness.

Thus, the data from all three realms together indicated that the complex environment manipulation facilitated learning and LTP, and additionally, cortical thickness. As such, these data provide evidence in support of the link between learning and LTP, and also in this case neuroanatomy. Although a powerful manipulation, as indicated by its global effects, the complex environment paradigm is lacking in the specificity of its underlying mechanism(s). It would not be altogether surprising to find multiple non-interacting mechanisms underlying the changes in the three different realms. As such, the NMDA blockade evidence provided by Morris and his colleagues, by having a more specific target of the manipulation is somewhat stronger in linking LTP and learning together.

In order to see how the three realms related to each other, and thus provide some insight into at least the possible multiplicity of underlying mechanisms, several relational

analyses were conducted between the behavioral, electrophysiological and neuroanatomical realms. Results from these analyses indicated that the learning measure taken from the acquisition phase of the water-maze, did not relate to any measure of LTP recorded at any time following LTP induction. These results were in agreement with similar findings from this laboratory (Cain et al., 1993). On the other hand both LTP and learning were found to be correlated to cortical thickness, albeit at different neuroantomical locations, relatively unassociated from each other.

Thus, these findings provide evidence against the link between LTP and learning, but support the idea that multiple and non-interacting mechanisms underly the relatively global changes seen after complex environment housing. Previous research has shown similar links between anatomy and LTP, as well as, anatomy and learning (Barnes 1979; 1983; Barnes and McNaughton, 1985).

In a way then, the results from the complex environment experiments contradict each other, depending upon the level and direction of analysis. The relational analysis carried out here also appears to contradict the findings of Barnes (1979) and Jefferey and Morris (1993), but LTP was induced in the present set of experiments in a single session, while both the former studies used multiple sessions, such that the amount of LTP may have been at, or near saturation levels. More analagous procedures were used in Cain et al. (1993), but findings still indicated no relation between LTP and learning.

Speaking particularly of the complex environment paradigm, it may in the future be necessary to further tease out the particular aspects or mechanisms, by which complex environment housing contributes to increased learning, LTP and cortical thickness. It has

already been shown that specific visual or motor tasks, affect selective neuroanatomical regions (Chang and Greenough, 1982; Greenough et al., 1985). Similarly, social housing conditions alone produce intermediate neuroanatomical effects (Rosenzweig et al., 1972). It has also been shown that simple exercise on a treadmill, or access to a running wheel can improve water maze acquisition (Fordyce and Farrar, 1991a; 1991b). In addition to these obvious differences in housing conditions, a variety of housing environments have been called "complex". The complex environment used in this set of experiments was similar to those of Green and Greenough (1986), Mohammed et al. (1986), Saari et al. (1983) and Silbert et al. (1989). Yet, other complex environments have ranged from enlarged cages in which a few objects were permanently placed (Forgays and Forgays, 1952; Forgays and Read, 1962; Forgus, 1954) to entire rooms with furniture and junk objects strewn about (Sharp et al., 1985; 1987). No doubt these various complex environments had differing effects, with some more effective at inducing behavioral, electrophysiological, or neuroanatomical changes than others. For example, although social housing has been shown to produce intermediate anatomical effects (Diamond, 1988) it does not appear to be requisite for behavioral-LTP effects (Sharp et al., 1985; 1987). Thus, a fuller examination of the critical factors that produce the behavioral, electrophysiolgical, and anatomical changes would be of great interest, and may again suggest a multiplicity of independent underlying mechanisms. As a method of isolating the individual mechanisms or clusters of mechanisms that give rise to the global differences observed after complex environment housing, it may be possible to set up an array of housing conditions, which are contrasted to each other. Obvious conditions

include the full complex environment, the opposite extreme of individual housing, and the potentially intermediate steps of social housing, and/or access to exercise.

Although the prior learning paradigm has produced little useful evidence linking LTP and learning, the complex environment has consistently been successful at inducing LTP-like changes. In lieu of the findings of Hargreaves et al. (1990) and Cain et al. (1993), it is highly doubtful that these changes are related to the complex spatial mileu that the rat moves through, as originally suggested by Sharp et al. (1985; 1987). However, these changes still exist and must relate to something. One possible method for determining what these factors might be is to take daily recordings from chronically implanted rats, while the levels of housing environment complexity are increased in a stepwise fashion in accordance with resultant changes, if any. In such an experiment one may start with individual housing in suspended cages, and move to individual housing in a complex environment void of objects or conspecifics, to social housing still void of objects, to a minimal complex environment in which only small manipulable objects exist, and finally to the full complex environment with a wide range of manipulable objects, lattice of ramps, and boxes or enclosed spaces.

As a side note, in the studies presented here, only male rats were examined. This was primarily done to avoid the fighting and rigid dominance hierarchies that occur in mixed colonies (Barnett, 1958). The effects of complex environments on the neuroanatomy of female rats has been examined and although the within rat inter-hemispheric pattern of cortical thickness is different in male and female rats, the effects of complex environment housing is the same (Diamond, 1985; 1987; 1988; Juraska,

1984; 1990). However, it is possible that EP recording difficulties would arise for female rats through the interaction of EPS, temperature changes, hippocampal anatomical changes, and the estrous cycle (Cain, Hargreaves and Boon 1993; McEwen, 1991; Moser, Mathiesen, and Andersen 1993; Woolley and McEwen, 1992). Not to mention the large literature that exists on sex-differences in maze learning. Teasing apart these potentially complex interactions however, is also an area worth future research.

As an end comment upon the complex environment paradigm, it has provided both positive, and negative evidence linking LTP and learning. Although this has been the most comprehensive assessment to date of the consequences of complex environment housing, there still remains the task of teasing apart the critical aspects of the environment, and how they may independently contribute to the various realms of changes observed here.

As a final comment upon the strategies outlined by Morris and Baker (1984) for exploring the links, if any, between LTP and learning, a single approach or strategy does not appear as powerful, as the use of multiple strategies. Had this series of experiments relied upon a single strategy, different findings would have been reported. Of the two strategies that have produced some results, the blockade/facilitation strategy should still search for other instances or manipulations that facilitate both LTP and learning, while the relational strategy should go beyond the traditional measures of LTP and examine, some of the less obvious parameters, such as induction threshold, optimal induction intervals, and saturation levels.

There is still much evidence to be gathered before a final pronouncement of LTP's relation or non-relation to learning is made. A close examination of the current literature, indicates that the field is far more optimistic than is warranted by the evidence. In carrying out the experiments that comprise this thesis, results have been found that support both sides of the LTP/learning issue, and thus, the field gains a little more knowledge of how complex the true answer may ultimately be.

APPENDIX A:
COMPARISON OF EP MEASURES AND VARIOUS BEHAVIORAL RECORDING CONDITIONS ACROSS INPUT/OUTPUT RELATIONS AND THE PHENOMENON OF LOW FREQUENCY POTENTIATION

A1: COMPARISON OF EVOKED POTENTIAL MEASURES AND VARIOUS RECORDING CONDITIONS ACROSS INPUT/OUTPUT RELATIONS

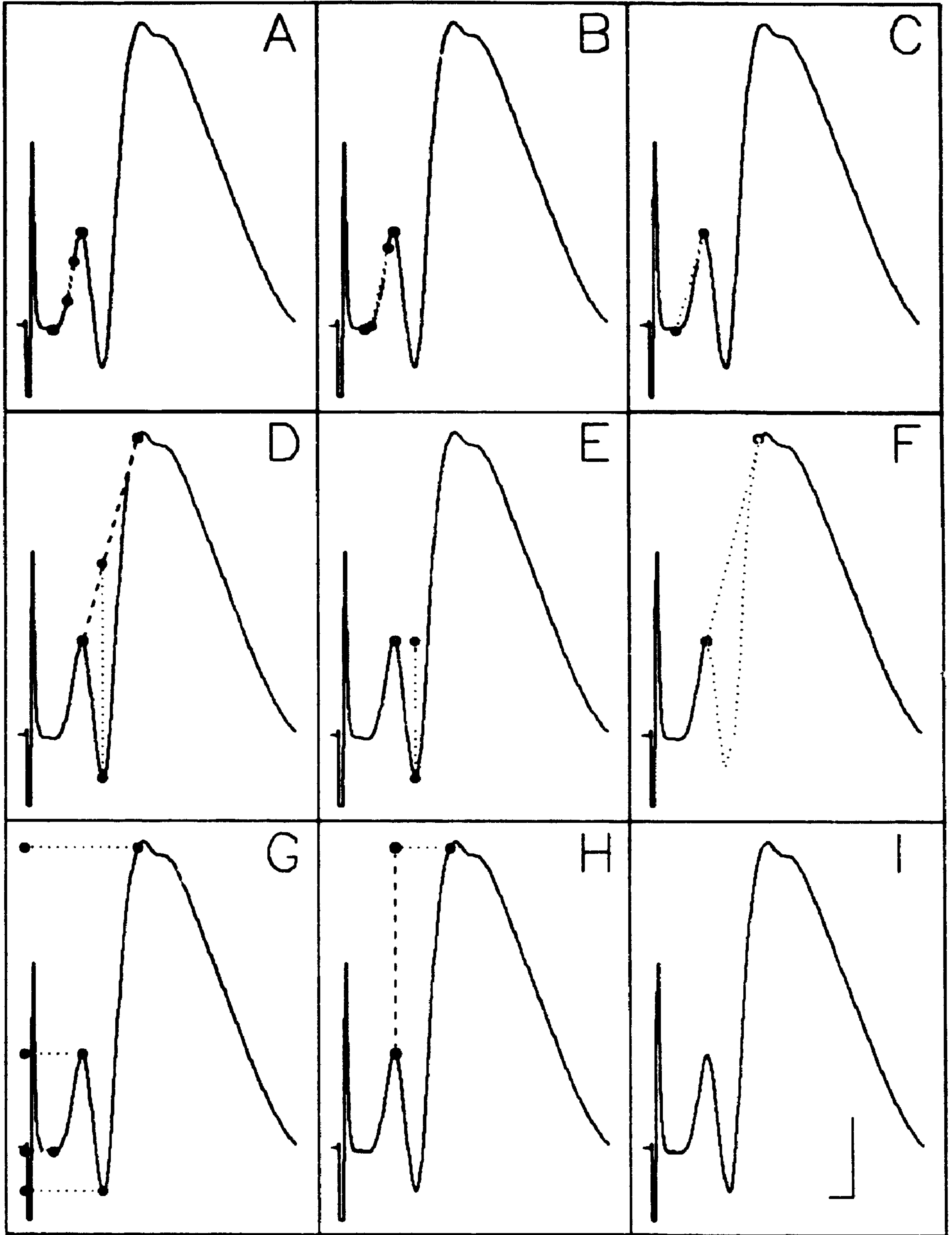
A1.1.1 Evoked Potential Measures:

A wide variety of perforant-path/dentate-gyrus EP measures have been used throughout the LTP, and LTP and learning literature. EP measures can be classified by the component or event of the EP that they measure. Most EP measures assess either the EPSP slope or the pop-spike, as described in Chapter 1. In addition to these components however, a number of researchers have examined the onset and offset latencies of these events. Thus, EP measures fall into one of three possible variable classes or variable clusters 1) EPSP slope measures 2) pop-spike measures 3) or latency of EP events measures. Large scale comparisons of the various EP measures have not been done. Thus, the sensitivity of these measures to different phenomena or recording conditions is largely unknown.

In the following analyses 11 EP measures were derived from the EPs or AEPs, of which three were EPSP measures, three were pop-spike measures, and the remaining four were latency of EP events. These EP measures were examined for their relatedness across a variety of recording conditions and stimulation intensities.

