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Environmentally-induced plasticity of hippocampal dentate gyrus evoked potentials in freely behaving rats

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ENVIRONMENTALLY-INDUCED PLASTICITY OF HIPPOCAMPAL DENTATE GYRUS EVOKED POTENTIALS IN FREELY BEHAVING RATS

by

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Abstract

ENVIRONMENTALLY-INDUCED PLASTICITY OF HIPPOCAMPAL DENTATE GYRUS EVOKED POTENTIALS IN FREELY BEHAVING RATS

by

Susan D. Croll

Advisor: Professor M. Elizabeth Bostock

Short-term exploratory modulation (STEM) is a recently described form of environmentally-induced hippocampal physiological plasticity. STEM is characterized by a rapidly-occurring increase in field EPSP magnitude accompanied by a decrease in population spike magnitude which occur after an animal is transferred into a different environment. The increase in EPSP magnitude and change in spike magnitude observed in STEM are also observed in a much better characterized form of hippocampal plasticity, long-term potentiation (LTP). This dissertation contains five experiments used to more fully characterize STEM by comparing it to LTP. The first experiment confirms that an NMDA receptor antagonist, MK-801, interferes with the induction of LTP in freely behaving rats. The second experiment demonstrates that MK-801 also interferes with STEM induction, suggesting that both phenomena are NMDA receptor-dependent. The third experiment demonstrates that EPSP to spike relationship shifts are observed after LTP in freely behaving rats, and that MK-801 interferes with, and even reverses, this shift. The shift is characterized by

smaller EPSPs being associated with larger spikes. The fourth experiment shows that an EPSP to spike relationship shift also occurs during STEM, and that MK-801 interferes with the shift. Unlike the shift observed in LTP, the shift during STEM is characterized by larger EPSPs being associated with smaller spikes. The final experiment shows that STEM occurs with transfers to both simple and complex environments, and that the EPSP enhancements decrease with repeated exposures to an environment. These experiments demonstrate that STEM and LTP share some common characteristics, such as reduction by MK-801 and alterations in EPSP to spike relationships, but are also different in many ways. In addition, they suggest that STEM occurs regardless of the nature of the environment into which an animal is transferred, but that the EPSP magnitude may depend on the animal's familiarity with the environment. This work suggests that while both LTP and STEM may reflect mechanisms underlying the hippocampus' response to stimulation (artificial or environmental), they are likely to represent two unique processes which may work together or separately to contribute to this response.

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This dissertation is dedicated to Paul Thomas Ripnick whose love and encouragement inspired me to begin my career as a neuroscientist, and whose tragic injury will continue to inspire me to fully understand the functioning and dysfunctioning of the nervous system.

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Chapter 1. Introduction

A. Specific Aims

In histological studies of the mammalian brain, the highly laminated hippocampus stands out as a beautifully swirled, intricate, and unique structure. Its jelly-roll shape challenges the three-dimensional abstraction skills of the most visuospatially gifted, as it persistently maintains its shape regardless of the plane of the sections. To look at it, even the most jaded of scientists is compelled to think, "this structure is special in some way." Closer anatomical examination reveals, however, that its nestled layers are merely a continuation of the neocortex (Swanson, 1983).

Primary sensory cortices are continuous with sensory association areas, which are continuous with polymodal association cortices, which continue into the hippocampus. Despite its continuity with the neocortex, one cannot deny that the hippocampus is situated in a unique anatomical position in the merging byways of the mammalian brain. It receives more highly integrated, refined, sensory information than any other part of the brain. Furthermore, its projections back to the neocortex are expansive (Swanson, 1983). Therefore, it seems that evolution has given the hippocampus the capacity to "see all" and "tell all." Perhaps, then, this beautiful structure does play a special role in the higher level processing of sensory

information in the brain. It is for this reason that the hippocampus has received so much scientific attention over the past two decades. Studies of hippocampal anatomy and physiology may help to more fully explain higher level information processing in those more evolutionarily advanced species which possess a hippocampus. More specifically, studies of changes in the hippocampus (hippocampal plasticity) in response to new information may help to unravel the mysteries of sensory information processing, learning, and memory.

This dissertation was designed to study one aspect of hippocampal physiological plasticity which may help us to better understand the hippocampus' role in information processing, learning, and memory. The most dramatic changes in hippocampal physiological functioning observed to date (except for those associated with pathological seizure states) are those associated with the long-term potentiation (LTP) of hippocampal synaptic efficacy (for review see Teyler & Discenna, 1987). Unfortunately, the methods used to induce long-term potentiation are artificial in nature, and hence may provide limited information about natural changes in hippocampal physiology. Recently, new forms of hippocampal physiological plasticity have been described which result from manipulations of an animal's environment. This dissertation will study one of these new phenomena,

short-term exploratory modulation (STEM). The specific aims of the dissertation will be to:

1) Determine if STEM shares common mechanisms of induction with LTP by studying the dependence of STEM on activation of the glutamate N-methyl-d-aspartate (NMDA) receptor subtype. The dependence of most forms of LTP on NMDA receptor activation is well documented (Teyler & Discenna, 1987).

2) Determine if STEM results in changes in synaptic efficacy. LTP has been demonstrated to result in increases in synaptic efficacy (Bliss & Lomo, 1973; Abraham, Bliss & Goddard, 1985; Kairiss, Abraham, Bilkey & Goddard, 1987).

3) Determine if STEM will be activated in any type of environment, regardless of its complexity. LTP results from a great deal of stimulation, whereas STEM has only been studied in simple environments with small amounts of stimulation (Sharp, McNaughton & Barnes, 1989; Green, McNaughton & Barnes, 1990). Animals exposed to complex environments across days show long-term changes in evoked potentials which more resemble LTP's profile in that the population spike becomes potentiated (Sharp, McNaughton & Barnes, 1985). By studying STEM in both simple and complex environments, it may be possible to determine whether STEM is more similar to LTP when animals are transferred into complex, rather than simple, environments.

To provide background information for the issues related to the experiments performed to meet these specific

aims, the introduction will review 1) hippocampal anatomy; 2) hippocampal chemistry; 3) hippocampal physiology; 4) theories of hippocampal functioning; 5) artificially-induced hippocampal plasticity (such as LTP); 6) NMDA receptor-dependency of hippocampal plasticity; 7) the relationship between LTP and learning; and 8) naturally-induced hippocampal plasticity (such as STEM).

B. Hippocampal Anatomy

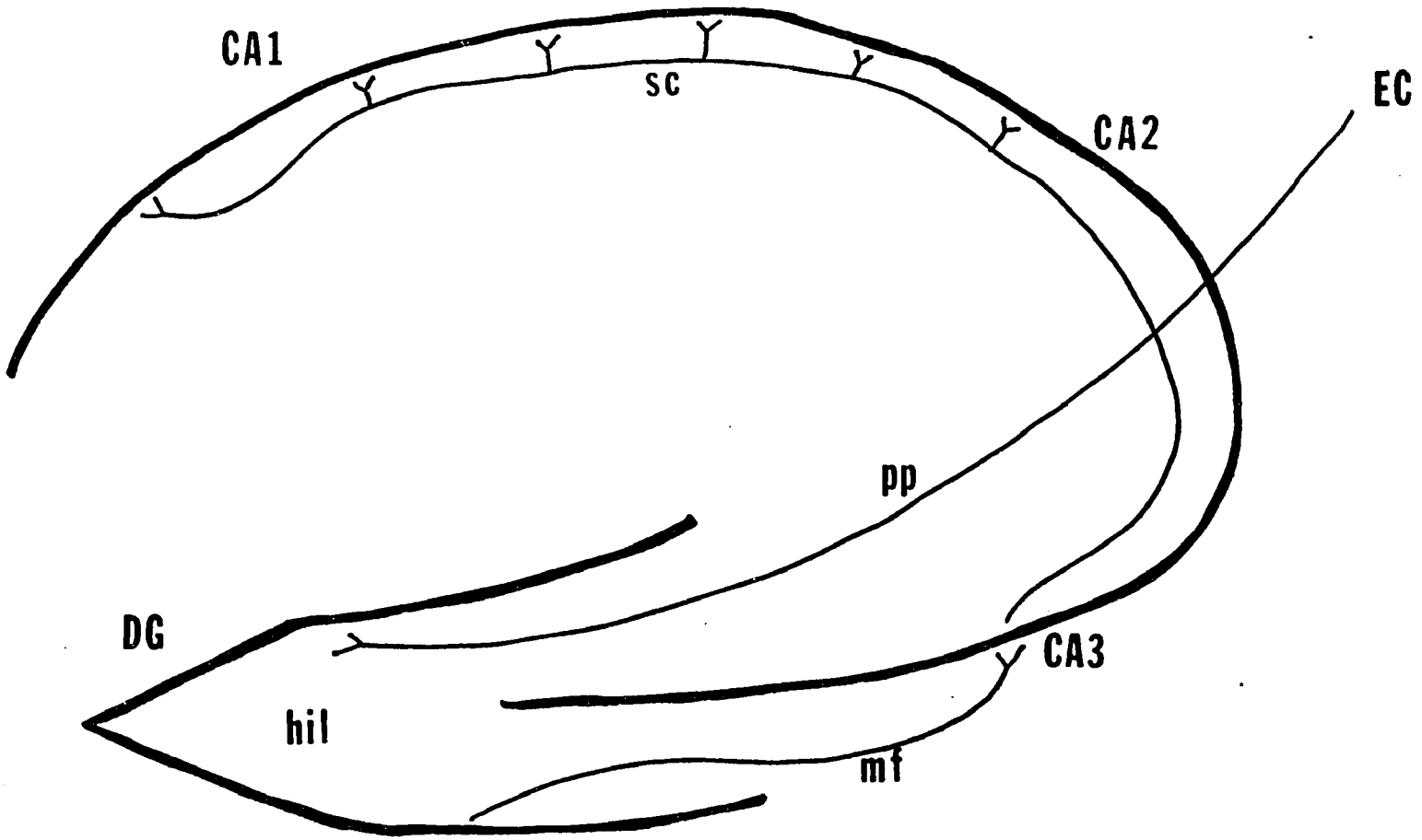
The hippocampal formation consists of the entorhinal cortex, the hippocampus proper, and the subicular areas (Brodal, 1981). The entorhinal cortex projects to the dentate gyrus of the hippocampus, which projects to Ammon's horn of the hippocampus, which then projects to the prosubiculum, subiculum, presubiculum, parasubiculum, entorhinal cortex, and perirhinal cortex (Swanson, Wyss & Cowan, 1978; Amaral, 1987; Hjorth-Simonsen, 1972; Raisman, Cowan & Powell, 1966; Chronister & White, 1971). The flow of information just described is unique for the brain because it is more unidirectional than generally observed in the brain. Each part of the hippocampal formation projects primarily to the next area in line, and has limited reciprocal connections back to the area which projects to it. The hippocampal circuitry is often described as the "trisynaptic circuit" because it has three major synapses:

entorhinal cortex to dentate gyrus, dentate gyrus to CA3/4 of Ammon's horn, and CA3/4 to CA1/2 of Ammon's horn (Swanson et al., 1978; Amaral, 1987; Brodal, 1981; Chronister & White, 1971). These circuits are illustrated in the schematic drawing of the hippocampus provided in Figure 1.

The entorhinal cortex sends its messages through the perforant path to the granule cells of the dentate gyrus. The perforant path has two major subdivisions, the medial and lateral, which send inputs to the dentate gyrus from the medial and lateral portions of layer II of the entorhinal cortex (Lorente de No, 1934; Steward, 1976; Hjorth-Simonsen, 1974; Hjorth-Simensen & Jeune, 1972). Fiber terminals from the medial perforant path synapse in the inner molecular layer of the dentate, close to the granular layer. Their synapses lie primarily on the proximal dendrites of the granules cells. The lateral perforant path synapses in the outer portion of the molecular layer in which the distal granule cell dendrites are found. Hence, the medial perforant path has more power to drive the firing of the granule cells than does the lateral perforant path.

The axons of the dentate granule cells make up the mossy fiber pathway which projects to the pyramidal cells in areas CA4 and CA3 of Ammon's horn (Lorente de No, 1934; Amaral, 1987; Andersen, 1975). CA3 and CA4 pyramidal neurons project to the pyramidal cells of areas CA1 and CA2 of Ammon's horn through the Schaffer collateral pathway.

Figure 1. Schematic drawing of the hippocampal tri-synaptic circuit. EC=entorhinal cortex, DG=dentate gyrus, hil=hilus of the dentate gyrus, CA1&3 of Ammon's horn are labelled as such, pp=perforant path, mf=mossy fiber pathway, sc=Schaffer collaterals


CA1
sc
EC
CA2
pp
DG
CA3
hil
mf

The pyramidal cell axons from CA3 and CA4 course along Ammon's horn from CA3/4 to CA1/2 with recurrent collaterals branching off along the way to synapse on the dendrites of those pyramidal cells located further along in Ammon's horn (Lorente de No, 1934; Amaral, 1987; Andersen, 1975). Although the axons of the pyramidal cells exit from the hippocampus diffusely, many of them exit through a ribbon-like pathway called the fimbria, which then gathers into a bundle called the fornix.