Figure 16 displays the 11 potential measures derived from each EP or averaged EP (AEP). The top row of panels defines the EPSP measures, while the middle row defines the pop-spike measures and the bottom row (first 2 panels) defines the latency of EP event measures. Figure 16I simply displays the AEP unobscured by any markings

Figure 16. Definitions of EP measures utilized throughout the present experiments. The top row depicts the EPSP slope measures, the middle row depicts the pop-spike measures, and the bottom row (panels G and H) depicts the latency of EP event measures. The specific measures employed are as follows (A: maximum slope, B double-ended roll-off slope, C: mean slope, D: peak-to-tangent pop-spike amplitude, E: peak-to-peak pop-spike amplitude, F: area of the pop-spike, G: event latencies from top to bottom... offset of pop-spike, onset of pop-spike, onset of EPSP, and peak of pop-spike, H: synchrony of pop-spike). Panel I displays the unobscured EP, with the horizontal calibration bar as 1 ms, and the vertical calibration bar as 5 mV. Open circles and dashed lines indicate demarcation points used to assist in defining the EP measures. Filled circles and dotted lines indicate the actual measures.


A
B
C
D
E
F
G
H
I

taken advantage of microcomputers and associated software packages that allow them to record and store a great many individual EPs, from which the measures can then be derived (Cain et al., 1993; Green et al., 1990; Hargreaves and Cain, 1991; Moser et al., 1993; Sharp et al., 1989). By recording individual EPs in this manner the variance of the derived measures can be evaluated under different recording conditions providing an additional dimension of information. As a result several of the following analyses examined the variability of individual EP measures across the I/O curve, and between the behavioral recording conditions of immobility and movement.

Further, since it is not known how much average measures derived from individual EPs differ from individual measures derived from AEPs, one of the following analyses examined this question across the I/O curve. This becomes a relatively important question, given that there are latency shifts among individual EPs, such that the onsets and offsets of the EP components may not coincide.

A1.1.8 Rationale of Experiments and Analyses:

Thus the purpose of this set of experiments and analyses was fourfold. First, to examine the relatedness of the different measures discussed above, across the I/O curve. Second, to describe the EP measures beyond standard maximum I/O curve stimulation intensities. Third, to compare EP measures derived from AEPs and average EP measures derived from individual sweeps. Fourth and finally, to examine the variability of the EP measures across the I/O curve and between Type I and Type II behaviors.

A1.2.1 Subjects:

20 Male hooded Long-Evans were used as subjects in these experiments and

1986; Sharp et al., 1985; Sharp et al., 1987; Sharp, McNaughton, and Barnes, 1989) and Robinson (1992). This measure uses the difference in amplitude between two fixed points of .5 to 1 ms apart. Morris and his colleagues have taken a different approach and run a linear regression through the EPSP slope for a .7 msec interval starting at approximately 1.8 msec after the stimulus artifact (Morris et al., 1986; Morris et al., 1989; Davis et al., 1992).

All the EPSP slope measures that have onsets demarcated at fixed latencies from the stimulus artifact are further susceptible to general shifts in the temporal axis of the EP, and thus changes in EPSP slope may be confounded with changes in latency. On the other hand, those EPSP slope measures that have onsets demarcated by the initial positivity of the EPSP are not susceptible to this contamination, as are the measures used here.

A1.1.3 Pop-spike Measures:

Figure 16D displays the definition of the the peak-to-tangent pop-spike amplitude. First, a tangent line is drawn from the pop-spike onset to the pop-spike offset. A vertical measure is then taken from the peak of the pop-spike to the point where it intersects the tangent line.

Figure 16E displays the definition of the peak-to-peak pop-spike amplitude. This measure is simply the difference in amplitude betweeen the onset of the pop-spike and the peak of the pop-spike.

Figure 16F displays the definition of the pop-spike area. This measure is derived by calculating the area beneath the tangent line drawn from the onset to the offset of the

pop-spike.

The pop-spike measures used in this thesis were more congruent with the literature, than the EPSP measures. This was largely due to the limited number of ways to measure the pop-spike, and partially due to the clarity with which these definitions have been described in the literature. The peak-to-peak pop-spike measure was used in the original LTP studies (Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973; Douglas and Goddard, 1975; Douglas, 1977). McNaughton and Barnes and colleagues have also previously used the peak-to-peak pop-spike amplitude (Sharp et al., 1985). However, this group now typically uses the pop-spike area, having found a correlation between the two measures of r=.90 (McNaughton et al., 1986; Sharp et al., 1987; Sharp et al., 1989; Castro et al., 1989). The peak-to-tangent pop-spike amplitude appears to have first been used by Robinson and Racine (1982), and continues to be used by Robinson and his co-workers (Robinson, 1992). A different method for assessing pop-spike changes has been employed by Skelton, while working in Phillips' laboratory (Skelton, Miller and Phillips, 1982; 1985; Skelton et al., 1987). This group measured the peak-to-peak pop-spike amplitude across a number of stimulation intensities, plotted the I/O curve based on this measure, and then calculated the area beneath this curve.

A1.1.4 Latencies of EP events measures:

Figure 16G displays the four latency of EP event measures. These measures simply denote the occurrence of specific EP events as latencies from the onset of the stimulus artifact. These events include the onset of the EPSP, the onset of the pop-spike (or the offset of the EPSP), the peak of the pop-spike, and the offset of the pop-spike.

The lattermost measure is defined by the point where the tangent line from the pop-spike onset intersects with the rising portion of the pop-spike occurring after the peak.

Figure 16H displays the synchrony of the pop-spike, and is calculated as time from the pop-spike onset to the pop-spike offset.

Bliss and Lomo (1973) were the first to mention that the onset latency of the pop-spike decreases, or occurs sooner, after the induction of LTP, but attached no significant meaning to this change. McNaughton and Barnes and their colleagues have also recently employed latency measures (Sharp, McNaughton, and Barnes, 1989; Green, McNaughton and Barnes, 1990). Additionally, Green et al. (1990) have employed a pop-spike threshold measure, defined as the relative amplitude of the EPSP slope at the time of pop-spike onset.

A1.1.5 Input/Output Relations Curves:

Integral to the field of LTP and related phenomena has been the use of input/ouput (I/O) relations or I/O curves. This method, as it applies to LTP, typically involves systematically changing the intensity of the test pulse over a range of values and recording the subsequent set of EPSP slopes, and where applicable, recording the pop-spike. The I/O curve allows for a more comprehensive examination of changes after experimental manipulations.

I/O curves also provide valuable start and endpoints for normalizing the highly heterogenous data that can result from slight variations in electrode placement. The typical starting points for I/O curves are those intensities that produce threshold values of either the EPSP slope or the pop-spike, such that intensity levels below the thresholds

do not produce these events.

The logical endpoints for I/O curves are those intensities that evoke the maximum values of the EPSP and/or pop-spike, such that intensities above this maximum evoke no change in these events or evoke values that are less than the maximum. However, explicit examinations of what constitutes a maximum I/O value have not been carried out, nor have values beyond any set maximum been substantially explored. Therefore one of the following analyses examined this question, recording AEPs generated by extremely high stimulation intensities.

A1.1.6 Hippocampal Slow wave Activity Patterns and EPs:

As discussed in chapter 1, both the endogenous slow wave activity patterns and EPs of the hippocampus vary with ongoing behavior. There are two common slow wave activity patterns endogenous to the hippocampus. Rhythmical slow activity (RSA) or theta, appears as an approximately sinusoidal wave form of regular amplitude with a frequency range of 6-12 Hz, while large amplitude irregular activity (LIA) is more variable in its amplitude and frequency and is sometimes accompanied by sharp waves that are 50-100 ms in duration and 2-5 times the amplitude of the background slow activity. In waking rats RSA has been associated with behaviors such as walking, running, rearing, head movements, and shifts in posture, which have been categorized as Type I or voluntary behaviors, while LIA has been associated with such behaviors as immobility, licking, tremor, and face washing, which have been categoried as Type II or automatic behaviors (Vanderwolf, Kramis, Gillespie, and Bland, 1975). These hippocampal ECG patterns, their underlying pharmacology, and the cellular mechanisms

that generate them have been the subject of numerous reviews (Bland and Colom, 1993; Buzsaki, Leung, and Vanderwolf, 1983; Stewart and Fox, 1990; Stumpf, 1965; Vanderwolf, 1988).

Hippocampal EPs, if produced by stimulating and recording from the standard afferent flow of the trisynaptic circuit are larger when LIA is present during Type II behaviors such as immobility, and smaller when RSA is present during Type I behaviors, such as walking (Leung, 1980; Buzsaki et al., 1981; Racine and Hafner, 1983; Brankack and Buzsaki, 1986; Green et al., 1990; Hargreaves et al., 1990). However, McNaughton and Barnes and their colleagues have previously reported no differences in the amplitude of EP measures recorded exploratory or Type I behaviors and immobility and grooming or Type II behaviors (Sharp et al., 1985; 1987). Their behavioral definitions were not very rigourous, and have been criticised as such (Hargreaves, et al., 1990). It is probable that the EPs in the studies that found no differences between behaviors were generated by fairly low stimulation intensities, while the EPs in the studies that did find differences between behaviors were generated by higher stimulation intensities. Thus, these differences may change across the I/O curve, such that at the higher intensities the differences between behavioral states may be more apparent. Such an examination took place in the analyses here, with a number of I/O curves being recorded under behavioral conditions of movement and immobility.

A1.1.7 Variability of EP Measures:

Traditionally, individual EPs have been averaged into a single EP, and the measures then derived from this AEP. More recently, a number of researchers have

taken advantage of microcomputers and associated software packages that allow them to record and store a great many individual EPs, from which the measures can then be derived (Cain et al., 1993; Green et al., 1990; Hargreaves and Cain, 1991; Moser et al., 1993; Sharp et al., 1989). By recording individual EPs in this manner the variance of the derived measures can be evaluated under different recording conditions providing an additional dimension of information. As a result several of the following analyses examined the variability of individual EP measures across the I/O curve, and between the behavioral recording conditions of immobility and movement.

Further, since it is not known how much average measures derived from individual EPs differ from individual measures derived from AEPs, one of the following analyses examined this question across the I/O curve. This becomes a relatively important question, given that there are latency shifts among individual EPs, such that the onsets and offsets of the EP components may not coincide.

A1.1.8 Rationale of Experiments and Analyses:

Thus the purpose of this set of experiments and analyses was fourfold. First, to examine the relatedness of the different measures discussed above, across the I/O curve. Second, to describe the EP measures beyond standard maximum I/O curve stimulation intensities. Third, to compare EP measures derived from AEPs and average EP measures derived from individual sweeps. Fourth and finally, to examine the variability of the EP measures across the I/O curve and between Type I and Type II behaviors.

A1.2.1 Subjects:

20 Male hooded Long-Evans were used as subjects in these experiments and

analyses. The rats were implanted with stimulating and recording electrodes in the perforant-path and dentate-gyrus respectively, using the procedures described in chapter 2. At the time of surgery the rats weighed between 280-480 g and were approximately 2-3 months old.

A1.2.2 Apparatus:

The recording setup was the same as described in chapter 2. Several Plexiglas boxes (approximately 30.5' cm), with a layer of bedding chips on the floor were used as recording chambers, one of which had an attached 35 cm running wheel. This latter chamber has been previously described fully in Hargreaves et al. (1990).

A1.2.3 Procedures:

After the rats had fully recovered from the surgical procedures, and had been tested for the appropriate EP configuration and stability, they were run in baseline I/O curve procedures. EPs were recorded from each rat at a minimum of 10 stimulation intensity levels, with the maximum number of stimulation levels being 16. The lowest stimulation intensity level recorded for each rat was 5-10 μa above the pop-spike threshold. All rats had a pop-spike threshold of 100 μa or less (mean: 38.75 $\pm$4.65). The lowest stimulation intensity used was 20 μa and the highest was 2000 μa. All rats were recorded at a test pulse frequency of .1 Hz or less. The test pulse recording frequency was sometimes less than .1 Hz, due to the technique of behavioral clamping, in which the experimenter would wait for the rat to exhibit the appropriate behavior. Potentials were either recorded during behavioral immobility, in which the rats were still, with their eyes open and heads held up against gravity, or during movement (walking/running in

the running wheel). EPs that did not meet the appropriate behavioral criteria were discarded from the analysis.

Typically each rat underwent only one recording procedure. However, a number of rats underwent a second I/O curve using monopolar stimulation as a control procedure (n=7). Most of the I/O curve recording procedures entailed collecting 10 sweeps per intensity level, during behavioral immobility, of which 6 curves were recorded as AEPs and 7 curves were recorded as individual EPs (although AEPs could also be generated from these latter 7 I/O curves). The remaining I/O curves (n=7) recorded 10 individual sweeps during each of behavioral immobility and walking/running, per intensity level. EPs for each of these behaviors were collected in a pseudo-random fashion, depending upon the ongoing behavior of the rat, although ultimately, some of the walking/running and immobility were induced, by a light tapping of the glass (for immobility) or by manually turning the running wheel (for walking/running).

A1.3.1 I/O Curve Description:

Figure 17 graphically displays the AEPs and EP measures in a single rat's I/O curve. Panel A of Figure 17 displays the AEPs, while panels B-D respectively depict the mean measure of the EPSP slope, the four latency of event measures, and the peak-to-peak measure of the pop-spike amplitude. The AEP recorded at the lowest intensity has a dual pop-spike evoked by the medial and lateral components of the perforant-path.

Figure 18 plots the values derived from the AEPs in Figure 17A against the stimulation intensity. Figure 18A displays the EPSP slope measures, while the middle and bottom panels (18B, 18C) respectively display the pop-spike and latency of event

Figure 17. The AEPs and graphic placement of selected EP measures that contribute to the construction of an I/O curve for an individual rat. A number of the AEPs elicited by stimuli of varying intensity (1-12) and the measures derived from them are shown. Panel A displays the AEPs that make up the I/O curve. The AEPs are based on 10 sweeps collected during behavioral immobility at a frequency of approximately .1 Hz or less. Horizontal calibration bar is 2 ms, and vertical calibration bar is 5 mV. Note the dual pop-spike at the lowest stimulation intensity, evoked by the medial and lateral divisions of the perforant-path (marked by **M** and **L**). Also note the secondary discharge occurring after the offset of the pop-spike at the high intensities. Panel B exhibits the mean EPSP slope measure as it changes across the I/O curve. Panel C exhibits the 4 latency of EP event measures across the I/O curve. These measures are labelled as **L1-L4**, indicating the onset of the EPSP, the onset of the pop-spike, the peak of the pop-spike and the offset of the pop-spike, respectively. Panel D exhibits the peak-to-peak pop-spike amplitude measure across the I/O curve. All four panels are on identical ordinate and abscissa scales. Generally as can be seen the measures shift right to left with increasing stimulation intensities.

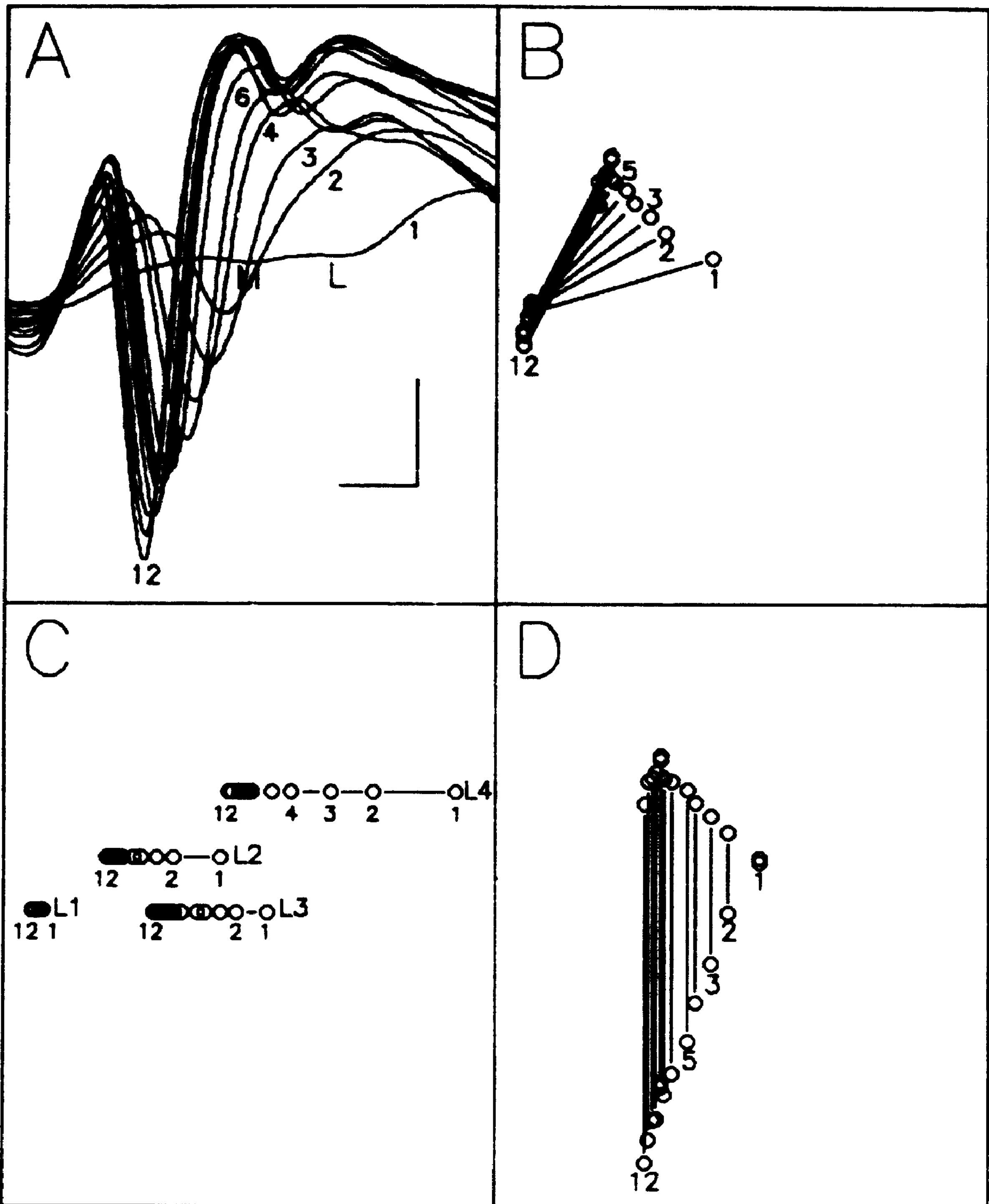
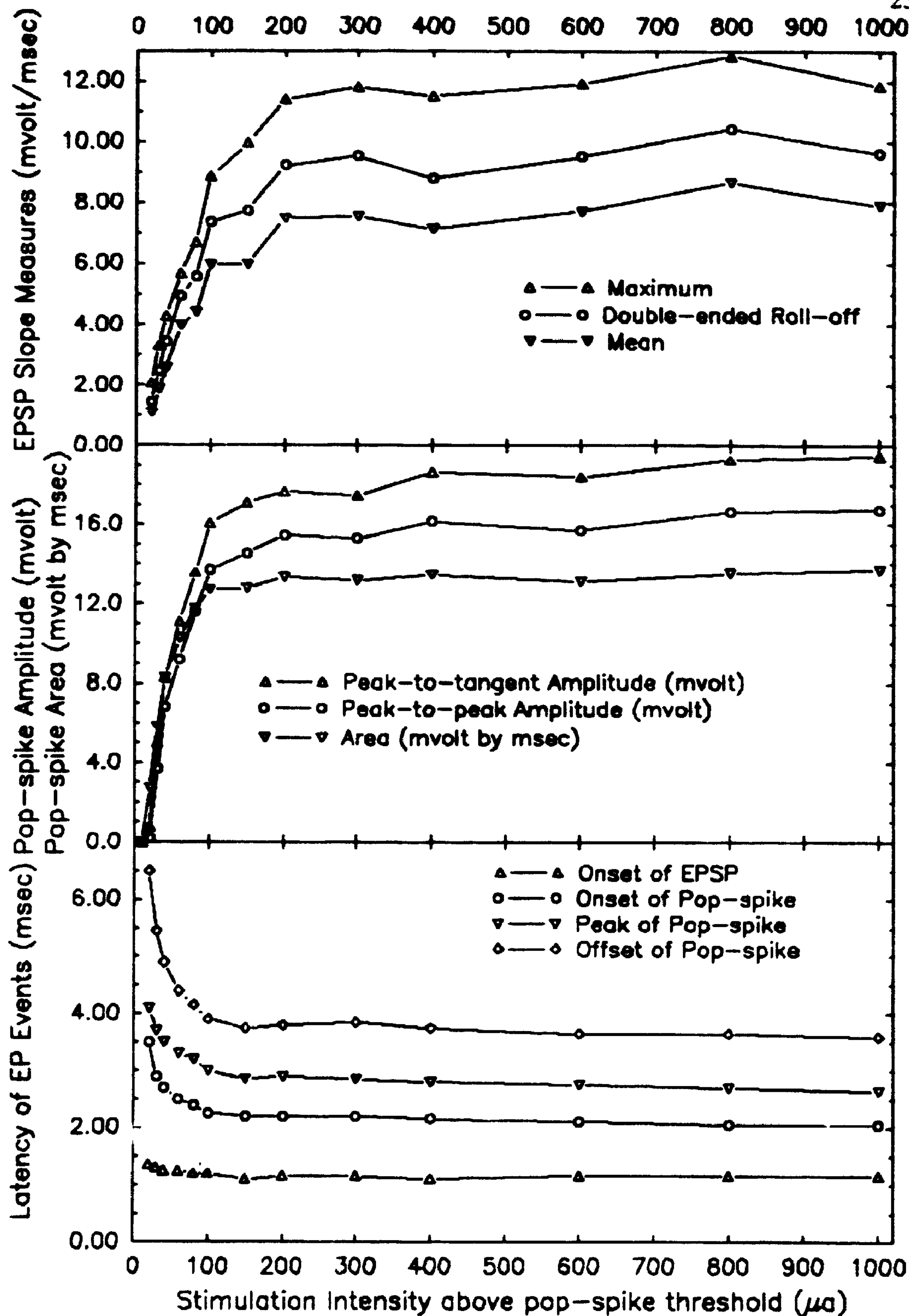

A
6
4
3
2
1
M
L
12
B
5
3
2
1
12
C
12
4
3
2
1
L4
12
2
1
L2
L1
12 1
12
2
1
L3
D
1
2
3
5
12

Figure 18. I/O curves for 10 of the possible 11 EP measures, constructed from the data in Figure 6. The top panel displays the EPSP slope measures, while the middle and bottom panels, respectively, display the pop-spike and latency of event measures. EPSP slope measures are displayed in rise over run or mV/ms units. Pop-spike amplitude measures are displayed in mV units or mV x ms units for the area of the pop-spike. Latency of EP event measures are displayed in ms units, measured from the onset of the stimulus artifact. Abscissa scale is stimulation intensity from 0 to 1000 μa and is unadjusted.


0 100 200 300 400 500 600 700 800 900 1000
EPSP Slope Measures (mvolt/msec)
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Maximum
Double-ended Roll-off
Mean
Pop-spike Amplitude (mvolt)
Pop-spike Area (mvolt by msec)
16.0
12.0
8.0
4.0
0.0
Peak-to-tangent Amplitude (mvolt)
Peak-to-peak Amplitude (mvolt)
Area (mvolt by msec)
Latency of EP Events (msec)
6.00
4.00
2.00
0.00
Onset of EPSP
Onset of Pop-spike
Peak of Pop-spike
Offset of Pop-spike
0 100 200 300 400 500 600 700 800 900 1000
Stimulation Intensity above pop-spike threshold (μa)

measures. This figure further indicates the individual measures, relations to each other within the variable clusters of EPSP slope, pop-spike, and latency of EP event measures. As can be seen in Figure 18A the maximum slope has the greatest EPSP values followed by the double-ended roll-off, and mean slope. Similarly, the peak-to-tangent measure has greater values than the peak-to-peak measure of the pop-spike amplitude. Further the area of the pop-spike appears to flatten out more than the other pop-spike measures. The latency of event measures exhibit proportionately greater change, the further away in time they are from the stimulus artifact. Additionally, it appears that these measures, and therefore the intervals between them remain fairly constant during the latter portion of the I/O curve.