The hippocampus has a laminated structure similar to, but not identical to, that of the cerebral cortex. Ammon's horn, which makes up the majority of the hippocampus, consists of seven layers (corresponding to the six cortical layers plus an epithelial zone)(Cajal, 1911; Lorente de No, 1934; Andersen, 1975). The first layer, which lies along the hippocampus' border with the lateral ventricle, is the epithelial layer. The second layer is a white matter layer called the alveus. This layer consists primarily of the myelinated axons of the pyramidal cells as they course into the fimbria/fornix. The third layer is the stratum oriens. This layer contains polymorphic cells. The fourth layer is the stratum pyramidale, which consists of large pyramidal cells. The axons of these pyramidal cells extend through the stratum oriens and into the alveus. The fifth layer is the stratum radiatum. The major components of this layer are the peripheral processes of the pyramidal cells from the

pyramidal layer. The sixth layer is the stratum lacunosum. This layer primarily consists of fiber bundles. The last layer, the stratum moleculare, contains the superior processes of the pyramidal cells from the pyramidal layer.

The dentate gyrus, unlike Ammon's horn, has only three layers (Cajal, 1911; Lorente de No, 1934, Andersen, 1975). These layers are wrapped around the end blade, or CA4 of Ammon's horn, in a semicircle. The upper and lower blades of the semicircle are often referred to as the outer and inner blades (Swanson et al., 1978). The concavity which contains CA4 and polymorphic cells is called the hilus.

The outermost layer of the dentate gyrus is the molecular layer (Lorente de No, 1934). It consists of some fibers and processes from cells in other layers, including the granule cells. Its primary composition, however, is of superficial pyriform, ovoid, or fusiform cells, deep triangular or stellate cells, and displaced granule cells from the granule cell layer. The second layer is the granule layer. This layer consists primarily of densely packed granule cells, characterized by the small size of their somata. These granule cells are considered the primary cells of the dentate gyrus, just as the pyramidal cells are considered the primary cells of Ammon's horn. The final layer is the polymorph layer. The polymorph layer contains many cell types, primarily with local contacts.

Ammon's horn has been divided into four fields, CA1, CA2, CA3, and CA4. CA1 and CA2 make up the uppper blade of Ammon's horn, CA3 makes up the lower blade, and CA4 makes up the end blade which extends into the hilus of the dentate gyrus. CA3 and CA4 together are often referred to as the regio inferior and CA1 and CA2 together are often referred to as the regio superior (Swanson et al., 1978). CA1 and CA3 are the largest and most well-studied fields of Ammon's horn, and are often subdivided into areas a, b, and c. Each of the fields has a slightly different anatomy than the others, particularly in terms of the characteristics of its pyramidal cells (Lorente de No, 1934; Andersen, 1975).

CA1 consists of the smallest pyramidal cells of Ammon's horn. The pyramidal cells in CA1 have no thorns (thick, short side-branches projecting from the dendrites), but give off fine side branches in the the stratum radiatum. CA3 consists of large pyramidal cells. The pyramids contain thick thorns used for contact with the mossy fibers from the dentate gyrus. In addition, the pyramidal cells of CA3 give rise to the Schaffer collaterals which synapse onto cells in CA2 and CA3 (Lorente de No, 1934). CA2 is a transitional area between CA1 and CA3. It has the thinnest pyramidal layer of all the fields of Ammon's horn. The pyramidal cells in CA2 are most similar in size to the pyramids of CA3. Unlike the cells in CA3, however, they do not have thick thorns, because they do not receive mossy fiber input,

and they do not give rise to any Schaffer collaterals (Lorente de No, 1934). The pyramidal cells of CA4, like CA3, do have thick thorns for contact with the mossy fibers, and they do give rise to Schaffer collaterals. Morphologically, however, the pyramidal cells are different from those in CA3. They are not true pyramids, but rather modified pyramids with an irregular form (Lorente de No, 1934; Andersen, 1975).

In addition to the cells already discussed, the primary cell layers of both the dentate gyrus and Ammon's horn contain numerous basket cells which function as local interneurons (Lorente de No, 1934; Andersen, 1975). These basket cells are thought to receive numerous excitatory inputs, but form inhibitory synapses onto the pyramidal and granule cells close to their cell bodies. Unlike most areas of the brain, the hippocampus has a low concentration of inhibitory interneurons relative to the number of pyramidal and granule cells (primary cells) (Buzsaki & Eidelberg, 1982).

In addition to connections between the different areas of each hippocampus, there are reciprocal commissural connections between contralateral hippocampi. There are several commissural pathways in the hippocampus, and that each is paralleled by an intra-hippocampal association pathway, such as those already described (Swanson et al., 1978; Blackstad, 1956; Fricke & Cowan, 1978). The major

hippocampal commissural pathways project from CA4 to the contralateral dentate, CA3 to the contralateral CA1 and subiculum, and from one dentate gyrus to the contralateral dentate.

The hippocampus receives projections from several areas of the brain other than the entorhinal cortex (Brodmann's area 28). The hippocampus also receives major inputs from the medial septum and diagonal band (through the fornix) and cingulate gyrus, and may receive inputs from the piriform cortex as well (Brodal, 1981; Haas, 1983; Amaral & Kurz, 1985; Baisden, Woodruff & Hoover, 1984; Meibach & Siegel, 1977a). In addition, it receives inputs from the locus coeruleus (Haas, 1983; Amaral & Cowan, 1980; Jones & Moore, 1977; Mason & Fibiger, 1979; Room & Groenewegen, 1986) and median raphe nucleus (Haas, 1983; Amaral & Cowan, 1980; Room & Groenewegen, 1986) in the mesencephalon and metencephalon.

The hippocampus sends efferents to areas other than the entorhinal and subicular cortices (Brodal, 1981; Irle & Markowitsch, 1982; Meibach & Siegel, 1977b; Swanson & Cowan, 1977; Raisman et al., 1966). Specifically, it projects through the fornix to the mammillary bodies, anterior thalamic nucleus, septal nuclei, preoptic cortex, lateral hypothalamus, periaqueductal gray, and pontine nuclei. The cingulate cortex appears to receive extensive indirect hippocampal input through the anterior thalamic nucleus as well as through the mammillary bodies to the anterior

thalamic nucleus. It is interesting that the hippocampus' projections are primarily to areas which are either association areas in the neocortex, or are areas thought to be involved in the regulation of motivated behavior. This anatomical profile is consistent with the notion that the hippocampus plays a role in the modulation and processing of higher level information in the mammalian brain.

C. Hippocampal Chemistry

The hippocampus has an unusually rich neurochemical profile. The primary excitatory synapses in the hippocampal trisynaptic circuit, as well as the commissure from CA3, are thought to use the excitatory amino acid glutamate as their neurotransmitter (Zalutsky & Nicoll, 1990; Collingridge, Kehl & McLennan, 1983; Cotman, Monaghan, Otterson & Storm-Mathisen, 1987; Jahr & Stevens, 1987). The hippocampus is rich in receptors for glutamate (Cotman et al., 1987; Monaghan, Bridges & Cotman, 1989; Cotman & Iversen, 1987). Currently, there are five subtypes of receptors described for glutamate (Monaghan et al., 1989; Barnes & Henley, 1992). These are the AP4 receptor, the kainate receptor, the metabotropic receptor, the AMPA receptor, and the NMDA receptor. The hippocampus contains all five subtypes of receptors. The role of these receptors in hippocampal function will be discussed in section 1F.

In addition to glutamate, many other neurotransmitters are found in the hippocampus. The hilar commissural pathways as well as most of the interneurons in the hippocampus are thought to use GABA as their neurotransmitter (Alger & Nicoll, 1982; Buzsaki & Czeh, 1981). These putative GABAergic neurons are located in the inhibitory circuits of the hippocampus.

The hippocampus is also rich in modulatory neuropeptides. Most notably, the lateral division of the perforant path contains enkephalin (Dahl, Burgard & Sarvey, 1990; Gall, Brecha, Karen & Chang, 1981) and the mossy fiber pathway contains dynorphin (McGinty, Henriksen, Goldstein, Terenius & Bloom, 1983). The hippocampus is also immunoreactive to a wide variety of other neuroactive substances such as cholecystokinin, vasoactive intestinal polypeptide (VIP) neuropeptide Y, vasopressin, galanin, and somatostatin (Kohler, Schultzber & Radesater, 1987; Kohler, Eriksson, Davies & Chan-Palay, 1987; Flood, Garland & Morley, 1990; Albeck, Smock, McMechen, Purves & Floyd, 1990).

Major inputs to the hippocampus consist of several of the classical neurotransmitter systems. The basal forebrain cholinergic system, especially the medial septum, sends an extensive cholinergic input into the hippocampus (Brodal, 1981; Haas, 1983; Amaral & Kurz, 1985; Baisden et al., 1984; Meibach & Siegel, 1977a). The locus coeruleus sends a

fairly extensive noradrenergic input into the hippocampus (Haas, 1983; Amaral & Cowan, 1980; Jones & Moore, 1977; Mason & Fibiger, 1979; Room & Groenewegen, 1986). Finally, the median raphe nucleus sends a serotonergic input to the hippocampus (Haas, 1983; Amaral & Cowan, 1980; Room & Groenewegen, 1986).

D. Hippocampal Physiology

Hippocampal EEG is divided into three major patterns (Vanderwolf, 1969; Leung, Da Silva & Wadman, 1982). These patterns include a brief desynchronized, high frequency, low amplitude pattern called small irregular activity (SIA), a pattern with intermittent spikes in a low frequency, high amplitude pattern called large irregular activity (LIA), and a rhythmic sinusoidal pattern (6 to 10 Hz in the rat) which has been termed rhythmical slow activity (RSA) or theta EEG. SIA appears to reflect brief periods of arousal or theta rhythm interruption. LIA has been observed during still, automatic behaviors such as quiet stillness, grooming, slow-wave sleep, gnawing, eating, species-specific behaviors, etc.

RSA, or theta, is highly correlated with active exploration (Vanderwolf, 1969; Sainsbury, Heynen & Montoya, 1987; Sinclair, Seto & Bland, 1982; Winson, 1974). Active exploration is defined by Vanderwolf (1969) as voluntary and

involving movement through space. Although sniffing rate in rats is similar to the rate of theta rhythm (Gray, 1971; Macrides, 1975), there has been little strong evidence that sniffing is reliably associated with or phase-locked to theta EEG. Finally, theta occurs during desynchronized sleep (Monmauer, 1982; Monmauer, Houcine & Delacour, 1979).

In addition to correlational studies which suggest that hippocampal theta EEG is associated with active exploration, there is experimental evidence suggesting that theta EEG may be necessary for the successful performance of spatial tasks. Lesions of the medial septum in rats disrupt hippocampal theta rhythm and disrupt learning (Winson, 1978; Mizumori, Perez, Alvarado, Barnes & McNaughton, 1990; Andersen, Bland, Myhrer & Schwartzkroin, 1979; Mitchell, Rawlins, Steward & Olton, 1982).

Although the hippocampus as a whole is a very physiologically active structure, individual neurons in the hippocampus are relatively quiet compared to individual neurons in the rest of the cortex. Their spontaneous firing rate is very low, if not non-existent (Ranck, 1973). Many primary hippocampal cells respond very specifically to a limited set of stimuli. Primary cells in the hippocampus are often divided into two physiological types, theta cells and complex spike cells (Ranck, 1973; Fox & Ranck, 1975; Fox & Ranck, 1981).

Theta cells fire primarily when the hippocampus is exhibiting a theta EEG pattern, and are generally phase-locked to the EEG. Studies of hippocampal physiology have suggested that theta cells can be found both in the dentate gyrus and Ammon's horn (Bland, Andersen, Ganes & Sveen, 1980; Ranck, 1973; Buzsaki, Leung & Vanderwolf, 1983; Rose, Diamond & Lynch, 1983). In CA1, these theta cells have been termed "displace" units, and fire when the animal is engaging in displacement or active movement through space (Ranck, 1973; O'Keefe and Nadel, 1978; McNaughton, Barnes & O'Keefe, 1983). In addition, some theta cells appear to be somewhat sensitive to "place", or an animal's physical location within its spatial environment (Kubie, Muller & Bostock, 1990). Most theta cells, especially in Ammon's horn, are interneurons. There are also theta cells located in the primary cell layer (granule cells) of the dentate gyrus. There is approximately a 180 degree phase shift in theta EEG from CA1 to the dentate (Ranck, 1973). That is, the theta cells in CA1 and the dentate fire alternately, so that the up-phase of CA1 theta falls on the down-phase of dentate theta and vice-versa.

Complex spike cells fire both in single spikes and in bursts, such that an occasional cluster of closely spaced action potentials can be recorded. The pyramidal cells of Ammon's horn of the hippocampus are complex spike cells (Ranck, 1973; Suzuki & Smith, 1985; Fox & Ranck, 1975; Fox &

Ranck, 1981). Some complex spike cells have been described in CA1/CA3 of the hippocampus which fire in response to exposure to certain places in the environment (the cell's firing field), and have been termed "place cells" (O'Keefe & Dostrovsky, 1971; O'Keefe, 1976; O'Keefe & Conway, 1978). The firing fields of these "place cells" are stable over time, and hence, may be providing some sort of sensory code to process spatial information. The firing field of each place cell is thought to be dependent on distal spatial cues, because rotation of spatial cues results in a rotation of the place cell's firing field (O'Keefe & Conway, 1978). When a set of spatial cues to which a cell was firing is removed, the cell continues to respond as though the cues were present. This phenomenon supports the speculation that the firing of the place cells reflects an internalized representation of the animal's environment (O'Keefe & Speakman, 1987). Although place cells have been noted for their stability, recent evidence suggests that even place cells can modify their firing fields over time as a result of experience with different cues (Bostock, Muller & Kubie, 1991).