A1.3.2 Results of the Examination of the Extended I/O Curves:

Figures 19 and 20 display the I/O curves recorded from 5 rats, whose maximal stimulation intensities were no less than 1500μa. The EP data were transformed as proportions of the maximum values, and the stimulation intensities were zeroed to the pop-spike threshold. Both the pop-spike measures and the latency of EP event measures exhibit a fair degree of stability even at stimulation intensities as high as 1500μa. The EPSP slope measures however, exhibit a marked instability that does not show any consistent trend either up or down at these extreme intensities. Two of the 5 animals' data presented here were further recorded at a stimulation intensity of 2000μa. The data from this stimulation intensity level were consistent with the data recorded at the lower intensity of 1500μa. No differences between the peak-to-tangent and peak-to-peak pop-spike amplitudes were observed. Similarly, no differences among the EPSP slope

Figure 19. Extended I/O curves for selected EP measures. Data are from 5 rats recorded at stimulation intensities of 1500 μa or higher. Recorded values of the EP measures have been converted to percentages of the maximal values for each measure. The abscissa scale of stimulation intensity is adjusted such that the pop-spike threshold is equal to zero stimulation intensity. The top panel exhibits the peak-to-tangent pop-spike amplitude, while the middle and bottom panels exhibit the area of the pop-spike and double-ended roll-off EPSP slope measures, respectively.

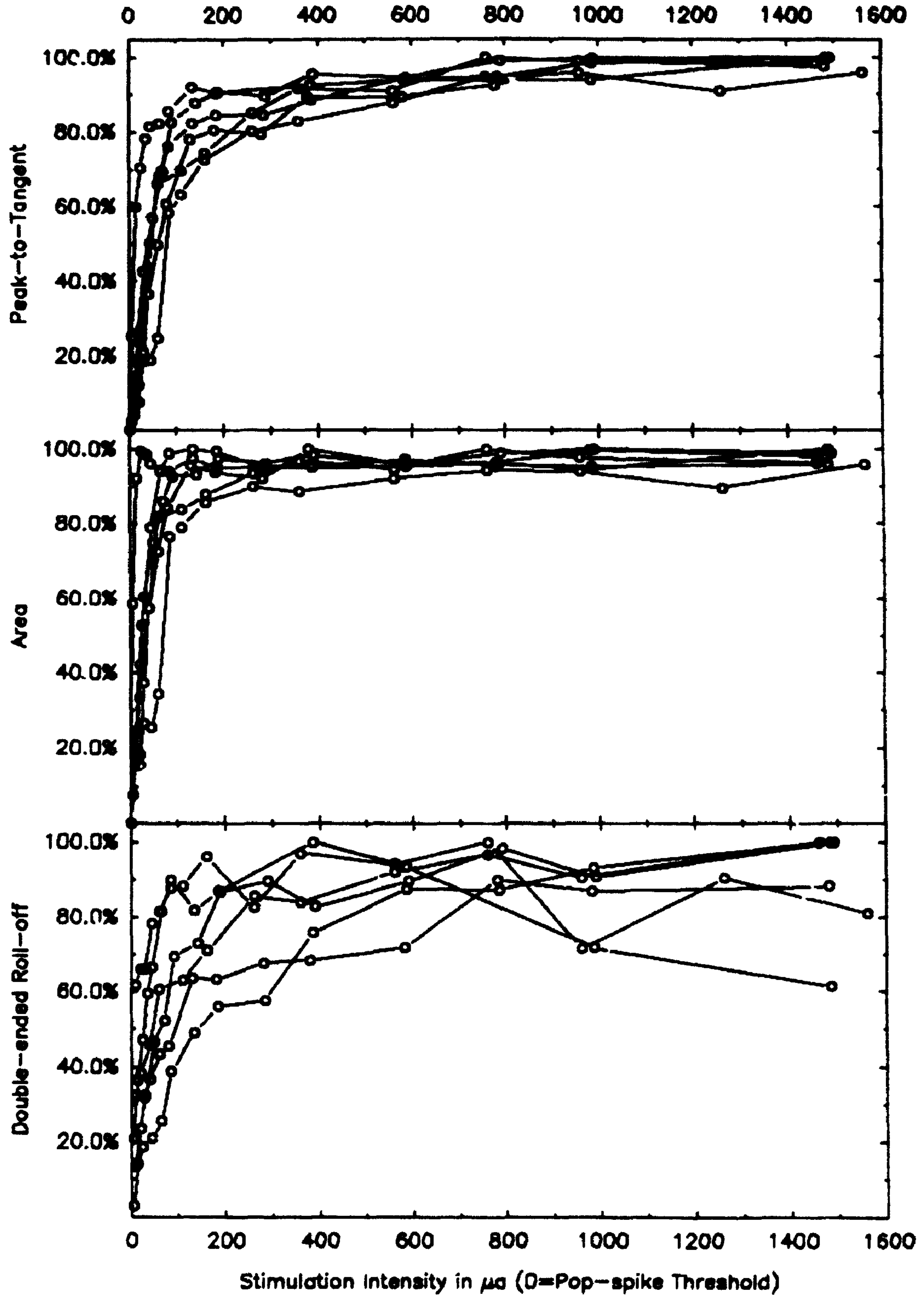
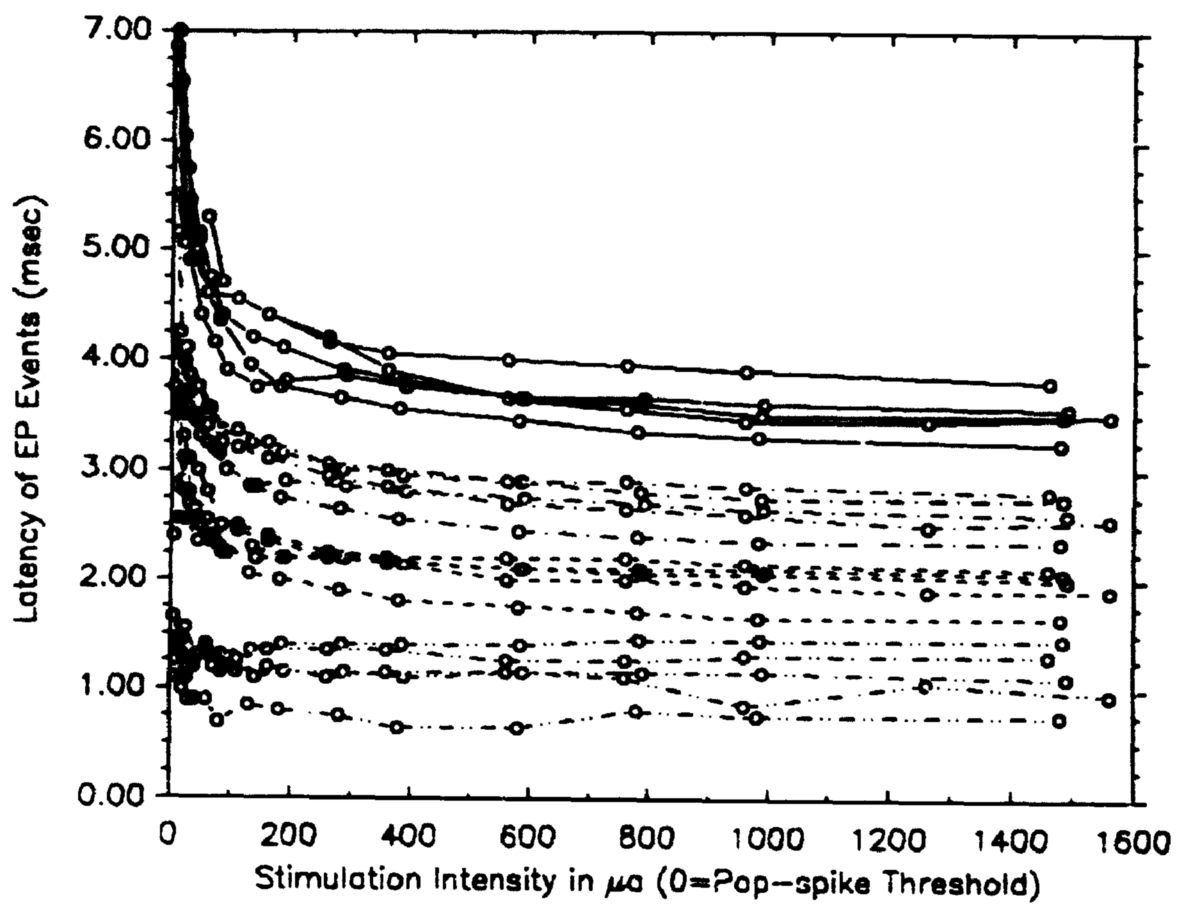

0
200
400
600
800
1000
1200
1400
1600
100.0%
80.0%
60.0%
40.0%
20.0%
Peak-to-Tangent
Area
Double-ended Roll-off
Stimulation Intensity in μa (0=Pop-spike Threshold)

Figure 20. Extended I/O curves for latency of EP event measures. Data from these 5 rats were recorded at stimulation intensities of 1500 μa or higher. Recorded values of the EP measures are in the original ms unit values. Latencies represent time from onset of the stimulus artifact. The abscissa scale of stimulation intensity is adjusted such that the pop-spike threshold is equal to zero stimulation intensity. The solid lines represent the offset of the pop-spike, the dash-dot-dashed lines represent the peak of the pop-spike, the dashed lines represent the onset of the pop-spike, and the dash-dot-dot-dashed lines represent the onset of the EPSP.


7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Latency of EP Events (msec)
0
200
400
600
800
1000
1200
1400
1600
Stimulation Intensity in μa (0=Pop-spike Threshold)

measures were observed. Observations made during the description of the I/O curve also held true here.

A1.3.3 Results of EP Measure Inter-correlations Across the I/O Curve:

I/O curves constructed from EP measures derived from the AEPs of all 20 rats were used in this analysis. Data in this analysis were untransformed. Correlation matrices for all the EP variables and the zeroed stimulation intensities were constructed for each of the 20 animals. These correlation matrices were then averaged across all animals and 99% confidence intervals calculated for each average correlation.

Results of this analysis are presented in Table XVIII. The average number of stimulation intensity levels that went into making the I/O curves was 12.3 $\pm$.417(sem). Generally, as can be seen from the table, the EP measures correlated well with each other, and the reliability of the coefficients was high, as indicated by the small 99% confidence bounds. The two exceptions to this were the onset of the EPSP slope and synchrony of the pop-spike latency measures. It is also worth noting that generally the intra-class correlations, were higher than the inter-class correlations, such that the EPSP slope measures related better among themselves than to the pop-spike measures. Further analyses of these relations did reveal non-linearities among the inter-class measures at the high-end of the I/O curves (analyses not shown).

A1.3.4 Results of Correlations Relating AEP Measures to the Average of Individual EP Measures:

Data for this analysis were derived from 7 rats that had the I/O curves recorded as individual sweeps during immobility. The AEPs and individual EPs were analysed

Table XVIII EP measure average correlation matrix (n=20) with 99% confidence intervals from the full I/O curves. The mean number of stimulation levels making up the I/O curves was 12.3 ±.417. The confidence intervals are based on a two-tailed t-distribution. The letters B through K represent the individual EP measures. EPSP Slope measures: **A** maximum, **B** double-ended roll-off, **C** mean, Pop-spike measures: **D** peak-to-tangent amplitude, **E** peak-to-peak amplitude, **F** area, Latency of EP event measures: **G** onset of the EPSP slope, **H** onset of the pop-spike, **I** peak of the pop-spike, **J** offset of the pop-spike **K** synchrony of the pop-spike. The within cluster correlations are higher than the between cluster correlations.

EPSP slope (A-C)			Pop-spike (D-F)			Latency of EP events (G-J)				
A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	
.979										**B**
.023										
.979	.995									**C**
.018	.005									
.914	.896	.902								**D**
.079	.079	.069								
.912	.894	.900	.999							**E**
.082	.079	.071	.001							
.879	.861	.866	.977	.975						**F**
.095	.089	.083	.021	.020						
-.579	-.496	-.495	-.561	-.557	-.570					**G**
.194	.195	.195	.213	.212	.218					
-.882	-.872	-.880	-.954	-.948	-.919	.582				**H**
.101	.092	.084	.034	.037	.079	.227				
-.886	-.873	-.879	-.942	-.939	-.900	.564	.969			**I**
.076	.073	.064	.043	.041	.063	.213	.030			
-.889	-.876	-.881	-.963	-.959	-.939	.595	.962	.961		**J**
.070	.071	.058	.019	.020	.028	.205	.023	.028		
-.678	-.669	-.666	-.738	-.738	-.706	.486	.698	.729	.819	**K**
.252	.256	.253	.280	.276	.287	.195	.278	.245	.223	

during separate sessions for all of the 11 EP measures. The individual EP measures for each animal at each intensity were then converted to average measures. This resulted in 2 identical I/O curve datasets for each animal, one based on EP measures derived from the AEPs, and one based on average EP measures derived from the individual EPs. Correlations for the matched variables in these two sets of data were then calculated for each rat's I/O curve. The subsequent correlation matrices were then averaged across the 7 animals and 95% confidence intervals, calculated for each average correlation, based on a two-tailed t-distribution. The results of this analysis are displayed in Table XIX. As can be seen, all the average correlations exceed .90, except the latency measure of the onset of the EPSP. One of the likely explanations for this finding is that this is the only measure manually set by the exerimenter. Although only the initial search onset is set manually, it is possible that the search criterion of .02 mV as the incline threshold of the EPSP onset may have been too stringent and therefore the inital search onset, placed by th_ experimenter and the automatic search criterion for the beginning of the EPSP are in the majority of cases identical.

A1.3.5 Results of the Variance of the EP Measures Recorded Under Behavioral Immobility and Movement Across the I/O curve:

Data for this analysis were derived from the 7 rats that had the I/O curves recorded as individual sweeps during both immobility and movement. The individual EP measures for each animal at each intensity were converted to average measures collapsed across the behavioral recording condition, such that these measures were based on data collected during immobility and movement averaged together. The maximum

Table XIX Correlations of the EP measures derived from AEPs versus average EP measures derived from individual EPs. Data was recorded from 7 rats during behavioral immobility. The number of cases was based on 92 stimulation intensity levels arrived at by summing across the intensity levels for each rat's I/O curve. Significance of correlations was evaluated using a one-tailed distribution.

Variable	r-value	Significance level
EPSP Slope Meaures		
Maximum	r= .998;	$p < .005$
Double-ended Roll-off	r= .973;	$p < .005$
Mean	r= .972	$p < .005$
Pop-spike Measures		
Peak-to-tangent	r= .998;	$p < .005$
Peak-to-peak	r= .997;	$p < .005$
Area	r= .998;	$p < .005$
Latency of Event Measures		
EPSP Onset	r= .424;	$p < .005$
Pop-spike Onset	r= .993;	$p < .005$
Pop-spike Peak	r= .994;	$p < .005$
Pop-spike Offset	r= .991;	$p < .005$
Synchrony	r= .938;	$p < .005$

values of each variable for each rat's I/O curve were selected as the basis to generate proportional scores for both the individual EP scores and the EP score averages. The proportional EP score averages were then subtracted from the appropriate individual proportional scores, thus producing proportional deviation scores of the 11 EP measures. These data were then matched for the approximate stimulation intensity level at which they were recorded and collapsed across all 7 rats, for each of the two behavioral recording conditions.

The EPSP slope measures all increased in their variability, as the stimulation intensity increases. The variance of the pop-spike measures displayed overall decrease, but only after an initial increase from the 1st to 2nd intensity levels. The latency of EP event measures are similar in their results to the pop-spike measures in that they display an overall trend towards decreasing variance across the I/O curve, but they differ in that the greatest variance in the latency measures occurs at the lowest recorded stimulation intensity. The latency measures further appeared to show no change in their variance over the last few stimulation intensities recorded at the high end of the I/O curves. The exception to this pattern was the onset of the EPSP latency measure, which exhibited no consistent pattern of variance change across the I/O curve.

I/O curves from the 7 rats, whose data were used to compare the AEP measures to the average measures from individual EPs (see above) were analysed in an identical fashion. The resulting patterns of variance change across the I/O curves for the different EP measures were identical in both analyses. Further, a subset of rats had second I/O curves recorded, using monopolar stimulation. Results from this analysis also exhibited

similar patterns of variance change across the I/O curve. These data were tested statistically by Cochran's C statistic (Max variance/Summed variance), for homogeneity of variance. Results indicated that all 11 EP measures were statistically non-homogeneous.

A number of the EP measures exhibited differences between the behavioral recording conditions. Additionally, the EPSP slope measures and some of the latency of EP event measures indicated that these differences changed across the I/O curves. It was not plausible to analyse this dataset with the appropriate mixed between/within subjects design. Consequently, the transformed data were analysed by a weaker fully between design, with individual rats, behavioral recording conditions, and stimulation intensities as factors. The results of this analysis indicated an overall effect of behavioral recording condition, indicating that EP measures were significantly lower when recorded during movement than when recorded during immobility [$F_{(10,1371)}=2.23$; $p=.013$]. The univariate tests however, revealed that this held for the EPSP slope and latency of event measures, but not for all of the pop-spike measures (Table XX). The interaction between the behavioral recording condition and the stimulation intensity was statistically significant, indicating that the observed difference in behavioral recording condition changed over the I/O curve. The univariate tests of this interaction again indicated that it applied to the EPSP slope measures and a few of the latency of event measures, but not to the pop-spike measures (Table XXI).

It was further observed that the variance in the EP measures recorded during movement appeared less than the variance recorded during immobility. This was tested

Table XX Main effect of behavioral recording condition of the variability analysis of the EP measures across the I/O curve during immobility and movement. The omnibus main effect of behavioral recording condition is reported below followed by the individual univariate F-ratios for the individual EP measures.

Variable	F-ratio	Significance level
Omnibus Effect of Behavior	$F_{(11,1370)} = 67.68$;	$p < .0005$
Univariate Effects		
Maximum	$F_{(1,1380)} = 506.26$;	$p < .0005$
Double-ended Roll-off	$F_{(1,1380)} = 526.96$;	$p < .0005$
Mean	$F_{(1,1380)} = 418.64$;	$p < .0005$
Peak-to-tangent	$F_{(1,1380)} = 1.13$;	$p = .288$
Peak-to-peak	$F_{(1,1380)} = 3.22$;	$p = .073$
Area	$F_{(1,1380)} = 5.57$;	$p = .018$
EPSP Onset	$F_{(1,1380)} = 4.56$;	$p = .033$
Pop-spike Onset	$F_{(1,1380)} = 16.82$;	$p < .0005$
Pop-spike Peak	$F_{(1,1380)} = 19.18$;	$p < .0005$
Pop-spike Offset	$F_{(1,1380)} = 98.24$;	$p < .0005$
Synchrony	$F_{(1,1380)} = 70.31$;	$p < .0005$

Table XXI Interaction of stimulation intensity and behavioral recording condition of the variability analysis of the EP measures across the I/O curve during immobility and movement. The omnibus interaction between stimulation intensity level and behavioral recording condition is reported below followed by the individual univariate F-ratios for the individual EP measures.

Variable	F-ratio	Significance level
Omnibus Interaction	$F_{(99,12802)} = 3.46$;	$p < .0005$
Univariate Effects		
Maximum	$F_{(9,1380)} = 5.84$;	$p < .0005$
Double-ended Roll-off	$F_{(9,1380)} = 6.50$;	$p < .0005$
Mean	$F_{(9,1380)} = 5.71$;	$p < .0005$
Peak-to-tangent	$F_{(9,1380)} = .90$;	$p = .520$
Peak-to-peak	$F_{(9,1380)} = .83$;	$p = .587$
Area	$F_{(9,1380)} = 1.66$;	$p = .095$
EPSP Onset	$F_{(9,1380)} = 1.69$;	$p = .033$
Pop-spike Onset	$F_{(9,1380)} = .35$;	$p = .958$
Pop-spike Peak	$F_{(9,1380)} = 5.20$;	$p < .0005$
Pop-spike Offset	$F_{(9,1380)} = 15.58$;	$p < .0005$
Synchrony	$F_{(9,1380)} = 16.32$;	$p < .0005$

statistically by comparing the variances recorded during immobility to the variances recorded during movement using three paired t-tests, one for each EP measure variable cluster. Results from this analysis indicated that the variance of the EP measures recorded during movement was less than that recorded during immobility for the EPSP slope measures [$t_{(29)}=5.62$; $p<.0005$], the pop-spike measures [$t_{(29)}=2.62$; $p=.014$], and for the latency of EP event measures [$t_{(49)}=3.29$; $p=.002$].

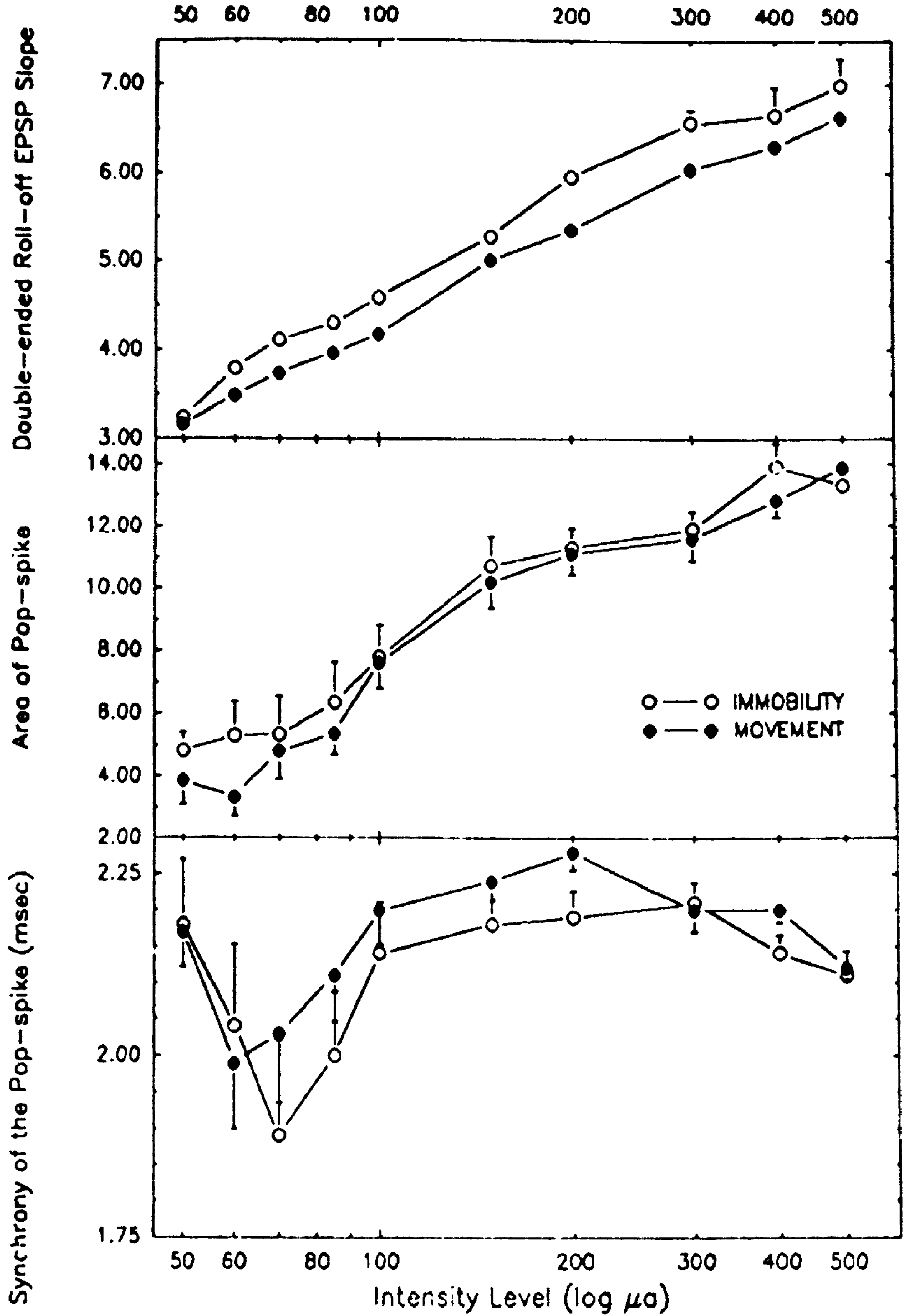
Finally, selected measures using the untransformed data from a single rat's I/O curve are presented in Figure 21, thus indicating the magnitude of these differences in real world units.