The principal cells of the dentate gyrus (granule cells) and of CA1/3 (pyramidal cells) have both been shown to be behaviorally relevant, but in different ways. For example, the neuronal responsiveness of the principal cells in the hippocampus depends, among other things, on the

behavioral state of the animal, but each type of cell has its own profile of responsiveness. The dentate gyrus is much more responsive during REM sleep and slow wave sleep than during still alertness, whereas CA1 cells were more responsive during slow-wave sleep (Winson & Abzug, 1977). Another experiment in rats showed that the dentate gyrus and CA1 respond differently to somatosensory stimulation provided to the animal (Herreras, Solis, Munoz, Martin del Rio & Lerma, 1988). Stroking of the animal's back resulted in an increase in hippocampal theta. This event led to an increased efficacy of dentate gyrus evoked potentials (from the perforant path), but a decreased efficacy of CA1 evoked potentials (from the Schaffer collaterals). The different regions of the hippocampus appear capable of working independently of one another despite their dense connections to one another. In fact, lesions of the dentate gyrus do not interfere with the place-specific firing of CA1/3 place cells despite their location farther "down the line" than the dentate in the hippocampal circuitry (McNaughton, Barnes, Meltzer & Sutherland, 1989).

The inhibitory GABAergic interneurons also play a role in the modulation of hippocampal activation. Modified basket cells can be found in association with both granule cells in the dentate gyrus and pyramidal cells in Ammon's horn. Evidence has been found for a role of the interneurons in both recurrent inhibition and feedforward

inhibition (Schwartzkroin & Knowles, 1983; Knowles & Schwartzkroin, 1981; Buzaki, 1984; Alger & Nicoll, 1982). That is, inhibitory interneurons are excited both by principal cells and by principal cell afferents to induce IPSPs on the principal cells. Because many theta cells are thought to be interneurons (Ranck, 1973; Konopacki, Bland, Colom & Oddie, 1992), it is likely that the inhibition of principal cells by interneurons is also mediated by the behavioral state of the animal. Therefore, most aspects of hippocampal physiology may be behaviorally sensitive. Thus, it is crucial to take into account the behavioral state of the animal, especially in terms of active, theta-associated behaviors, when undertaking any study of hippocampal physiology in intact animals (Hargreaves, Cain & Vanderwolf, 1990).

E. Theories of Hippocampal Function

Based solely on its expansive anatomical connections and its position as an advanced polymodal association area, one might guess that the hippocampus processes integrated sensory information for storage or behavioral responses. Combining this unique anatomy with its behaviorally relevant physiology, it seems that the hippocampus might be involved in the higher level processing of environmental information for later use. Although there are several different major

theories about hippocampal functioning (O'Keefe & Nadel, 1978; Olton, Becker & Handelmann, 1979; Sutherland & Rudy, 1989; Mishkin, 1991), they all agree that the hippocampus plays some role in memory processing of certain types of information.

Initial clues about the hippocampus' role in memory came from clinical studies of patients with medial temporal lobe damage which included hippocampal damage. Patients with traumatic lesion or surgical resection of the hippocampus and associated structures show memory impairments (Scoville & Milner, 1957; Milner, Corkin & Teuber, 1968; Milner, 1968; Milner, 1966). In addition, patients with anoxic damage, to which the CA1 pyramidal cells in the hippocampus are especially vulnerable, have difficulties learning new information (Gilman, 1965; Volpe & Hirsh, 1983; Volpe, Pulsinelli & Davis, 1985; Squire & Shimamura, 1986). Finally, Alzheimer's disease, a neurodegenerative disease which is characterized by severe memory impairments, is characterized by hippocampal deterioration (Hyman, Van Hoesen, Damasio & Barnes, 1984; Ball, 1988).

The most studied clinical case has been that of H.M., a patient who suffered from memory impairments after having large portions of his medial temporal lobe removed to alleviate intractable epilepsy (Scoville & Milner, 1957; Milner, 1968; Milner, 1966). R.B., a patient who sustained

specific damage to CA1 of the hippocampus following an ischemic episode, has recently been studied to localize similar memory impairments more specifically to the hippocampus (Zola-Morgan, Squire & Amaral, 1986). Both of these patients showed a severe anterograde amnesia, such that they could not remember most information presented to them after the time of the injury. Their amnesia suggested that the medial temporal lobe, including the hippocampus, plays a role in memory formation or retrieval. Despite the severe anterograde amnesia, H.M. and R.B.'s memories of certain types of information were relatively spared. They were able to learn new procedural skills, though they had no verbal memory of having learned or performed these skills. In addition, they were able to retain information in short-term memory, as long as they continued to rehearse it. The sparing of certain types of memory suggests that the hippocampus is involved in only certain types of memory.

In addition to his anterograde amnesia, H.M. showed a graded retrograde amnesia. Specifically, he was able to recall details from experiences farther back than a year prior to injury with no difficulty. From a year before his damage until just prior to the injury, he had a graded memory loss such that the closer to the injury an event occurred, the less likely he was to recall it. Because hippocampally-damaged patients were able to recall remote events, it was thought unlikely that the hippocampus was

necessary for long-term memory storage, and was therefore not the "site" of permanent memory stores. It seemed likely, however, that the representation of recently learned information was retained within the hippocampal-medial temporal circuitry because its recall was dependent on the integrity of these structures.

Experiments conducted in rats and monkeys served to confirm that the hippocampus is crucial for many forms of learning and memory such as those measured in humans (Zola-Morgan & Squire, 1985; Squire & Zola-Morgan, 1983). The tasks which appear to be most sensitive to hippocampal damage are tasks which are spatial in nature. Animals with either hippocampal plus amygdala or selective hippocampal damage perform poorly on memory tasks (Mahut, Moss & Zola-Morgan, 1981; Mahut, Zola-Morgan & Moss, 1982; Mishkin, 1978), and especially tests of spatial memory such as mazes (Morris, Garrud, Rawlins & O'Keefe, 1982; Jarrard, 1978, 1983; Gage, 1985). In fact, human amnesic patients fail on human versions of the same tasks designed to test memory impairments in nonhuman primates (Squire, Zola-Morgan & Chen, 1988). As would be predicted based on observations of H.M. and other patients with hippocampal damage, rats show little or no impairment in recall of well-learned tasks (Jarrard, 1978; Gage, 1985). Sutherland, Arnold, and Rodriguez (1987) showed that rats receiving lesions at various time points after learning a spatial task will

demonstrate a graded retrograde amnesia. Recently, Zola-Morgan and Squire (1990) have shown graded retrograde amnesia in hippocampally damaged monkeys for learning paired associates, consistent with Sutherland et al.'s results and clinical observations of H.M and R.B.

The data suggest that although hippocampal integrity is not necessary for the retrieval of information from long-term memory, it is certainly necessary for the processing of certain types of information for long-term storage. In addition, this processing must take time because hippocampal damage interferes with the recall of information from the recent past. This time-dependent mnemonic processing has been termed "consolidation", and is traditionally thought to represent the transfer of information from short-term memory into long-term memory. Although the role of the hippocampus in learning and memory is likely to be much more complex than merely the transfer of information from short to long-term memory stores, this theory provides a useful starting point from which to theorize about hippocampal function.

Many of the earlier theories of hippocampal function dealt primarily with the idea that the hippocampus was responsible for the consolidation of information from short-term to long-term memory. The more popular recent theories (O'Keefe & Nadel, 1978; Olton et al., 1979; Sutherland & Rudy, 1989; Mishkin, 1991) have started with the assumption that the hippocampus is involved in some sort of time-

dependent memory processing, but have expanded this to deal either with the specific types of memory which the hippocampus processes, or with how the hippocampus processes them.

One influential theory that deals with the types of memory that the hippocampus processes is the cognitive map theory proposed by O'Keefe and Nadel (1978). They divide spatial memory processing into two major systems, a taxon system and a locale system. The taxon system is responsible for goal-driven spatial behavior, and is involved in the development of routes. A route, as defined by O'Keefe and Nadel, is a transitory navigational tool for orienting an animal towards a specific spatial goal. A route is also very rigid, and generally confined to the context in which it is planned. In contrast, the locale system is responsible for curiosity-driven spatial behavior. This behavior includes exploration of environments and the identification of novel or changed stimuli in environments. The locale system is used to form cognitive spatial maps of places. These maps are flexible and can change across time to reflect changes in stimuli, contexts, or perspectives.

O'Keefe and Nadel (1978) propose that the hippocampus controls the locale system. That is, it is responsible for the development and modification of cognitive maps. They point out that the two spatial processing systems do not act entirely independently of one another. That is, animals

could obtain information necessary for the building of an internalized spatial map of an environment while engaging in goal-directed behaviors. Perhaps more importantly, animals are likely to draw on information from their spatial maps to plan routes. In other words, the taxon system may rely on maps formed by the locale system for more efficient navigation. Therefore, their theory predicts that hippocampal damage will destroy the locale system, leaving the taxon system intact. However, even functions which normally rely on the taxon system will be compromised to some extent following hippocampal damage because of the loss of mapping and flexibility.

Shortly after O'Keefe and Nadel (1978) proposed their cognitive map theory, Olton et al. (1979) proposed that O'Keefe and Nadel were correct in stating that the hippocampus played a special role in certain types of memory, but were incorrect in identifying which type of memory it was responsible for. Specifically, Olton et al. reexamined the literature and pointed out that although spatial memory appeared to be particularly vulnerable to hippocampal damage, hippocampectomized animals also had difficulty with certain non-spatial tasks as well. They claimed that all memory could be divided into reference versus working memory. Reference memory involves memory for the general logistics of a task, or the more procedural elements of the task. In one classic maze task, for

example, an animal learns that there are eight baited goal arms on a radial maze, and that to receive maximum reward most efficiently the animal must visit each arm once and only once. This information is part of the animal's reference memory because it involves the general procedure or strategy with which the animal completes the task during every trial.

In contrast, working memory is the memory for the specific information necessary to perform the task successfully within a trial. This memory involves the type of information which not only does not need to be remembered beyond task completion, but should not be remembered past task completion, because it would be likely to interfere with future trials if it were. An example of working memory would be the animal remembering which goal arms it had already retrieved reward from, so that it would not re-visit the empty arms.

Olton et al. (1979) point out that most lesion studies which show that hippocampectomized rats cannot perform spatial tasks properly had used spatial tasks, such as the one described above, which require working memory. They further point out that the few studies which show that non-spatial learning is disrupted after hippocampal damage use tasks with a substantial requirement for intact working memory processes. In addition, they propose that the clinical evidence from studies of human amnesics with

hippocampal damage (such as H.M) supports their view by drawing a parallel between the working versus reference memory taxonomy and the declarative versus procedural memory taxonomy (discussed by Winograd, 1975). As previously discussed, humans with hippocampal damage have difficulty with declarative memory, but have intact procedural memory. If working memory is thought of as declarative memory because it involves retaining specific pieces of information through a learning trial, then their use of human studies as suppport for their theory is valid. It is difficult, however, to divide any learning task into strictly working versus reference memory components, and even more difficult to divide human memory into these elements.

More recently, Sutherland and Rudy (1989) proposed the configural association theory of hippocampal function. This theory picks out what is common between O'Keefe and Nadel's locale system and Olton et al.'s working memory. Both types of memory rely on the successful association between groups of stimuli in some context. Their theory states that the hippocampus takes multiple stimuli coming in from the environment and links them into a single stimulus representation, which Sutherland and Rudy term a configuration. This theory succeeds at explaining the hippocampus' special role in the processing of spatial information. Because spaces (or places) are defined by what cues lie where, and more importantly, where they are in

relation to each other, it would be necessary to link cues together relationally to form an internalized spatial representation.

Unlike theories which are entirely spatial in nature, however, Sutherland and Rudy`s theory allows the hippocampus to claim a critical role in any other type of memory processing which requires the association of cues in time or space into one stimulus configuration. In addition, it claims that any type of context-dependent learning requires hippocampal integrity because of the need to link elements of the learning situation with elements of the context.

Many of the newer theories of hippocampal function agree with configural association theory to some extent, but deal more with the process by which these associations are formed. Renewed attention has been given to the role of the Hebb synapse in the formation of associations necessary for learning and memory to occur. Hebb (1949) proposed that associative learning may occur due to strengthening of synapses after inputs from two different cells or cell assemblies repeatedly meet at the same synapse. He proposed that after this repeated simultaneous activity the two cells or assemblies, and thus the stimuli that they represent, become associated.

Mishkin (1991) has dealt with the issue of hippocampal-cortical interaction by discussing Hebb's (1949) original notion of the involvement of cell assemblies in more complex

forms of associative learning. Hebb proposed that networks of cells could work together in certain patterns to code for more complex types of information, and that groups of these networks could fire onto common synapses to cause complex associations. Miskin discusses this in terms of the hippocampus. He proposes that the hippocampus is the site of the overlap of the networks of cells called cell assemblies. That is, memories of complex stimuli (such as environments) are stored as cell assemblies in the cortex. When new information comes in, the hippocampus somehow activates these cell assemblies and links them with new information, or links them with each other, if a point of common interest has been found. In this model, the hippocampus is given a role as a sort of ultimate polymodal associator. Anatomically, the hippocampus is well suited for this role because of its position as the highest level polymodal association area, and because of its elaborate reciprocal connections with the cerebral cortex.

Mishkin's new theory accounts not only for the role of the hippocampus in memory storage, but for the form of memory storage as well. Ever since Lashley's (1929, 1950) cortical lesion studies first demonstrated that memory is not stored within certain cerebral locations, but is retrieved by mass action of large areas of cortex, memory researchers have been reluctant to continue the search for the physical site of the "engram" or memory trace. Recent

memory theories tend to consider the engram as a pattern of multiple cell firing, rather than as a single locus within the complex circuitry of the cortex. Mishkin's theory explains memory storage in terms of its firing patterns rather than locus, while still accounting for the data which have given one specific locus, the hippocampal formation, a special role in memory processing.

In addition to the many theories of hippocampal function which have been proposed, many models of hippocampal functioning have been offered as explanations of specific circuit interactions within the hippocampus. Many models have been proposed as explanations of specific hippocampal phenomena which have been described empirically. Others deal more generally with hippocampal and/or cortical circuit interactions. A discussion of several models which deal either with general hippocampal function or specific dentate gyrus function will be briefly presented here.

Marr (1971) introduced a model of cortical information processing based on the principles used for computer processing of information. He based his neural network model on the suspected relationships between excitatory and inhibitory neurons within the cortical layers, and on Hebb-like modifiable synapses. As part of his discussion, he specifically mentions the hippocampus as a structure which fits well into his proposed model.

Marr's model suggests that stimuli entering the cortex are classified, diagnosed, and interpreted based on features shared with other members of their stimulus class. These features include both physical features and functional or consequential features relevant to the survival or behavioral repertoire of the aninal. In addition, he proposes that stimuli are grouped together not only based on their classification, but also based on their context, an idea not far removed from the configural associations of Sutherland and Rudy (1989).

His specific model proposes that synapses in the neocortex (especially those from granule cells onto pyramidal cells) are modifiable. He suggests that these synapses are similar to those proposed by Hebb (1949), except that he proposes that one of the excitatory inputs into the output cells is not modifiable while another excitatory input is (McNaughton & Morris (1988) later applied this idea to the hippocampus). In addition to these two excitatory inputs, he proposes that each output cell receives two inhibitory inputs, one on the dendrites and one on the soma. The inhibitory synapses onto the dendrites are used for a subtraction function in the output cell for all of the inputs its dendrites receive from their afferents. The inhibitory synapses onto the soma perform a division function to allow only a certain fraction of the spikes generated by the spike generator to be transmitted.

Finally, Marr (1971) proposes a third type of inhibitory synapse. This type consists of synapses from local interneurons onto codon cells. These are the cells which filter the evidence about each stimulus for its diagnosis into a certain classification.

Marr (1971) proposes that the inhibitory circuits set the threshold for firing of the codon cells. Marr suggests that within the circuitry of the afferent fibers, the codon cells, and the output cells, the synaptic weightings can be altered depending on the nature of the inputs and outputs that travel through the circuitry.

McNaughton and Morris (1988) bring the ideas first proposed by Marr (1971) to a discussion of synaptic strengthening as a mechanism for memory formation in the hippocampus. In this model they propose that multiple representations can be coded within the same set of cells by use of distributed processing in a cellular "correlation matrix." In this model, synapses have an initial strength equal to zero. If two inputs, each with a strength of one, simultaneously fire onto the synapse, it will then obtain a strength of one. In addition, they propose that there may be less modifiable detonator synapses to drive neurons, a feedback from each principal neuron back onto itself, and a feedforward inhibitory input to the principal neurons which serves to divide the excitation onto a cell by the number of excitatory inputs received.

These elements add several properties to the correlation matrix. One property is the ability to reverberate. That is, information could be held in the matrix for longer than a simple longitudinal firing system could be expected to hold it. This element is particularly important because of the suspicion that information is at least partially retained within the hippocampal circuitry for fairly long periods of time. Another property is the ability to inhibit post-synaptic firing. The proposed feedforward inhibition allows for the cell to be in variable states when the excitatory input arrives, as Marr (1971) first proposed, such that it will be more or less likely to fire. This phenomenon not only gives the matrix a greater degree of flexibility, and hence of specificity in its coding, but also allows it both to clear or reset itself, and to respond very differently to small versus large amounts of input. McNaughton and Morris (1988) specifically propose that the inhibitory inputs are activated much more easily than the excitatory inputs, and at a much lower threshold, such that postsynaptic firing may actually be inhibited when the stimulation is only minimal. Thus, the hippocampal circuitry can silence parts of its own output matrix, allowing only the most salient associations to be expressed in the output.

McNaughton and Morris go on to point out that the hippocampus, and particularly the dentate gyrus of the

hippocampus, possesses the physiological profile necessary for the proposed correlation matrix to be in effect. The dentate gyrus has a large number of granule cells which all receive multiple inputs from cells in layer II of the entorhinal cortex such that all entorhinal inputs are distributed across a matrix of granule cells. In addition, each cell receives at least two separate types of input, proximal inputs from the medial perforant path, and more distal (and hence, less effective at inducing output) inputs from the lateral perforant path. Some medial perforant path inputs produce larger EPSPs (10 to 20 times larger) than the rest of the inputs, and thus may serve as the detonator synapses. Finally, the dentate gyrus is rich in inhibitory basket cells which have feedforward inputs to the principal neurons. It seems physiologically feasible, then, that McNaughton and Morris are correct in proposing that the hippocampus processes information by means of a reverberatory distributed correlation matrix.

Miller (1989) expands upon the correlation matrices proposed by McNaughton and Morris by suggesting that these matrices are too readily saturable (a point clearly admitted by McNaughton and Morris). He suggests that another element needs to be added to the model, and that is a consideration of the variable state of the hippocampus during theta EEG. Because the hippocampus typically shows a theta EEG rhythm during exploratory behaviors (in which spatial information

is likely to enter the matrix), he suggests that the hippocampus may activate loops with the cortex that are phase-locked with theta rhythm. His idea deals less with the activation of synapses within the hippocampus, and more with its interactions with cortical structures. As activation from theta circulates through the loops within the hippocampus and between the hippocampus and cortex, the "up" phase of the theta rhythm will hit different areas at different times, throwing each point of intersection between afferents (which he terms nodes) into variable states of baseline activation. He suggests that those stimuli that cause maximal activation in phase with the oscillations produced by the theta EEG will cause selective strengthening of the nodes. In this way, the loops will become self-organized based on which connections are being strengthened in phase. His ideas allow hippocampal theorists to think about how the hippocampus gets information in and out of the cortex. This point is important because, as already mentioned, the hippocampus only organizes information, it does not store information for long periods of time. In addition, Miller accounts for the role of theta EEG in the processing of complex environmental information in the hippocampus.

F. Artificially-Induced Hippocampal Plasticity

If the hippocampus is involved in learning and memory, it needs to be highly plastic to encode or process changes in information. Over the past two decades, many forms of hippocampal plasticity have been described. The most well-studied and most fully described are those that are artificially induced. That is, the changes measured in these forms of plasticity are not caused by changes in the animal's natural experiences or behavior, but rather by alterations in electrical or chemical stimulation introduced into the hippocampus by researchers. The best characterized of these forms of artificially induced plasticity is long-term potentiation (LTP).

LTP is an artificially induced form of plasticity, best characterized in the hippocampus, in which high frequency stimulation of afferents results in an increase in magnitude of the field excitatory post-synaptic potential (EPSP) and an even greater increase in the magnitude of the population spike (Bliss & Lomo, 1973; Bliss & Gardner-Medwin, 1973; Andersen, Sundberg, Sveen & Wigstrom, 1977; Dunwiddie & Lynch, 1978; McNaughton, 1982). LTP is a relatively long-lasting form of plasticity, and has been demonstrated to last for several days (Bliss & Gardner-Medwin, 1973) to several weeks (Barnes, 1979).

LTP has been demonstrated in all three synapses in the trisynaptic circuitry of the hippocampus. LTP was first demonstrated in the perforant path-dentate gyrus circuit (Bliss & Lomo, 1973; Bliss & Gardner-Medwin, 1973). Since 1973, LTP has been extensively studied in all three hippocampal synapses in hippocampal slices (Andersen et al., 1977; Lynch, Halpain & Baudry, 1982; Malenka, Madison & Nicoll, 1986; Yamamoto & Sawada, 1981). It has also been studied extensively in anesthetized animals (Bliss & Lomo, 1973; Abraham & Mason, 1988; Bliss, Lancaster & Wheal, 1983) and in freely behaving animals for the first of these synapses, the perforant path to dentate gyrus synapse (Bliss & Gardner-Medwin, 1973; Barnes, 1979; Gilbert & Mack, 1990; Morris, Anderson, Lynch & Baudry, 1986). Electrical trains delivered to the medial perforant path in the 200 to 400 Hz range are generally effective in producing LTP of the dentate granule cells.

An additional characteristic of LTP, reflective of the greater effect on spike values than EPSP values, is a leftward shift in the EPSP to spike (E-S) relationship (Bliss & Lomo, 1973; Abraham et al., 1984; Kairiss et al., 1987). This shift represents an increase in synaptic efficacy such that the same EPSP value will be associated with a larger population spike value after LTP induction than before LTP induction. This shift has also been reported to be associated with a decreased slope of the E-S

relationship under some conditions (Abraham et al., 1984; Kairiss et al., 1987).

In addition to LTP induced by high frequency trains of stimulation, a form of LTP which is induced by the pairing of stimuli from sequentially activated or co-active afferents has been described (McNaughton, Douglas & Goddard, 1978; Dunwiddie & Lynch, 1978; Rose & Dunwiddie, 1986; Winson & Dahl, 1986). This method of inducing LTP involves sending a single pulse through one pathway onto the post-synaptic cells, and shortly afterwards sending a train of stimulation through another pathway to the post-synaptic cell. This form of LTP induction was an exciting discovery on one hand because it is induced at frequencies much more similar to frequencies (i.e. lower frequencies) that go through the pathway normally in animals. On the other hand, it demonstrated a synaptic cooperativity of a Hebbian nature, hence providing support for Hebb's model of synaptic strengthening.

G. NMDA Receptor Dependency of Hippocampal Plasticity

The mechanism which has received the most attention in hippocampal plasticity is the excitatory amino acid receptor system in LTP. As previously mentioned, the hippocampus is rich in receptors for the excitatory amino acid glutamate (Cotman et al., 1987; Monaghan et al., 1989; Cotman &

Iversen, 1987). Two of the receptor subtypes, the AP4 and kainate receptors, have no demonstrated role in LTP. The AP4 receptor is most often pre-synaptic, and is thought to function pre-synaptically as an autoreceptor (Monaghan et al., 1989; Barnes & Henley, 1992). Although post-synaptic AP4 receptors have been described, especially in the retina, it appears that AP4 receptors in the hippocampus all function as autoreceptors. Kainate receptors may also be pre-synaptic, and are associated with ion channels (Monaghan et al, 1989; Barnes & Henley, 1992).

Although little is known about the role of the metabotropic receptor in LTP, it is possible that this receptor plays some role. The metabotropic receptor is linked to the phosphotidylinositol second messenger system, and therefore has further reaching and longer-term effects on the post-synaptic cell where it is located (Monaghan et al., 1989; Barnes & Henley, 1992). These characteristics of the metabotropic receptor make it a good candidate for part of the system which induces and maintains changes associated with LTP.

The remaining two receptor subtypes are those that have demonstrated roles in LTP (Muller, Joly & Lynch, 1988). The AMPA receptor is involved in normal synaptic transmission in the hippocampus (Sommer, Keinanen, Verdoorn, Wisden, Burnashev, Herb, Kohler, Takagi, Sakmann & Seeburg, 1990). The kinetics of the receptor are rapid, and the duration of

receptor action is brief (Sommers et al., 1990, Monaghan et al., 1989). If AMPA receptor activity is blocked, normal transmission in the hippocampus is blocked as well. Activation of the AMPA receptor opens its channel for monovalent cations, which can then flow freely across the post-synaptic membrane to depolarize the cell.

In contrast to the actions of the AMPA receptor, the NMDA receptor plays no apparent role in non-tetanic synaptic transmission in the hippocampus. When NMDA receptors are blocked, non-tetanic transmission occurs normally (Dingledine, 1983; Collingridge et al., 1983; Wigstrom, Gustafsson & Huang, 1986). The kinetics of the NMDA receptor are slow, and its action is longer term (Monaghan et al., 1989). The nature of these kinetics suggests that the NMDA receptor plays a more important role in longer term changes in hippocampal neurons. Perhaps the most interesting property of the NMDA receptor is that it is both ligand and voltage-dependent (Dingledine, 1983; Herron, Lester, Coan & Collingridge, 1986). That is, glutamate and other NMDA receptor agonists applied to NMDA receptors fail to activate the receptor unless the post-synaptic membrane is already depolarized. This voltage-dependency prevents the NMDA receptor from being active during normal glutamate release. The NMDA receptor is a receptor complex which has sites for glutamate and glycine binding nestled around a calcium channel. Magnesium ions sit in the calcium channel,

blocking calcium from flowing through into the post-synaptic cell. When the post-synaptic cell is depolarized, the magnesium blockade is released, allowing glutamate binding to open the calcium channel for calcium to pass through into the cell. Therefore, NMDA receptor activation requires the presence of both a receptor ligand and a depolarized post-synaptic membrane.