A1.4.1 Discussion of the EPSP Slope Measures:

In comparing the maximum and mean EPSP slope measures, data from the maximum slope appear more stable, or less sensitive to fluctuations in the EP recording conditions. This is not surprising given the number of factors that can influence both the onset of the EPSP and the offset of the EPSP (onset of the pop-spike), which are integral to the definition of the mean slope, but which do not necessarily affect the maximum slope.

The double-ended roll-off EPSP slope measure has advantages over both the maximum and mean slope measures. The EPSP slope itself fluctuates in its duration, depending upon the stimulation intensity; at low stimulation intensity levels the EPSP slope has a longer duration than at high stimulation intensity levels. The maximum slope measure is fixed in its duration, and therefore does not accommodate the expansion and contraction of the EPSP duration. The mean slope measure avoids this problem by being

Figure 21. Means and standard errors of the mean of the untransformed data recorded during immobility and movement, from selected EP measures drawn from a single animal. Stimulation intensity that the EPs were recorded at is represented in a log scale along the abscissa.



50
60
80
100
200
300
400
500
Double-ended Roll-off EPSP Slope
7.00
6.00
5.00
4.00
3.00
Area of Pop-spike
14.00
12.00
10.00
8.00
6.00
4.00
2.00
IMMOBILITY
MOVEMENT
Synchrony of the Pop-spike (msec)
2.25
2.00
1.75
Intensity Level (log μa)

bound to the EPSP onset and offset. However, this introduces a contamination brought about by the onset of the pop-spike, particularly at lower intensity levels that are still above the pop-spike threshold. At these stimulation intensity levels the EPSP slope gradually rolls off to the pop-spike onset reflecting an amplitude at this point that is largely influenced by the gradual increase in the number of granule cells firing. Thus, the mean slope measure under these circumstances is possibly an inaccurate estimate of granule cell depolarization.

The double-ended roll-off slope compensates for the fixed duration by proportionately matching the duration of the EPSP slope, as defined by the onset and offset interval. This measure also avoids the influence of the pop-spike onset by rolling back from this point in proportion to the length of the EPSP slope. As a result, the double-ended roll-off slope measure rolls back a greater amount at the lower stimulation intensity levels, and rolls back a lesser amount at higher stimulation intensity levels. Further, by proportionately rolling-off from the onset of the EPSP this measure tends to capture the EPSP slope at a constant rise function, or where it is most linear, as opposed to the rapidly changing rise function, present at the start of the EPSP slope, or where it is curvilinear. As such, the double-ended roll-off measure optimizes the stability/sensitivity trade-off lying between the maximum and mean slope measures on this ratio.

The original EPSP slope measure employed in the pioneering studies of Bliss and Lomo (1973), Bliss and Gardner-Medwin (1973), Douglas and Goddard, (1975), and Douglas (1977) not only detects changes in EPSP slope, but is also susceptible to the

general shifting of the EP along its temporal axis, or change in latency, which may function independently of EPSP slope changes. So too is the measure employed by McNaughton and Barnes and their colleagues. This EPSP slope measure can further be criticized for its fixed duration, as with the maximum slope measure used in the present series of experiments. Robinson (1992) used a similar slope measure to that of McNaughton and Barnes, except that the onset is demarcated by the initial positivity of the EPSP and its duration is 1 ms. Consequently, this measure is not susceptible to temporal shifts in the EP.

Morris and his colleagues have taken a different approach and run a linear regression through the EPSP slope for a .7 msec interval starting at approximately 1.8 msec after the stimulus artifact (Morris et al., 1986; Morris et al., 1989; Davis et al., 1992). This measure also employs a constant duration, but has the distinct advantage of using a regression approach, and therefore may be more accurate at estimating the rise portion of the slope than the other measures that simply use two points to estimate the rise portion. Another advantage of this measure is that a 1.8 msec onset of their slope measure places it on a portion of the EPSP slope where the rise function should be relatively constant. However, since this measure employs a fixed onset latency, it will be susceptible to temporal shifts in the EP, a problem which appears to be common to many of the EPSP slope measures used outside of this thesis.

1.4.2 Discussion of Pop-spike Measures:

The pop-spike measures used in the present series of studies are more congruent with the literature, than the EPSP measures. The tangent line used in the peak-to-tangent

pop-spike amplitude and in the calculation of the pop-spike area attempts to provide an estimate of where the EPSP slope would have been, had the pop-spike not been present. Thus, the peak-to-tangent pop-spike amplitude measure becomes a theoretically truer estimate of the number of cells firing than the peak-to-peak pop-spike amplitude, by dropping the amplitude from the point where the EPSP slope would have been. However, there are difficulties in identifying the offset of the pop-spike, and whether it represents a cessation of granule cell firing, or an active inhibition component (Andersen et al., 1971a; Lomo 1971a). This issue is more fully discussed under the section of EP latency events. Thus, the peak-to-peak pop-spike amplitude measure may be a more concrete measure of the number of cells firing by basing its values on identifiable demarcation points, as opposed to interpolated ones. This advantage is particularly heightened in the presence of secondary pop-spikes triggered by the lateral perforant-path (McNaughton and Barnes, 1977; McNaughton, 1980; Abraham and McNaughton, 1984). In this situation the tangent line drawn is not an accurate estimate of the EPSP slope. However, there are occasions, particularly at the lower stimulation intensities, where the pop-spike is present, but not strong enough to produce a downward deflection of the EP. In these cases, the peak-to-tangent pop-spike amplitude can still assess the pop-spikes, since the markers of onset, peak and offset of the pop-spike can still be detected as changes in the upward deflections of the EPSP slope. However, without a downward deflection of the pop-spike the peak-to-peak measure will record the value as negative or null.

The area of the pop-spike may be a truer estimate of the number of cells fired than either of the other two pop-spike measures employed. It also has the advantage over

the other two measures of being able to adjust to changes in the synchrony of the cells firing, whereas the other two measures may record changes in synchrony as changes in the number of cells firing (Green et al., 1990). As such, when the cells fire more synchronously, as indicated by a smaller width of the pop-spike, the summation of the cells firing at the peak of the pop-spike may be greater, without the overall number of cells that fire changing. In the case of dual pop-spikes being fired by both the medial and lateral perforant-path fibres, this measure still provides an overall estimate of the number of granule cells discharged. However, since it utilizes the tangent line, the area measure has disadvantages similar to those of the peak-to-tangent pop-spike measure, associated with estimating the EPSP slope and the accuracy of the pop-spike offset. As with the peak-to-tangent pop-spike amplitude measure however, the area measure is still able to assess pop-spikes that have peak amplitudes greater than the amplitude of the EP at the pop-spike onset.

A1.4.3 Discussion of the Latency of EP Event Measures:

The latency of event measures demarcate the start points, end points, and peaks of the EPSP slope and pop-spike. The onset of the EPSP slope reflects the first detectable indication that the granule cells are beginning to depolarize.

The onset of the pop-spike or offset of the EPSP indicates the latency at which a noticeable number of granule cells begin to fire. This onset is typically defined as the highest point on the EPSP slope before a downward deflection towards the pop-spike peak begins. However as mentioned before, the pop-spike is sometimes only indicated by a damping of the EPSP slope.

The latency of the peak of the pop-spike reflects the latency at which the modal number of granule cells fire, and is typically defined as the lowest amplitude after the onset of the pop-spike. Yet, as the onset of the pop-spike is not always indicated by a downward deflection of the EP, so too is the peak not always identifiable as the most negative point following the pop-spike onset.

The offset of the pop-spike should reflect the near cessation of granule cell firing. However, it is probable that other inhibitory influences or other cell populations are firing that affect this offset. Thus, it has been suggested that some of the late EP components occurring immediately following the pop-spike offset may be the near synchronous discharge of the CA4 and/or CA3 pyramidal cells (Buzsaki et al., 1983; Racine, personal communication; Winson and Abzug, 1978), or the synchronous activation of granule cells on the more distal blade of the dentate gyrus by fibres of the lateral perforant-path (Corcoran, personal communication), or secondary discharges of the granule cells themselves (Lomo, 1971a; Andersen et al., 1971a). Finally, the pop-spike synchrony represents an estimate of the interval during which the granule cells discharge in response to perforant-path stimulation. The smaller this value, the more synchronous the firing of the granule cells is thought to be.

In the future it would be more profitable to compare the EPSP slope measures most used in the literature with those currently analysed. That this was not done here, has much to do with the lack of good descriptions of EPSP slope measures in the literature. However, with the more recent advent of commercially made software packages working definitions have been easier to obtain. Also as a result of these

software packages the future measures will probably become fewer and therefore, as a result the literature more uniform in its uses of these measures.

Of the phenomenon examined here the intra-class EPSP and pop-spike measures did not differ from each other, and essentially gave the same results across the I/O curve, between the different behavioral recording conditions, and in the pattern of variance change across the I/O curve. However, within each class some measures were slightly more sensitive to changes than others. Yet, for the phenomena examined here, these slight differences in sensitivity did not greatly affect the outcome of any statistical analyses, except for the pop-spike measures, on the differences between behavioral recording coditions. Here, it was found that the pop-spike area was significantly different between the different behavioral recording conditions, whereas the peak-to-peak pop-spike amplitude only exhibited a trend, and the peak-to-tangent pop-spike amplitude exhibited no difference. The intra-class correlations of the latency of EP events were also high, with the exception of the EPSP onset. This may have had to do with the particular way in which this measure was operationally defined, or simply due to its lack of variability across the various recording conditions tested.

McNaughton and Barnes (1977) have reported a correlation of .94 between the latency of the peak amplitude and the width or duration of the EPSP at half its peak amplitude. Neither of these EPSP measures were recorded here, nor were they recorded across the I/O, nor at intensities above which the pop-spike occurred. However, this correlation still provides an estimate of the within variable cluster measure relationships. Similarly [illegible]rnes (1979) reported a correlation exceeding .97 between pop-spike area and

peak-to-peak amplitude. Again, this correlation was between animals and not across a range of stimulation intensities.

A number of differences existed between the different classes of EP measures. First, although there was substantial linear relatedness across the I/O curve, as evidenced by the inter-class correlations (with the exception of the latency of the onset of the EPSP) there were non-linearities at the high-end of the I/O curve. Wilson (1981) and Chavez-Noriega, Bliss, and Lomo (1989) have also observed similar non-linearities in portions of the I/O curve between EPSP slope measures and pop-spike measures. The non-linearities observed by Wilson (1981) and by Chavez-Noriega et al. (1989) were found at both the high end and low end of the I/O curve. The discrepency between the results presented here and those of Wilson (1981) and Chavez-Noriega et al. (1989) is probably due to their use of stimulation intensities below the pop-spike threshold.

Second, the EPSP slope measures increased in their variability across the I/O curve, while both the pop-spike and latency of EP events decreased. McNaughton, Barnes and Andersen (1981) have also observed an increase in variability of the intracellular EPSP measures over an I/O curves that were below pop-spike threshold. Decreases in the variability of other measures across the I/O curve have not been previously identified or discussed.

Third, the EPSP slope measures exhibited strong differences between the behavioral recording conditions of movement and immobility, while pop-spike and latency of event measures either exhibited no differences, or weaker ones. Previous research has either found differences between the two behavioral recording conditions on

the measures examined (Brankack and Buzsaki, 1986; Buzsaki et al., 1981; Hargreaves et al., 1990; Leung, 1980; Racine and Milgram, 1983) or not found differences (Sharp et al., 1985; 1987; Winson and Abzug, 1978) Data indicating that the EPSP slope measures are more sensitive to this difference have not been published previously.

Fourth, and finally, differences between the two behavioral recording conditions became greater across the I/O curve for the EPSP measures, but the differences for the pop-spike and latency of EP event measures, either do not change or diminish.

Thus, the evidence suggests that choice of intra-class measures is not that critical. However, arguments can be made irrespective of the data for which measures are more appropriate, as for the pop-spike measures in the case of pop-spike peaks, which do not deflect downwards, or in case where two pop-spikes are present evoked by both the lateral and medial components of the perforant-path. Although similarities exist between the different components of the EP, differences also exist, and therefore more than a single class of measure should be employed.

A1.4.4 Discussion of the Extended I/O Curves:

Identifiable true maximum values were not readily apparent in any of the EP measures. The display of the 5 animals recorded at intensities higher than 1000 μa in Figure 11 indicated that while some EPSP values declined, others increased. The pop-spike measures and the latency of event measures, continued to change across the full duration of the I/O curve. Even the 2 rats that had EPs recorded at stimulation intensities of 2000 μa continued to show changes from the immediately previous stimulation intensity. It is worth noting however, that the observed changes at the high end of the

I/O curve were miniscule from one stimulation intensity to the next. Of the 11 EP measures none showed a change greater than 5% over the last 500 μa stimulation intensity, with the exception of the EPSP slope measure, which could change in either direction. As such, if the maximum value was arbitrarily determined to fall anywhere within this immediate range of stimulation intensities, the I/O curve would not be greatly affected. Therefore it may be more valuable to indicate a minimal proportional increase in the EP measures which a given stimulation intensity must evoke in order to continue the I/O curve.

A1.4.5 Discussion of Correlations of AEP Measures with Averages of Individual Measures:

Not surprising were the close correlations between the EP measures derived from AEPs and average EP measures derived from individual EPs, as presented in Table II. As indicated earlier, the low correlation for the onset of the EPSP slope, may have to do with its implementation, although the low correlation may also be attributable to this measure's lack of variance. Winson and Abzug (1978) in a similar analysis found that the amplitude of AEPs recorded in the CA1 pyramidal layer were not different from the average amplitude of the individual EPs making up the AEP. However, this comparision was only indicated for one intensity and the number of animals involved was not reported. Thus, measures derived from either AEPs or averaged measures derived from individual EPs are virtually interchangeable with each other, and the concern over EP latency shifts unwarranted.

A1.4.6 Discussion of the Variance of the EP Measures Across the I/O Curve Under the Different Behavioral Recording Conditions:

Differences between the behavioral recording conditions of immobility and movement were found for the EP measures overall, with some of the variable clusters indicating greater differences than others, as already discussed above. In all of the EP measures that did exhibit differences, the EPs recorded during movement had lower values than those recorded during immobility. This finding is in agreement with previous work (Brankack and Buzsaki, 1986; Buzsaki et al., 1981; Hargreaves et al., 1990; Leung, 1980; Racine and Milgram, 1983). Studies that have not found these specific differences between movement and immobility (Sharp et al., 1985; 1987; Winson and Abzug, 1978) may have used different definitions of movement and immobility, an argument which has been suggested before (Hargreaves et al., 1990; Leung, 1980). One of the main rationales for doing this analysis, as discussed in the introduction of this appendix was that the negative findings of Sharp et al. (1985; 1987) may have been due low stimulation intensities. However, given the findings here that certain EP measures show the greatest difference at the lower intensities, this earlier suggestion is probably incorrect.

Winson and Abzug (1978) found that the differences in AEPs between SWS and the remaining behavioral categories increased across the I/O curves. Leung and Vanderwolf (1980) in their examination of the effect of atropine on the CA1 AEP examined the differences between movement and immobility across a few intensities. Although the EPSP slope amplitudes were recorded only below the pop-spike threshold,

their control I/O curve indicates that the difference in the EPSP measure between the two recording conditions, may have increased with the stimulation intensity, while differences observed for the pop-spike amplitude remained constant.

The variance of the EP measures recorded during movement was always less than that recorded during immobility, regardless of the stimulation intensity. This finding should not be surprising given that the variability of the amplitude of the underlying hippocampal ECG patterns of LIA and RSA are also supportive of the data in that there is greater amplitude variation during LIA than there is during RSA.

These studies continue to show the importance of controlling for behavior, when recording EPs from whole, unaesthetized preparations.

A2: LOW FREQUENCY POTENTIATION AS A RESULT OF I/O CURVE RECORDING

A2.1 Low Frequency Potentiation:

LTP is typically assessed by test pulses that are similar to the pulses making up the trains that induce potentiation, differing only in the intensity and frequency used. Consequently, it becomes a question of some importance as to what test pulse frequency to use when studying LTP and/or its related phenomena. Douglas and Goddard (1975), found potentiation effects in the absence of AD, after the delivery of trains at 0.2 Hz, which consisted of 120 pulses, but not after trains of 16 pulses at the same frequency.

Skelton, Miller and Phillips (1985) specifically set out to examine the question of low-frequency potentiation. Rats in this study received a constant ascending input/output train of stimulation from 10 to 400μa, three pulses per intensity, in approximately 10-14 steps, at either 0.2 Hz, 0.1 Hz, or 0.04 Hz. Seven of these trains were delivered over several days, with the results indicating clear potentiation at the 0.2 Hz frequency, no potentiation at the 0.04 Hz frequency and mixed results at the 0.1 Hz frequency. Therefore, it was not clear from the results of Skelton et al. (1985), whether a stimulating frequency of 0.1 Hz had a potentiating effect or not. Further, the intervals between the seven I/O trains were inconsistent in their duration, being either .5 hr or 24 hrs, with the last I/O interval being 24 hrs. However, the initial six I/O curves were not applied in a systematic fashion, which obscured any duration/decay effects (Skelton et al., 1985). Thus, the issue of low-frequency potentiation was deemed to be worth re-examining, since extensive I/O curves formed an integral part of this thesis.

Anecdotal observation and initial pilot work indicated that low-frequency potentiation had a relatively short decay time, and therefore was traced only at a single post potentiation time, approximately 20-25 minutes after I/O curve completion. Thus the phemomenon examined here may fall into Racine and Milgram's (1983) definition of short term potentiation phenomena.

The properties of the low frequency potentiation phenomenon examined here were assumed to be similar to that of LTP. Accordingly, it was predicted that if any potentiation was found it would be stronger at the low intensity than at the high intensity and that it would be better represented in the pop-spike measures than in the EPSP slope measures.

A2.2 Procedures:

Nine rats were implanted with the standard recording arrangement with a bipolar stimulating electrode placed in the perforant-path and a recording electrode placed in the hilus of the dentate gyrus, as outlined in chapter 2.

Upon recovery I/O curves of 8-14 ascending intensity levels were collected, with 10 sweeps at each intensity. The lowest intensity was less than or equal to 100μa, and evoked a pop-spike just above threshold. The maximum intensity evoked a pop-spike that was greater than the immediately previous intensity by less than 5%. and was either 1000μa or 1500μa. Of the 9 I/O curves analysed for this experiment 6 were recorded during behavioral clamping and therefore the inter-pulse frequency approximated .1 Hz with the occasional EP being recorded with an interval longer than 10 seconds. For the remaining 3 I/O curves the inter-pulse interval was fixed at 10 seconds, with each pulse

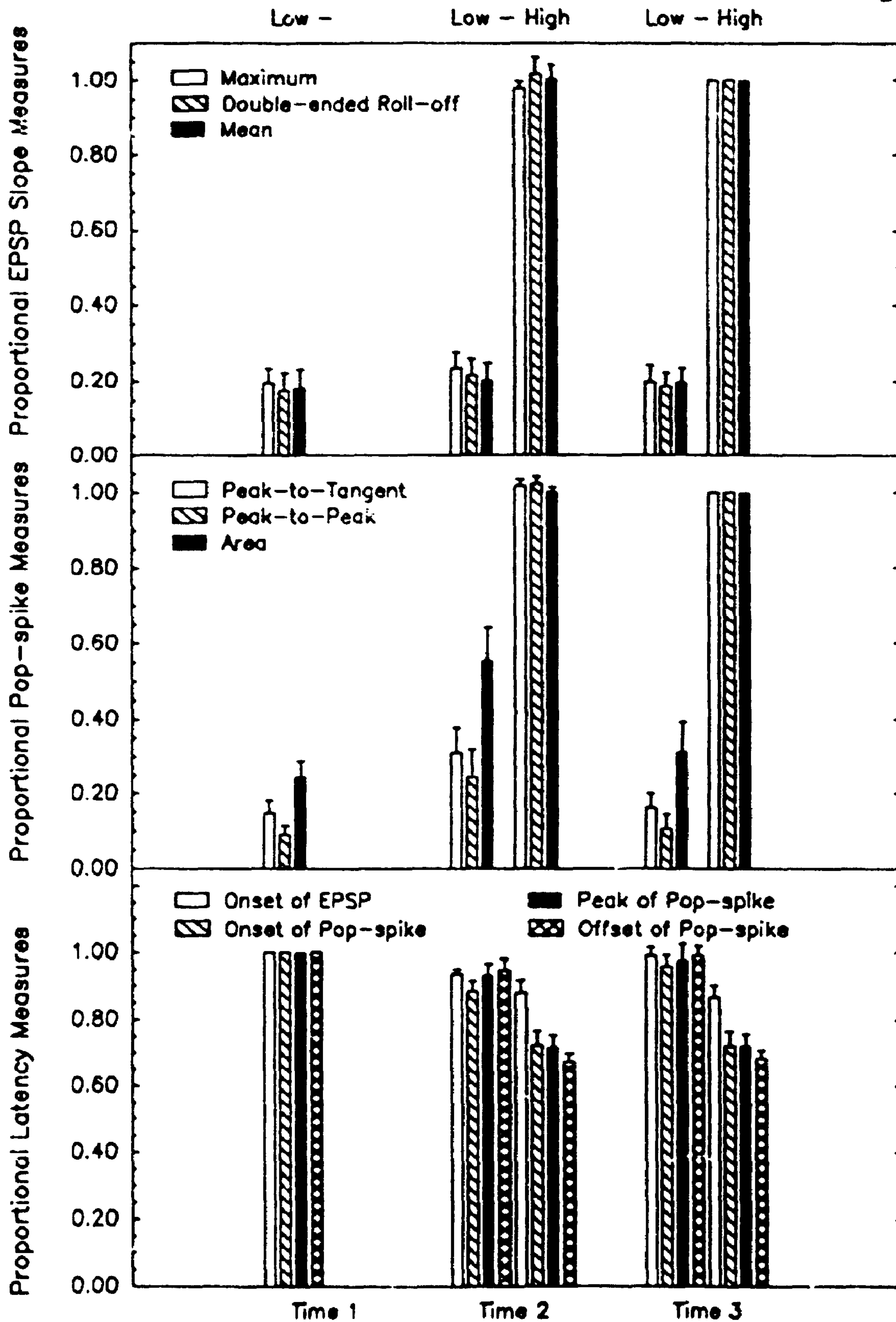
being delivered according to a stopwatch, regardless of the behavior of the rat. Immediately following completion of the I/O curve, an AEP of the lowest recorded intensity was collected again. After a 20 to 25 min interval had lapsed the lowest intensity AEP was recorded for a third time, followed by an AEP recorded at the highest intensity.

The differences between the first low intensity recorded at the begining of the I/O curve and the second low intensity AEP recorded immediately after measured the full impact of the potentiation induced by the low frequency stimulation used in generating the I/O curve. The differences between the third low intensity AEP recorded 20-25 min after the I/O curve and the second low intensity AEP recorded immediately following the I/O curve measured the amount of decay that had occurred during the 20-25 min interval. The differences between the first high intensity AEP recorded at the end of the I/O curve and the second high intensity AEP recorded 20-25 min later, measured the amount of decay that had occurred during the 20-25 min interval.

A2.3 Analyses and Results:

The 11 measures were derived from the AEPs and were analysed using within subjects repeated measures designs and thus the raw form of the data were used. However, for the purposes of display and description the data were transformed into proportions of the values evoked by the maximal intensity recorded at 20-25 min after the I/O curve. The transformation of the latency measures was performed using time 1 of the low intensity, since this would produce the maximal latency values. These data can be seen in Figure 22 and are described below.