The currently proposed sequence of receptor events in LTP involves an interaction between the effects of glutamate release on the post-synaptic activities of the AMPA and NMDA receptor subtypes. The initial stimulation(s) will not open the NMDA receptor channel for calcium influx because of the magnesium blockade. It will, however, activate the AMPA receptor, allowing cations to enter the cell and depolarize the post-synaptic membrane. Because the stimulations are very close together for LTP-inducing stimuli, the next stimulation(s) will cause the release of glutamate onto the post-synaptic cell while the cell is still depolarized. Because the cell is depolarized, the NMDA receptor channel can open, allowing an influx of calcium ions. These calcium ions can set off a long chain of events which might contribute to the induction and maintenance of LTP. One of these events directly affects the AMPA receptor subtype. Specifically, there are two known forms of the AMPA receptor, flip and flop (Sommer et al., 1990). These two forms are distinct receptors coded by two different

transcripts. Flip has a higher efficacy than flop. It appears that changes initiated by calcium influx through the NMDA receptor channel eventually lead to an increased number of flip versus flop AMPA receptors on the post-synaptic membrane. This change in the AMPA receptor causes the post-synaptic cell to have an enhanced response to stimulation, such as the enhanced responses measured during LTP (Sommer et al., 1990; Muller et al., 1988; Izumi, Miyakawa, Ito & Kato, 1987).

LTP in two areas of the hippocampus, the dentate gyrus and CA1, depends on NMDA receptor activation (Collingridge et al., 1983; Harris, Ganong & Cotman, 1984; Wigstrom, Gustafsson & Huang, 1986; Morris et al., 1986; Errington, Lynch & Bliss, 1987) whereas in CA3 it does not (Zalutsky & Nicoll, 1990). Evidence that LTP depends on NMDA receptor activation comes from the finding that the spike and EPSP enhancement normally observed during LTP is suppressed when NMDA receptor antagonists are present. Results from studies of the effects of MK-801, a non-competitive NMDA receptor antagonist which blocks LTP <u>in vitro</u> (Coan, Saywood & Collingridge, 1987), on LTP induction <u>in vivo</u> in the dentate gyrus have been inconsistent. Abraham and Mason (1988) have reported that MK-801 does significantly block spike enhancement following LTP induction in the dentate gyrus of anesthetised rats, but only in the very highest dose used, 1.0 mg/kg. They observed no significant reduction in spike

amplitude at 0.05 or 0.10 mg/kg MK-801, and additionally, they measured no significant reduction in EPSP amplitude. Halliwell and Morris (1987) have also found no interference with LTP after MK-801 administration in vivo. More recently, Gilbert and Mack (1990) report that 1.0 mg/kg and 0.10 mg/kg MK-801 both block spike potentiation in the dentate gyrus of freely behaving rats 24 hours after LTP induction. Fifteen minutes after LTP induction, this reduction was only observed in the 0.10 mg/kg MK-801 group. Interestingly, Gilbert and Mack report no potentiation of the EPSP in freely behaving rats after induction of LTP, even in the vehicle condition.

H. LTP and Learning and Memory

Evidence has been found for some relationship between LTP and behavioral learning. First, induction of LTP has been demonstrated to enhance learning in animal studies of associative learning (Berger, 1984; Laroche, Doyere & Bloch, 1989). Second, saturating levels of LTP have been reported to intere with spatial learning (Barnes, 1978; McNaughton, Barnes, Rao, Baldwin & Rasmussen, 1986; Castro, Silbert, McNaughton & Barnes, 1989), although recent studies have failed to replicate this finding (Korol, Abel, Church, Barnes & McNaughton, 1992). These two pieces of evidence suggest that learning and LTP may rely on plasticity in the

same circuitry. Third, NMDA receptor blockade, in addition to interfering with LTP induction, has been shown to inhibit hippocampally-mediated spatial learning in rats (Robinson, Crooks, Shinkman, and Gallagher, 1989; Morris et al., 1986). LTP has been discussed as a candidate learning mechanism in the mammalian hippocampus (Teyler & Discenna, 1984; Collingridge, 1987). The NMDA receptor, on which LTP depends, is Hebbian by nature. That is, the NMDA receptor is not activated unless the cell receives post-synaptic stimulation at least twice; once to depolarize the cell, allowing the NMDA receptor channel to be opened, and a second time to actually open it. That is, the NMDA receptor will not function unless the post-synaptic cell is already depolarized. It is this dependence on repetitive stimulation for cell depolarization that makes the NMDA receptor so important for plasticity as opposed to normal transmission in the nervous system (Collingridge & Singer, 1991). LTP's role as a candidate learning mechanism is strengthened by the fact that its activation is dependent on the NMDA receptor, and is therefore Hebbian in nature, as associative learning is hypothesized to be.

In order to further strengthen the claim that LTP might be closely linked to learning processes, attempts have been made to establish mechanistic links between LTP and learning. The demonstration of interference of both LTP and learning by NMDA receptor blockade is one such link. The

difficulty that has arisen in drawing conclusions from these studies is that there is little evidence for the blockade of both learning and LTP in vivo by the same doses of the same NMDA receptor antagonist (Keith & Rudy, 1990). Robinson et al. (1989) have demonstrated learning impairment in rats with low doses of MK-801. It is necessary to test learning using low doses of MK-801 because at higher doses, animals experience severe motor impairments which make the performance of most learning tasks nearly impossible (Clineschmidt, Williams, Witoslawski, Bunting, Risley, & Totaro, 1982). Unfortunately, most studies of MK-801 blockade of LTP induction have used much higher doses of MK-801. Fortunately, Morris et al. (1986) were able to demonstrate learning deficits as well as interference with LTP induction using comparable doses of AP-5. Further demonstrations of similar dose-response curves for the two phenomena will be necessary, however, if a parallel is ever to be drawn between LTP and hippocampally-mediated learning.

I. Naturally-Induced Hippocampal Plasticity

Some forms of naturally-occurring physiological plasticity have also been discovered which resemble LTP in that there are changes in synaptic efficacy. Not unexpectedly, these forms of natural plasticity depend on exposure to or changes in configural environmental cues.

The first form was characterized by Sharp, McNaughton, and Barnes in 1985. In this form of naturally-induced plasticity, rats exposed to complex environments (i.e. with toys and social interactions) demonstrate an less reliable (Sharp, Barnes & McNaughton, 1989) increased field EPSP and more consistent increased population spike magnitude in perforant path-dentate gyrus evoked potentials. Although the increase in magnitude is not as dramatic as in LTP, it does have the same physiological profile, and it does appear to be relatively long-lasting. A common characteristic of the two phenomena was recently described when a rapid increase in the induction of an immediate early gene, zif-268, was demonstrated in the hippocampus of animals exposed to complex environments (Withers, Wallace, Weiler & Greenough, 1991). LTP has been shown to also result in a rapid increase in zif-268 levels (Cole, Saffen, Baraban & Worley, 1989). Therefore, LTP and exposure of animals to complex environments share at least one common biochemical correlate.

A more transient form of environmentally-induced plasticity, termed short-term exploratory modulation (STEM) (Barnes, McNaughton & Erickson, 1991) has been characterized (Sharp et al., 1989; Green et al., 1990). In this form of plasticity, rats will demonstrate a rapid and dramatic increase in granule cell field EPSP magnitude accompanied by a decrease in population spike magnitude throughout the

first ten to fifteen minutes that an animal is placed in a different environment. The observed changes in synaptic efficacy do not appear to be due to handling, EEG, or motoric factors, but rather reflect the recent exploratory history of the animal (Green et al., 1990). The fact that the value of the EPSP at any time during a recording session can be predicted with fairly good accuracy by knowing past EPSP values and the animal's recent history of exploratory behavior (Sharp et al., 1989) suggests that this change in efficacy may be a result of short-term information processing about the new environment. In addition, this change occurs each time the animal is placed back in the environment, even if the animal has had past experience with the environment, suggesting that the animal might be "orienting" itself in space using the available spatial information.

An alternative explanation for STEM has recently been proposed (Moser, Mathiesen & Andersen, 1993). The EPSP enhancement observed during STEM can be induced not only by active exploration, but also by warming the brain to increase brain temperature. This experiment suggests that the EPSP enhancement may be caused by an elevation in brain temperature which accompanies active exploration. Their explanation fails to account for the change in spike amplitude. Correlations between brain temperature and spike depression were only detected during exploration. Passive

warming of the brain did not induce spike depression. Therefore, it is possible that the EPSP enhancement is temperature-dependent, but that the spike depression is more closely related to exploration and information processing in the animal. The current dissertation will reveal several dissociations between the EPSP enhancements and spike depressions observed during STEM. These dissociations lend support to the notion that the two components of the STEM phenomena may rely on different mechanisms.

In addition to morphological changes occurring within existing neurons, recent work has suggested that the adult mammalian hippocampus exhibits a form of plasticity rarely seen in adult nervous systems. The hippocampal dentate gyrus of adult rats exhibits neurogenesis, or ongoing neuron birth (Cameron, Gould, Daniels & McEwen, 1991), and ongoing cell death as well (Gould, Woolley & McEwen, 1990). Although the functional significance of these findings is currently unclear, cell turnover in the adult dentate gives the structure the potential for fairly dramatic changes in circuitry.

J. Rationale

To determine exactly how the hippocampus functions to initiate changes in circuitry leading to long-term memory storage, it is necessary to understand the mechanisms by

which it changes. Much is now understood about the changes that occur during LTP. LTP is, however, an artificially induced form of plasticity. In many experiments, the frequencies used to induce LTP are not firing frequencies encountered normally by animals. Therefore, in order to understand how the hippocampus works normally to process information for long-term memory storage, one must understand the mechanisms and characteristics of naturally-occurring changes in the hippocampus.

The experiments in this dissertation were designed to study the mechanisms and characteristics of one of the forms of naturally-occurring plasticity in the hippocampus, STEM. It will study the characteristics of STEM, which occurs in the dentate gyrus, primarily by comparing its characteristics to those of dentate LTP. This comparison will allow a better understanding about whether LTP versus STEM are artificial versus natural forms of similar processes, or whether STEM represents a different and unique form of hippocampal plasticity.

Because it is well-established that LTP in the dentate gyrus is dependent on NMDA receptor activation, and because hippocampally-mediated learning also appears to depend on NMDA receptor activation, the first two experiments were designed to determine whether or not STEM is also dependent on NMDA receptor activation for its induction.

The first experiment confirmed that LTP can be blocked by behaviorally relevant doses of the NMDA receptor antagonist, MK-801, in freely behaving rats. This confirmation of results first obtained by Gilbert and Mack (1990) was important because low doses of MK-801 need to be used to test the NMDA receptor dependence of STEM. As previously mentioned, doses of MK-801 above .10 mg/kg result in motor impairments so severe, that animals are not able to move. Because STEM is dependent upon an animal actively exploring its environment (Sharp et al., 1989), it was crucial that animals retain this ability. It was important to replicate Gilbert and Mack's results before testing MK-801 in STEM, because they have been the only researchers to successfully interfere with LTP induction with this dose of MK-801 in vivo. Abraham and Mason (1988) found that this dose of MK-801 does not interfere with LTP in vivo. Abraham and Mason's rats were anesthetised, however, whereas Gilbert and Mack's were awake.

The second experiment assessed the effects of MK-801 on STEM. Three doses of MK-801 were used, the .10 mg/kg tested for LTP, and two lower doses, .05 and .08 mg/kg MK-801, to examine the dose dependency of the effect. These doses were selected based on pilot studies examining the behavioral effects of MK-801 at different doses. This experiment was important because it allowed a direct comparison between the mechanisms of artificially-induced LTP and naturally-induced

STEM. The finding that MK-801 interferes with STEM demonstrated that it may share a commonality of mechanism (i.e. NMDA receptor dependence) with LTP.

The third experiment used a stimulus intensity series to confirm that the leftward shift in the E-S relationship normally observed during LTP is found in freely behaving rats. Thus, an increased EPSP associated with similar spikes results from LTP in this preparation. This increased EPSP from stimulation of afferents could reflect an increased synaptic efficacy due to changes at the primary afferent synapse onto the cell population or the increased efficacy of another pathway in the circuit which influences the response of the synapse. In addition, the third experiment examined the effects of .10 mg/kg MK-801 on the E-S relationship shift. This experiment clarified the role of the NMDA receptor in the increased synaptic efficacy observed during dentate LTP.

To draw further comparisons between artificially induced LTP and naturally induced STEM, Experiment 4 examined whether or not STEM, like LTP, is associated with an alteration of the E-S relationship. Using stimulus intensity series, Experiment 4 examined the E-S relationship in animals first transferred into a different environment, and measured it again three and 20 minutes after environmental transfer. Because STEM is developing by three minutes after transfer, and has fully developed by twenty

minutes after transfer, changes in the E-S relationship during STEM were measured by comparing the relationship at these time points to that measured immediately after transfer. Similarities and differences between STEM and LTP were observed based on their unique profiles of E-S relationship shifts, and the NMDA receptor dependencies of these shifts.

The final experiment, Experiment 5, set out to determine if the properties of STEM change with variable amounts of stimulation or remain stable regardless of the amount of stimulation. LTP is induced by larger amounts of stimulation than the amount presumed to induce STEM. If the two phenomena are similar, or if STEM is simply a "mild" form of LTP, then STEM would be expected to alter its phenomenology to appear more similar to LTP with increased amounts of stimulation. As previously mentioned, exposure to large amounts of environmental stimulation results in spike enhancements more reminiscent of LTP (Sharp et al., 1985). If STEM and LTP are unique, and underlie different behavioral processes, then we would expect to see STEM change its phenomenology in some way which does not resemble LTP, or to maintain its integrity regardless of the amount of stimulation given to the animal.

The experiments in this dissertation will provide evidence that although STEM and LTP may share some commonality of mechanism, they are also different in many ways.

Chapter 2. General Methods

Subjects

Male Fischer-344 rats (9-11 month old retired breeders), similar to those used in past STEM experiments, were used as subjects for all experiments. Subjects were housed in the Queens College New Science Building animal facility with food and water available ad libitum. Colony rooms were maintained on a 12 hour light-dark cycle (lights on 0700 hours).

Surgery

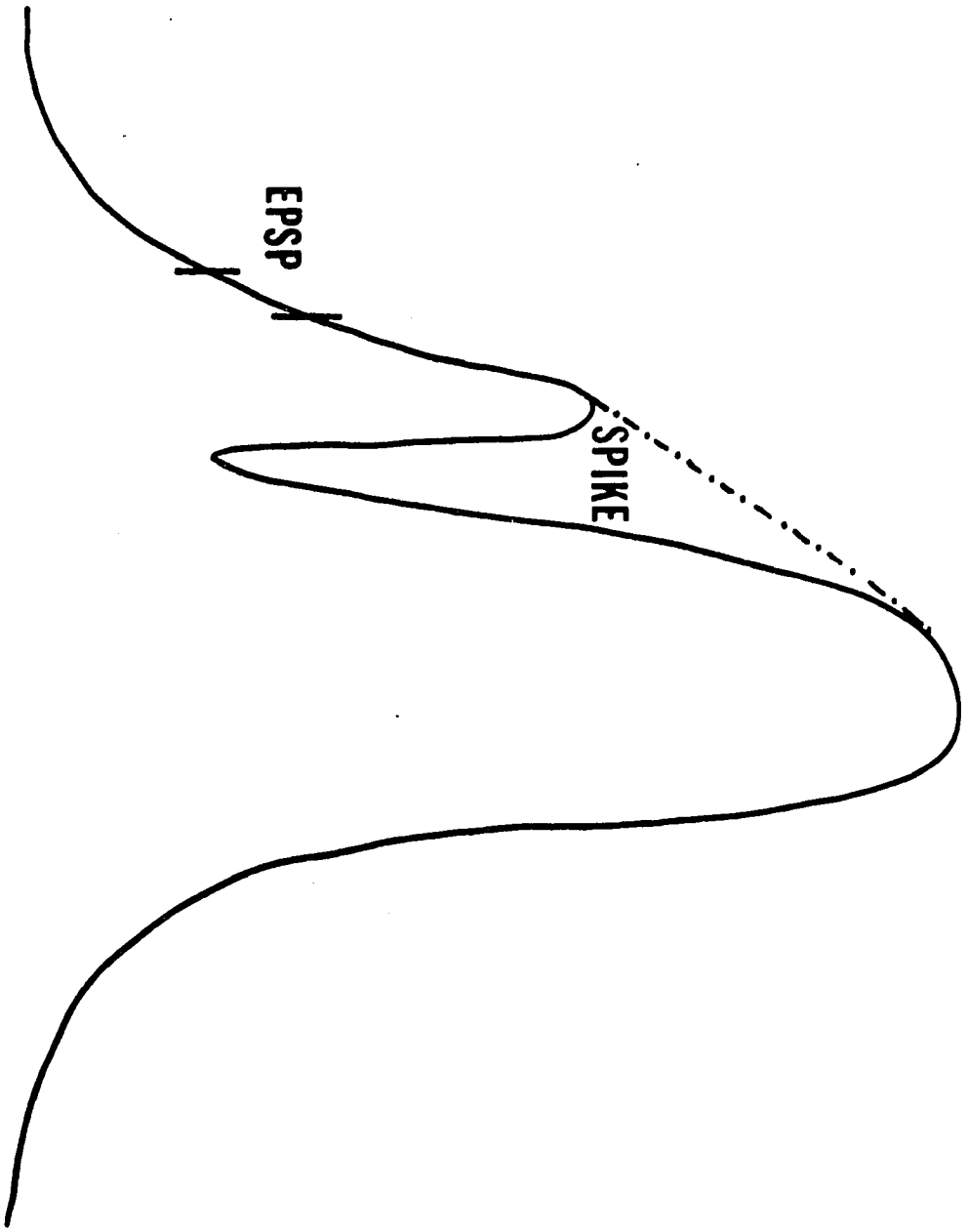
Rats were chronically prepared (under 75 mg/kg Nembutal anesthesia) for recording of perforant path-dentate gyrus evoked potentials (as described by Sharp, Barnes, & McNaughton, 1987). Teflon insulated, stainless steel, monopolar electrodes were stereotaxically implanted at approximately 4.4 lateral, 8.1 posterior (referenced to bregma) for stimulating in the angular bundle of the medial perforant path and 2.4 lateral, 3.9 posterior (referenced to bregma) for recording in the hilus of the dentate gyrus bilaterally. Electrode depths and exact electrode placements were adjusted using physiological criteria. The electrodes were connected via gold connector pins to an

Augat connector plug, and were chronically secured to the skull using dental acrylic. Ground screws with connector pins were used as current return and to anchor the head piece to the skull. Rats were given at least one week of post-surgical recovery before recording began.

Recording and Stimulating Equipment

During recording, rats were connected to the stimulating and recording equipment through a lead wire connected to the Augat connector plug on their skull caps. The lead wire hung freely from a wooden frame suspended above a standard colony cage, allowing the animal free movement. Single pulse square wave stimuli were delivered under the control of a Televideo 386 personal computer at .1 Hz (except where noted) through Grass S44 stimulators and Grass stimulus isolation units. The stimulus intensity remained constant throughout each experiment for each channel (with the exception of the stimulus intensity series in Experiments 3 and 4), and was set prior to the first day of recording at the value which evoked the minimum reliable spike (ranging from 2 to 15 volts in different channels). Concurrent with each stimulus pulse, the evoked response was amplified through a x1 FET and a x10 amplifier to an A/D connector box from which the computer sampled evoked responses at 20kHz for 25 msec. Data collection and

Figure 2. Schematic representation of measurement of EPSP and population spike values for a typical granule cell evoked potential. EPSP is measured as the change in magnitude (i.e. slope of the curve) between two fixed time points after stimulus onset (points indicated by slashes on rising phase of waveform). Population spike is measured by the area between the curve and a tangent line connecting the points of spike onset and offset (indicated by slashed line).


EPSP
SPIKE

analysis was performed using Brainwave Systems'(Broomfield, CO) "Experimenter's Workbench" software.

Measurement of Physiological Responses and Data Analysis

As illustrated in Figure 2, the field EPSP, or the synaptic component of the response, was measured as the amplitude of the waveform between two fixed time points on the rising phase of the waveform (roughly representing the 'slope' of the curve). The population spike (which is thought to result from granule cell firing), was measured as the area between the waveform and a tangent line connecting the points of spike onset and offset (as described by Barnes, 1979). EPSP and spike values were automatically generated by the computerized waveform analysis program after the two points on the rising phase of the waveform used to extract the EPSP value were selected by the experimenter. Throughout the five experiments, EPSP values are expressed as mV change per .4 milliseconds after a 10x amplification of the potential. Spike values are represented by a number generated by the computer program to quantify the area between the tangent line and the curve. Although these spike values can be used to compare spikes from various waveforms and animals, they do not themselves represent an established unit of measurement. EPSP and spike values were measured this way because prior

experiments with STEM had quantified EPSP and spike values in a similar manner using the same software. Use of this system of measurement ensured that results of these experiments could be directly related to previous findings.

For each experiment, baseline measurements were taken at the start of each recording session for both EPSP and population spike values (five to 30 values were averaged, as indicated for each experiment). Subsequent EPSP and population spike values were normalized against the baseline values so that changes in magnitude could be readily assessed. Baseline values were set at 1.0, so that subsequent normalized values represented the proportion of change in the evoked potential magnitude. All analyses were performed on the normalized values, except where noted otherwise.

Sample waveforms exhibiting the characteristic EPSP and spike enhancements normally observed during LTP (Figure 3) and STEM induction (Figure 4) are included.

Except when otherwise stated, all analyses were two or three-way analyses of variance (ANOVAs) performed on normalized spike and EPSP values. For each experiment, mixed Factorial ANOVAs were performed to examine differences across time as well as differences between groups of animals. Group comparisons of treatment conditions, including drug dose (Experiments 1-4), stimulation type (Experiments 1 and 3), and environmental complexity

(Experiment 5), were between groups factors with each animal being exposed to only one manipulation. Within groups variables included days (Experiment 5) and time within a recording session (all experiments), with each animal being measured at each time point.

Figure 3. Sample waveforms from one animal given high frequency stimulation (15 400 Hz trains of stimulation - each train separated by 10 sec). The solid waveform represents the pre-induction evoked potential, and the dashed waveform represents the potentiated post-induction evoked potential. (scale bars = 1ms, 3.9 mV)

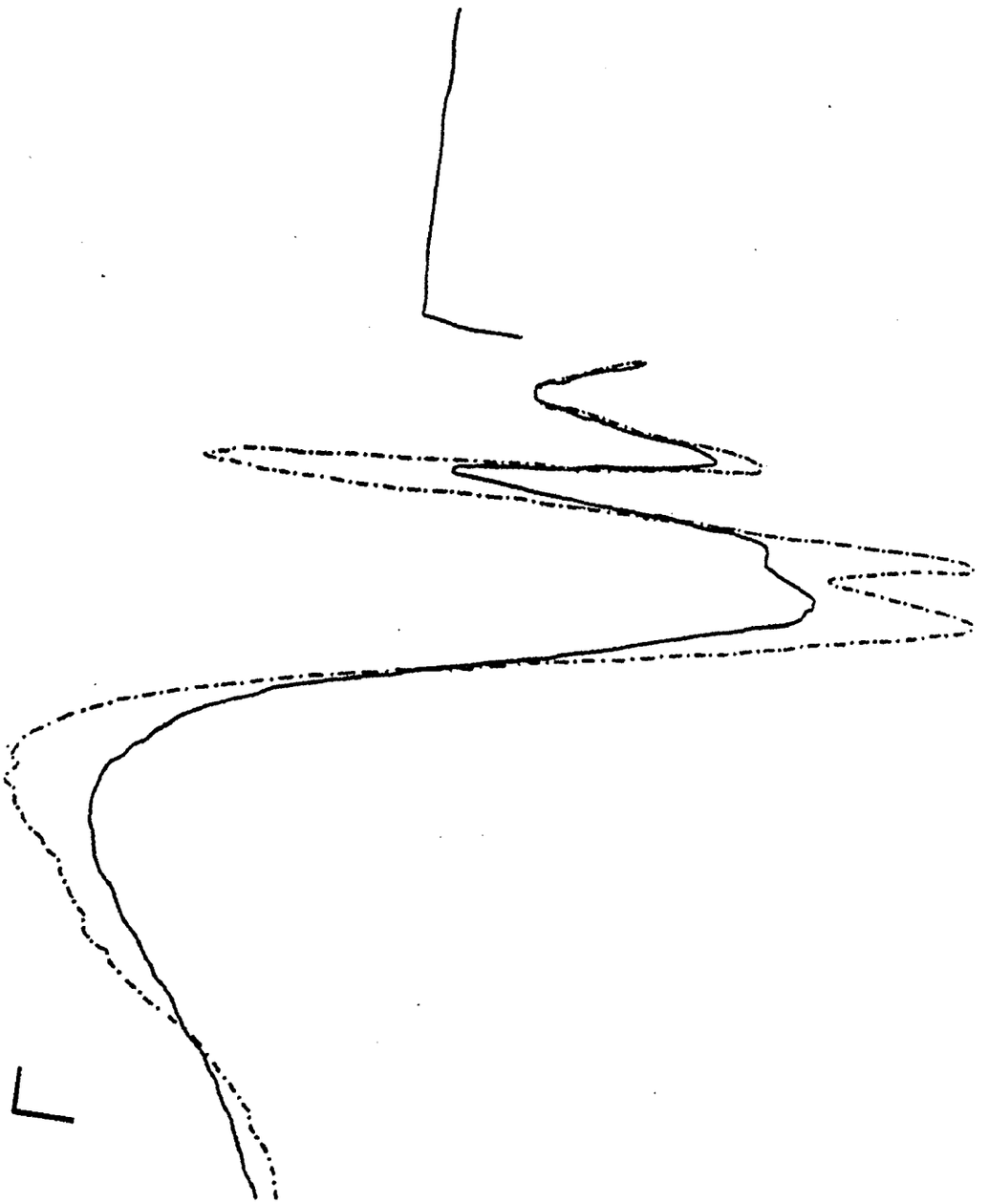
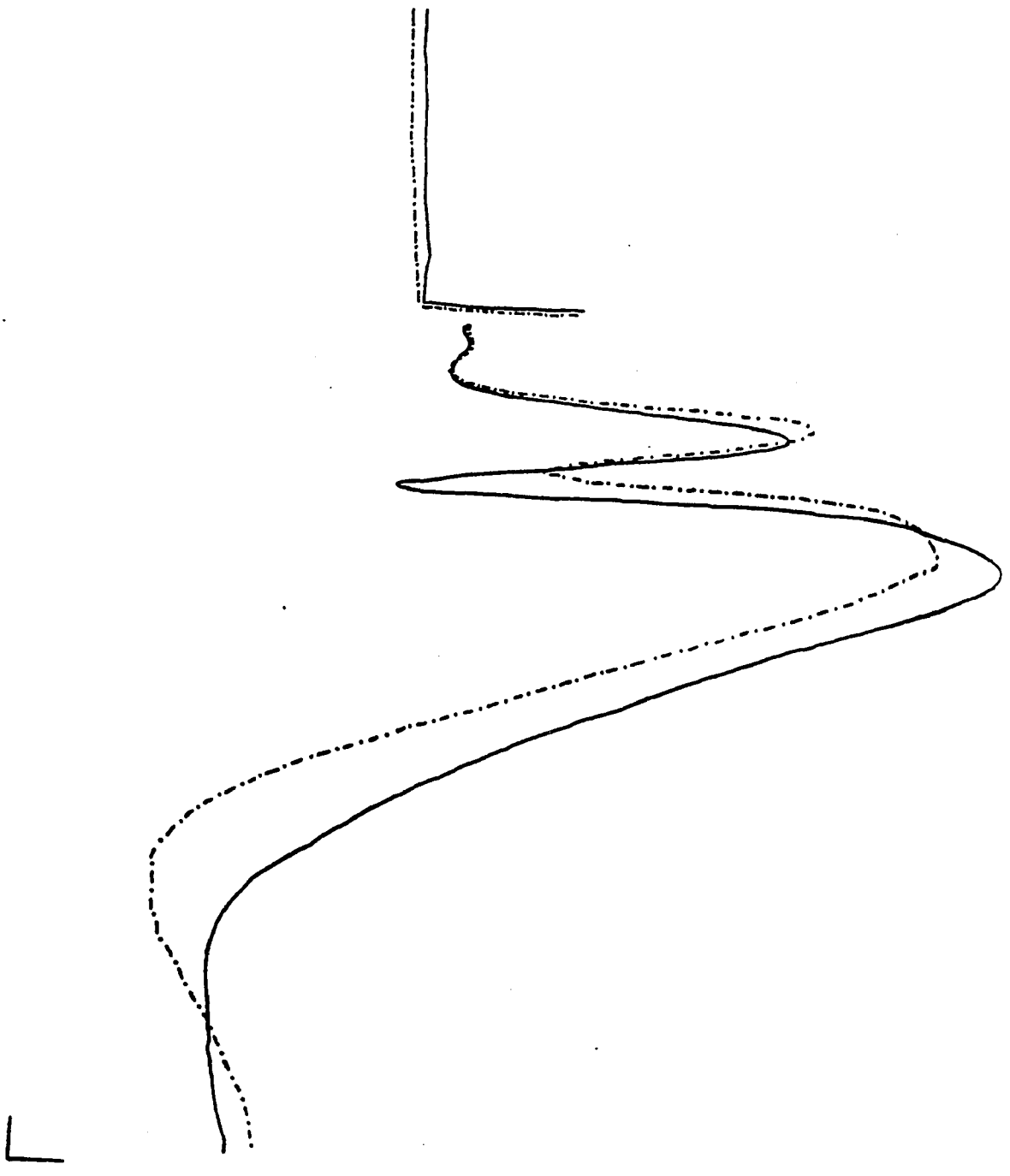