Figure 22. Means and standard error of the means, of the 11 AEP measures, at time 1, time 2, and time 3, for both the low and high intensities. Time 1 occurred at the start of the I/O curve, time 2 at the completion of the I/O curve, and time 3 occurred 20-25 minutes after the completion of the I/O curve. Measures are graphically grouped top to bottom into variable clusters representing the EPSP slope, pop-spike, and latency of events. Measures evoked by the low stimulation intensities are located to the left of the time demarcations, and measures evoked by the high intensities are located to the right of the time demarcations. Data are proportions based on values from the the high intensity recorded at time 3, or the low intensity recorded at time 1 for the latency of event measures.


Low -
Low - High
Low - High
Proportional EPSP Slope Measures
Maximum
Double-ended Roll-off
Mean
1.00
0.80
0.60
0.40
0.20
0.00
Proportional Pop-spike Measures
Peak-to-Tangent
Peak-to-Peak
Area
1.00
0.80
0.60
0.40
0.20
0.00
Proportional Latency Measures
Onset of EPSP
Onset of Pop-spike
Peak of Pop-spike
Offset of Pop-spike
1.00
0.80
0.60
0.40
0.20
0.00
Time 1
Time 2
Time 3

The low intensity data were analysed across the 3 recording times, at the begining, end, and 20-25 min after the I/O curve, using a series of repeated measures designs for each of the AEP variable clusters of the EPSP slope, the pop-spike, and the latency of EP event measures. These results indicated differences over time for both the EPSP slope [$F_{(6,30)}$=4.46; p=.002] and pop-spike [$F_{(6,30)}$=3.18; p=.016] measures, but not for the latency measures.

The high intensity data were analysed across the 2 recording times, at the end, and 20-25 min after the I/O curve, using a series of repeated measures designs for each of the AEP variable clusters of the EPSP slope, the pop-spike, and the latency of EP event measures. These results indicated no differences between these recording times for any of the AEP variable clusters.

All further pairwise comparisons of interest were accomplished by paired t-tests, evaluated using a one-tailed probability distribution. These comparisons indicated that the low intensity contrasts comparing the AEP recorded at the begining of the I/O curve to the AEP recorded immediately following the I/O curve, the maximum and double-ended roll-off EPSP slope measures, all of the pop-spike measures, and all of the latency measures with the exception of the offset of the pop-spike displayed significant LTP-like shifts (Table XXII). Of the low intensity contrasts comparing the AEP recorded immediately after the I/O curve to 20-25 min after the I/O curve, only the double-ended roll-off slope measure, all of the pop-spike measures, and only the onset of the pop-spike latency measure indicated significant decay from time 2 (Table XXIII).

These results are shown in Figure 22, where the EPSP slope at the low intensity

Table XXII Analyses of the AEP data from the low frequency potentiation experiment. Pairwise comparisons of the low intensity potentiation data (time 1 to time 2) were made using paired t-tests, and evaluated with a one-tailed distribution. The comparisions were performed on the individual EP measures.

Variable	t-value	Significance level
EPSP slope measures		
Maximum	$t_{(8)}$ =-2.00;	p=.041
Double-ended Roll-off	$t_{(8)}$ =-1.93;	p=.045
Mean	$t_{(8)}$ =-1.30;	p=.115
Pop-spike measures		
Peak-to-tangent	$t_{(8)}$ =-2.66;	p=.015
Peak-to-peak	$t_{(8)}$ =-2.17;	p=.031
Area	$t_{(8)}$ =-5.05;	p<.0005
Event latency measures		
EPSP Onset	$t_{(8)}$ = 4.86;	p<.0005
Pop-spike Onset	$t_{(8)}$ = 3.63;	p=.004
Pop-spike Peak	$t_{(8)}$ = 2.07;	p=.036
Pop-spike Offset	$t_{(8)}$ = 1.37;	p=.103

Table XXIII Analyses of the AEP data from the low frequency potentiation experiment. Pairwise comparisons of the low intensity decay data (time 2 to time 3) were made using paired t-tests, and evaluated with a one-tailed distribution. The comparisions were performed on the individual EP measures.

Variable	t-value	Significance level
EPSP slope measures		
Maximum	$t_{(8)} = 1.26$;	$p=.122$
Double-ended Roll-off	$t_{(8)} = 1.86$;	$p=.050$
Mean	$t_{(8)} = 1.11$;	$p=.149$
Pop-spike measures		
Peak-to-tangent	$t_{(8)} = 3.09$;	$p=.008$
Peak-to-peak	$t_{(8)} = 2.29$;	$p=.026$
Area	$t_{(8)} = 4.68$;	$p=.001$
Event latency measures		
EPSP Onset	$t_{(8)} = -1.61$;	$p=.073$
Pop-spike Onset	$t_{(8)} = -2.34$;	$p=.024$
Pop-spike Peak	$t_{(8)} = -1.38$;	$p=.103$
Pop-spike Offset	$t_{(8)} = -1.05$;	$p=.162$

shows a nominal increase of approximately 4%[3] for the maximum and double-ended roll-off measures, whereas the pop-spike displays a more robust 16%, increase for the peak-to-tangent, and peak-to-peak measures, and a 30% increase for the area measure. The latency measures at the low intensity indicate approximately a 6% decrease, except the onset of the pop-spike which exhibited an 11% decrease from time 1 to time 2. All the AEP measures at the high intensity displayed a decay of 2% or less, indicating no real change or decay.

A2.4 Discussion of the Low Frequency Potentiation:

These data and analyses indicate that low frequency potentiation can occur using a test pulse frequency as low as 0.1 Hz. However, as predicted, the potentiation effects were stronger for the pop-spike measures than they were for the EPSP slope measures, and occur in greater magnitude at the low test pulse intensities than at the high test pulse intensities. Further, the effects appear to be short term and had largely decayed by the end of the 20-25 min interval.

These results support the findings of Douglas and Goddard (1975), although a number of procedural differences exist between the two studies. The data and results presented here, also reinforce and extend those of Skelton et al. (1985), and to some extent clarify the decay issue.

Since there did appear to be a non-significant pattern of residual potentiation 20-25 min after completion of the I/O curve it is difficult to judge, whether the low frequency potentiation examined here should be defined as a short term or long term

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[3]These values are all based on the maximal AEP evoked at time 3 as representing 100%.

phenomenon. Racine and Milgram (1983) examined short term potentiation phenomena in a number of limbic forebrain sites, including the perforant-path to dentate gyrus system. The longest potentiation component in this system had a median decay constant of 389 seconds, such that approximately 6.5% of the original potentiation would remain after 25 minutes. It should be noted however, that Racine and Milgram (1983) induced their post-tetanic potentiation through the delivery of a minimum of 6 brief 400 Hz trains spaced 10 minutes apart, using a final intensity of 2400μa. Thus, the comparability of decay times between these two phenomena may not be be appropriate.

Skelton et al. (1985) also used an I/O curve procedure to induce their potentiation, and the interval between the sixth and seventh I/O curve treatments was 24 hrs for all I/O curve test pulse frequencies. Their results indicated that I/O curve recorded at .2 Hz showed an increase over this last interval, while the curve recorded at .1 Hz showed a decrease. The latter decrease however, was still above the original baseline value recorded several days earlier.

Recent results from this lab, indicate that significant potentiation can be present, 24 hrs after a test session, in which abbreviated I/O curves consisting of three intensities, 10 pulses per intensity were repeatedly delivered (Cain et al., 1992). Consistent with the findings here however, is that the potentiation observed by Cain et al. (1992) occurs only for the pop-spike measures and exhibits the greatest effect at the low intensity. Several replications of these findings were made using the same animals, with most of these replications exhibiting residual potentiation (Cain, et al., 1992).

The degree or occurrence of low frequency potentiation may be dependent upon the intensity of the test pulses as well as the frequency, such that higher intensities can induce the phenomenon at lower frequencies, and conversely, low intensities may not induce potentiation until higher frequencies are used. In support of this relationship are a number of studies that have employed .1 Hz test pulse stimulation frequency over prolonged periods at low stimulation intensities without resulting in any potentiation of the pop-spike (Sharp et al., 1989; Green et al., 1990).

In summary, the data collected in this exeriment show that, as a result of extensive I/O curve recording, low frequency potentiation occurs. Further, this potentiation shows a differential effect at low intensities and acts selectively on the pop-spike measures. Data concerning the duration of this phenomenon are not yet complete. Thus, it is advisable to use lower than .1 Hz test pulse frequencies, when recording I/O curves until the stimulation intensity/frequency interactions can be examined systematically. As a result of these findings the recording procedures carried out in chapter 4 of this thesis had substantial intervals between the recording of the formal I/O curves and the LTP procedures. Additionally, during the LTP procedures only abbreviated I/O curves were recorded. Finally, the minimal interval between test pulse intervals was 10 s, with many intervals being greater than this due to behavioral clamping.

APPENDIX B:
VERIFICATION OF ELECTRODE PLACEMENT

B: VERIFICATION OF ELECTRODE PLACEMENT IN THE COMPLEX ENVIRONMENT EXPERIMENTS

Due to the histological procedures used, in which only every 10th section was saved, complete electrode placement verification could not be accomplished in all cases. Specifically, verification of the depth coordinate was unobtainable for both recording and stimulating electrodes in 7 of the 18 rats used in the complex environment housing experiment. However, an approximate anterior-posterior coordinate and an exact medial-lateral coordinate were obtained for all cases. In some cases the depth could be determined by the squared off shape of the bottom end of the electrode tract, in combination with gliosis surrounding the bottom of the tract. Table XXIV lists all the coordinates that could be determined for the recording and stimulating electrodes. Coordinates for the placements were determined from Paxinos and Watson (1986).

Of the 7 complete recording placements, all were in the granular or hilar regions of the dentate gyrus. Of the remaining incomplete recording placements all were appropriately aimed at the dorsal hippocampus. Medial-laterally a number of the placements extended over the CA4 pyramidal cells. Anterior-posteriorly, the recording electrodes were overall slightly shifted anterior to the targeted coordinate. Finally, one recording electrode whose final depth could not be determined, had passed through the hilus and into the granular layer of the inferior blade of the dentate gyrus.

Of the 6 complete stimulating placements only one appeared to have one of the

staggered tips placed beneath the white matter of the perforant-path. Of the remaining incomplete stimulating placements all would have come into contact with the perforant-path if the final depth were also correct. As with the recording electrodes, the anterior-posterior placements of the stimulating electrodes exhibited a pattern of an overall forward shift from the targeted coordinate.

Table XXIV Anterior-posterior (AP), Medial-lateral (Lat), and Depth (Dep) coordinates of the recording and stimulating electrodes of the rats used in the complex environment housing experiment. Both AP and Med coordinates are distances from bregma in mm, with bregma and lambda in the same horizontal plane. Dep coordinates are distances from the surface of the skull. *Denotes the two rats from the complex environment from which the electrophysiological recordings were unobtainable.

	Record			Stimulate		
Litter/Housing condition	AP	Lat	Dep	AP	Lat	Dep
A/Complex	-4.16	2.4	--	-7.64	4.4	--
	-3.80	2.2	3.4	-7.80	4.0	3.6
•	-3.80	1.8	--	-7.64	3.9	3.6
A/Individual	-4.16	2.0	3.4	-7.64	4.0	--
	-3.90	2.0	--	-7.80	4.2	3.8
	-4.52	2.0	--	-8.00	4.2	4.0
B/Complex	-3.80	2.4	--	-7.64	4.0	--
	-3.80	2.4	--	-7.64	4.0	--
	-3.64	2.6	3.4	-7.64	4.4	--
	-3.80	2.6	3.4	-7.64	4.6	4.4
B/Individual	-3.80	2.2	3.3	-7.64	4.1	4.4
	-3.80	2.4	--	--	--	--
	-3.64	2.6	3.3	-7.80	4.4	--
	-3.64	2.4	--	-7.64	3.9	--
C/Complex*	-4.16	2.4	3.6	-7.64	4.6	--
C/Individual	-4.16	2.6	--	-7.8	4.4	--
D/Complex	-4.30	2.2	--	-7.8	4.4	--
D/Individual	-4.16	2.4	--	-7.8	4.3	3.8

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