Figure 4. Sample waveforms from one animal transferred into a different environment. The solid waveform represents the pre-induction of STEM evoked potential, and the dashed waveform represents the post-induction of STEM evoked potential. (scale bars = 1ms, 11.9 mV)



Chapter 3. Experiment 1: The Effects of MK-801 on EPSP and Spike Magnitudes during Long-Term Potentiation in Freely Behaving Rats

A. Introduction

The first experiment tested the effects of a low dose of MK-801 (0.10 mg/kg) on LTP induction in freely behaving rats. Abraham and Mason (1988) observed no significant effect of this dose of MK-801 on LTP induction in anesthetised rats. Gilbert and Mack (1990), however, reported a significant interference with the spike potentiation in unanesthetised rats after MK-801 administration. Experiment 1 will examine Gilbert and Mack's (1990) finding that low doses of MK-801 interfere with LTP induction in unanesthetized rats. The results of this experiment can be found in Bostock, Croll & Sharp (1990).

B. Methods

Procedures

Rats were injected sub-cutaneously with 0 mg/kg (vehicle) or .10 mg/kg MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate, or

dizocilpine, supplied by Merck & Company and dissolved in saline). Thirty minutes after the injection, each animal was placed in an opaque carrying box and transported to the recording room. Fifteen minutes of baseline evoked potentials were collected. In addition to providing a baseline measurement, this fifteen minutes allowed the animal's physiology to stabilize (i.e. any changes resulting from STEM should have reached asymptotic levels). Following the 15 minute baseline, 15 high frequency (400 Hz) pulses of 20 msec duration were delivered through both stimulating electrdes simultaneously. For ten minutes immediately following stimulation evoked potentials were collected and animals were monitored for seizure activity. No seizure activity was recorded from any animal. From the ten minutes of post-stimulation recording, two minute samples of evoked potentials were averaged from the first two minutes to establish average EPSP and spike values for immediately after stimulation, and from minutes eight to ten to establish values for ten minutes after stimulation. Additional two-minute samples of evoked potentials were taken at the 20, 30, and 40 minute post-stimulation time points to observe the time course of physiological changes following high frequency stimulation.

Data Analysis

For the purpose of analyses, the magnitudes of evoked potentials were examined at six time points, one prior to LTP and five following the induction of LTP. The magnitude of the evoked potential at the first time point was taken as the average of 30 baseline sweeps taken just prior to LTP induction. The 30 baseline sweeps (i.e. the five minutes just prior to stimulation) provided a good estimate of the animal's pre-stimulated baseline EPSP and spike values. The magnitude of the evoked potentials at five subsequent time points measured following LTP induction consisted of the average of the twelve sweeps immediately following LTP induction, and twelve sweeps each at 10, 20, 30, and 40 minutes after high frequency stimulation. Fewer sweeps were averaged in order to calculate averages for more precise time points post-stimulation. Each of the five subsequent time points were normalized against the initial evoked potential values as described in the general methods.

C. Results

As can be seen in Table 1, baseline EPSP and population spike values were not significantly different in the vehicle versus MK-801 groups. Following high frequency stimulation, there was a significant overall increase in EPSP values

across time periods ($F(5,70)=4.27$, $p<.002$) (see Figure 5). Animals receiving vehicle injections showed a mean 28% potentiation of the EPSP at 40 minutes after LTP induction, as compared to a mean potentiation of 18% in animals receiving MK-801. The decreased EPSP potentiation observed in animals receiving MK-801 was not significant ($F(1,14)=2.15$, $p>.165$).

There was also a significant potentiation of the population spike over time following high frequency stimulation ($F(5,55)=13.94$, $p<.0001$) (refer to Figure 6). MK-801 administration significantly reduced the population spike potentiation observed following high frequency stimulation ($F(1,11)=5.81$, $p<.035$). Animals receiving MK-801 showed no further spike potentiation after the initial small potentiation induced by the high frequency stimulation. Vehicle-injected animals, however, showed a continued increase of the population spike throughout the 40 minute follow-up period. Animals receiving vehicle injections had a mean potentiation of 277% 40 minutes after LTP induction, whereas animals receiving MK-801 showed a mean potentiation of only 95%.

D. Discussion

In Experiment 1, 0.10 mg/kg of MK-801 significantly interfered with LTP of the population spike induction in

Table 1. Baseline (averaged from 30 pre-stimulation sweeps) EPSP and spike values (mean + SEM) for vehicle versus MK-801 animals.

Group	baseline EPSP	baseline spike
Vehicle	64.30 ± 18.70	50.57 ± 14.10
MK-801	44.40 ± 13.50	78.75 ± 20.46

Note: Baseline values for animals injected with MK-801 did not differ significantly from those of animals injected with vehicle (baseline EPSP values ($t(14)=.864$, $p>.40$); baseline spike values ($t(11)=1.16$, $p>.27$)).

Figure 5. Normalized EPSP values (proportion of baseline EPSP) for vehicle versus MK-801 groups as a function of time after high frequency stimulation

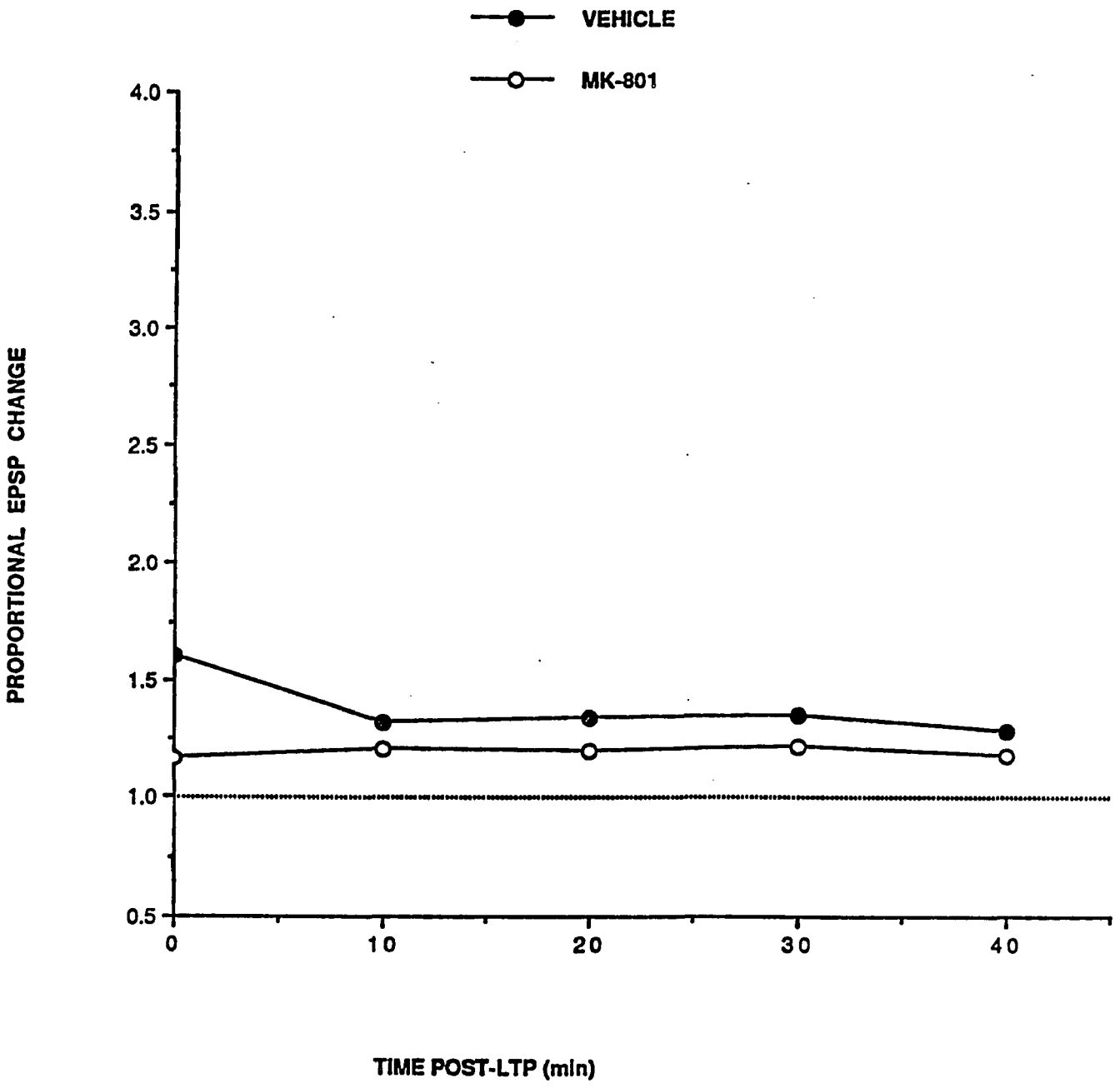
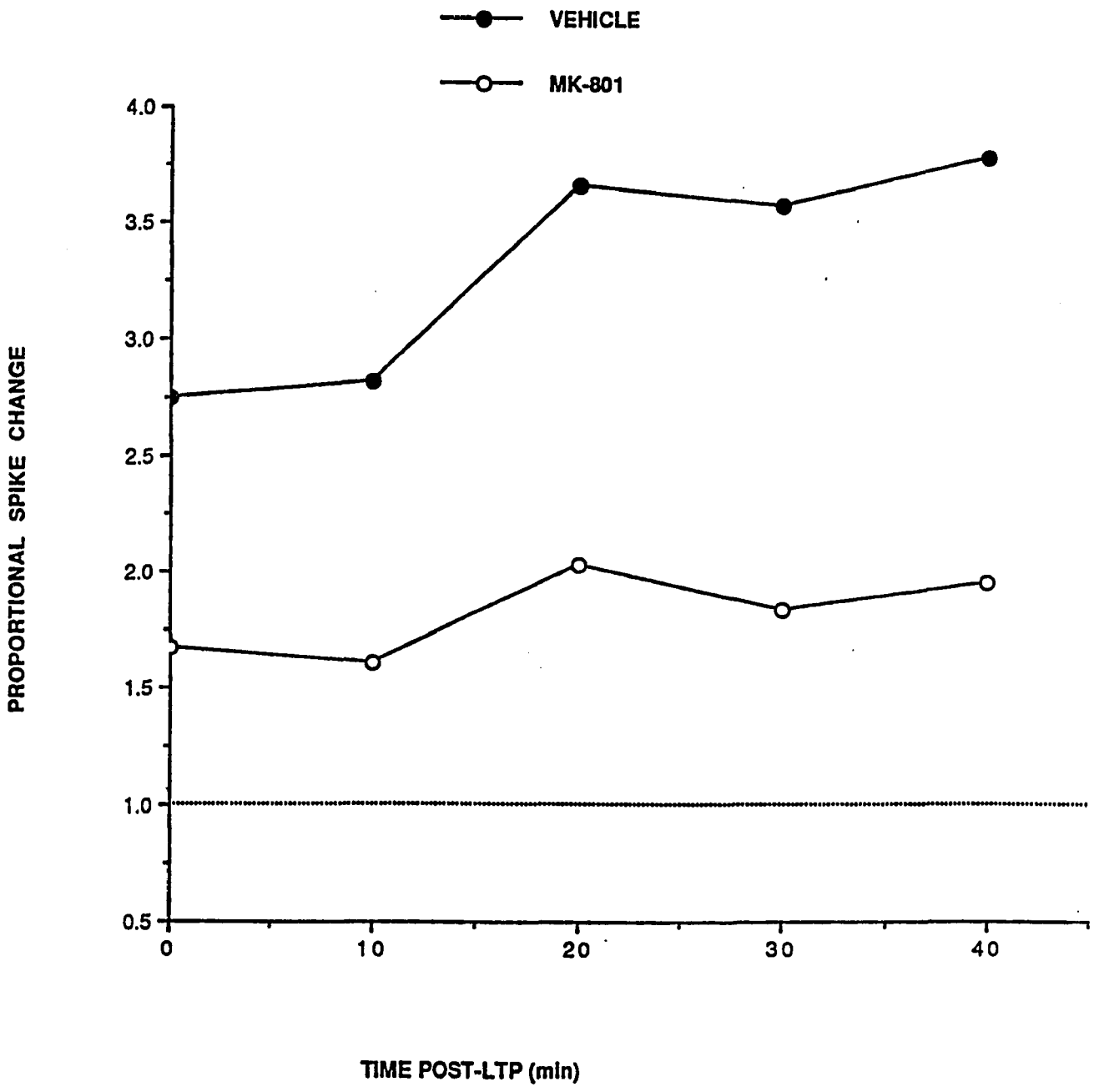

VEHICLE
MK-801
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
PROPORTIONAL EPSP CHANGE
0
10
20
30
40
TIME POST-LTP (min)

Figure 6. Normalized population spike values (proportion of baseline spike) for vehicle versus MK-801 groups as a function of time after high frequency stimulation.


VEHICLE
MK-801
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
PROPORTIONAL SPIKE CHANGE
TIME POST-LTP (min)

vivo. This result agrees with Gilbert and Mack's (1990) findings, but disagrees with Abraham and Mason's (1988). The experimental conditions in the present study differed from Abraham and Mason's (1988) in two important ways. First, and most significantly, Abraham and Mason (1988) tested MK-801 in urethane-anesthetized rats. Experiment 1, as well as Gilbert and Mack's (1990) study, tested MK-801's effects on LTP in unanesthetized rats. It is possible that the urethane anesthesia altered dentate physiology or peripheral mechanisms in such a way that low doses of MK-801 were not able to exert an effect on LTP induction at this synapse. Secondly, Abraham and Mason (1988) injected animals intraperitoneally, whereas in the current study animals were injected subcutaneously. This difference is not likely to account for the different results, since Gilbert and Mack (1990) used intra-peritoneal injections in their study as well.

The results of the present study confirm that LTP induction in the perforant path-dentate gyrus circuit is dependent on NMDA receptor activation. This finding has been well-documented in hippocampal slice studies, but has received less support in vivo. It now appears likely that NMDA receptor activation is necessary in vivo as well, even in freely behaving rats. Perhaps even more importantly, it appears that MK-801 is able to interfere with LTP induction at doses which interfere with the acquisition of learning

tasks in rats (Robinson et al., 1989). Although these results cannot provide direct evidence that LTP or LTP-like mechanisms are involved in learning, they at least provide information which is consistent with the notion that *in vivo* LTP and some forms of learning rely on similar mechanisms.

Chapter 4. Experiment 2: The Effects of MK-801 on EPSP and Spike Magnitude During Short-Term Exploratory Modulation

A. Introduction

Little is known about the mechanisms by which STEM is induced in rats. Dentate LTP is similar to STEM in that it is an experience-dependent change in synaptic efficacy. Much is now known about the mechanisms involved in the induction and maintenance of dentate LTP. It is not known whether STEM relies on some of the same processes for its induction as LTP does. Experiment 2 evaluated the effects of NMDA receptor antagonism on the development of STEM. NMDA receptor antagonists interfere with LTP induction in the dentate gyrus (Morris et al., 1986; Coan et al., 1987; Errington et al., 1987; Gilbert & Mack, 1990). Therefore, if LTP and STEM rely on similar receptor mechanisms for their induction, STEM should also be blocked or reduced in magnitude in the presence of NMDA receptor antagonists. Three doses of the NMDA receptor antagonist, MK-801, were used in this experiment. These three doses, .05, .08, and .10 mg/kg, were selected based on pilot studies which suggested that .05 mg/kg is the highest dose at which there is no behavioral evidence of a rat being under the influence of MK-801, and .10 mg/kg is the lowest dose at which motor impairments are still minor, such that rats can explore and

move effectively. The final dose, .08 mg/kg, was selected as an intermediate dose. In addition, these doses are similar to those used to observe learning impairments in rats (Robinson et al., 1989). The results of this experiment have been published in Croll, Sharp & Bostock (1992).

B. Methods

Procedures

After recovery from surgery, evoked potentials were recorded from 13 animals for one 20-minute period per week each for four consecutive weeks. All rats were rapidly transported (60-90 seconds) in an opaque carrying box from their home cages to the recording chamber where evoked potential recording started immediately. Because the EPSP enhancements associated with STEM can be predicted from an animal's recent history of exploratory behavior, observations of the rats' behavior were taken simultaneously with each evoked potential. A trained observer watched each rat throughout the recording session. Concurrent with each stimulus pulse, the observer entered a symbol directly into the computerized data acquisition system signifying the type of behavior in which the animal was engaging at the moment the pulse was delivered. The observer classified behaviors

into three categories. These categories were theta behaviors, grooming and other active non-theta behaviors (such as chewing or gnawing), or still alertness. Theta behaviors included any behaviors which are normally associated with theta EEG. These behaviors are exploratory in nature, and include rearing, walking, and sniffing. At the end of the 20 minute recording session, each animal was promptly returned to its home cage.

Thirty minutes prior to each recording session, animals were injected sub-cutaneously with MK-801 in doses of either .00 mg/kg (vehicle), .05 mg/kg, .08 mg/kg, or .10 mg/kg (dissolved in 0.5 ml saline). Each rat received only one of the four doses of MK-801 each week. In addition, each rat received each of the four doses during the course of the four week experiment. Half of the rats received the drug doses in an ascending dose series order and the other half of the animals received a descending dose series order.

Data Analysis

For the purposes of analyses, the 20 minute recording session was broken into six time bins. The first, or baseline, time bin consisted of the first five sweeps. Only the first five sweeps were included in the baseline average because of how rapidly changes in EPSP and spike values begin during STEM. Subsequent time bins included sweeps 21-

40, 41-60, 61-80, 81-100, and 101-120. EPSP and spike values at each time bin were normalized against the initial time bin. The between groups factor for the ANOVA was dose series order, such that each rat was either ascending or descending. The within groups factors were drug dose and time bin, such that all rats were evaluated at all doses and at all time points.

C. Results

Data from 22 hippocampi were available for the EPSP analysis (11 each in the ascending and descending dose series), and 17 were available for the population spike analysis (10 in the ascending dose series and seven in the descending dose series).

Baseline responses for the population EPSP and population spikes are given in Table 2. For the baseline EPSP values there was no effect of order ($F(1,19) = 0.36$, $p>.55$) or drug dose ($F(3,57) = 2.3$, $p>.09$) and no significant interaction between the two ($F(3,57) = 1.20$, $p>.3$). The trend toward an effect on baseline EPSPs as a function of drug dose ($p>.09$) is highly unlikely to account for the effects found in this experiment, because if MK-801 had an effect on baseline EPSPs, it was to increase these values. For the baseline population spike values there was also no significant effect of dose series order ($F(1,15) =$

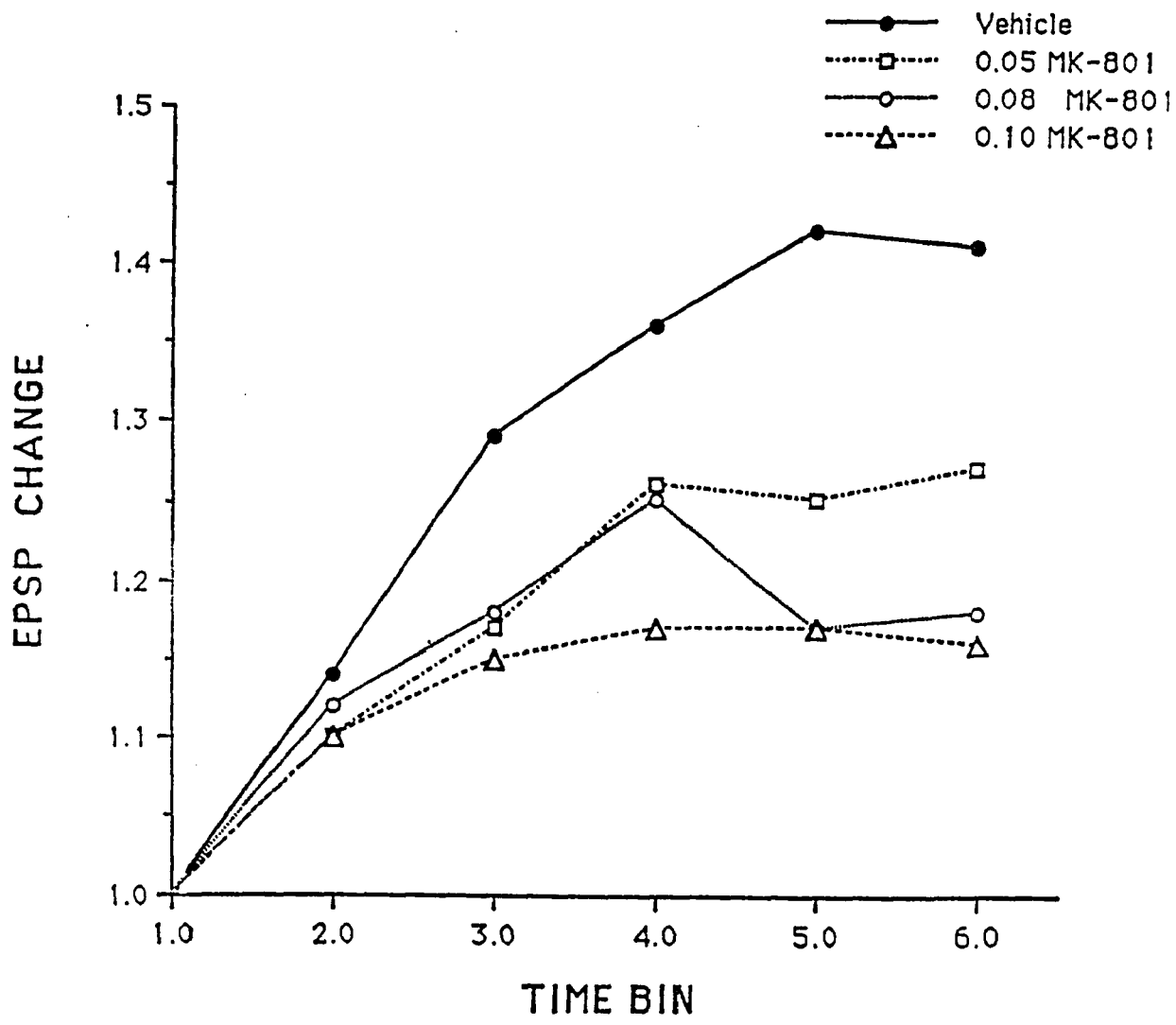
0.42, p>.5) or drug dose (F(3,45) = 1.8, p>.16) and no significant interaction between the two (F(3,45) = 0.41, p>.7). The stimulus intensities used for the two groups were 6.7 $\pm$ 1.1 V and 6.7 $\pm$ 0.8 V (mean $\pm$ SEM) for the ascending and descending dose series order groups, respectively.

Consistent with previous findings (Sharp et al., 1989; Green et al., 1990), the analysis revealed an immediate and dramatic enhancement of population EPSP magnitude (main effect of time bin, F(5,100) = 10.15, p<.0001) which took place over the first 10-15 minutes after animals were placed in the recording chamber (see Figure 7). MK-801 reduced this population EPSP growth 13 to 26 percent (F(3,60) = 2.65, p <.057). A Tukey HSD test showed that population EPSP enhancement values at all doses of MK-801 were significantly different from vehicle (p <.05, two-tailed), although the different doses of MK-801 were not significantly different from one another. Vehicle sessions showed a greater field EPSP enhancement than was seen during drug sessions. Although the main effect of MK-801 on field EPSP enhancement only approached significance, there was a significant drug dose x time bin interaction (F(15,300) = 2.14, p<.008). This interaction reflects the fact that MK-801 reduces field EPSP enhancement more dramatically during later time bins within the session than during initial time bins (see Figure 7). For the field EPSP, there was no

Table 2. Baseline population EPSP and population spike values for ascending dose series, descending dose series, and both groups combined for 4 doses of MK-801 (mean ± SEM).

Group	.00 mg/kg	.05 mg/kg	.08 mg/kg	.10 mg/kg
EPSP				
asc.	55.1 ±7.5	52.5 ±8.8	59.6 ±9.5	61.5 ±11.9
desc.	38.6 ±10.9	50.2 ±10.9	57.0 ±11.6	48.3 ±11.1
comb.	46.4 ±7.7	51.3 ±7.8	58.2 ±7.7	54.6 ±8.1
SPIKE				
asc.	102.5 ±13.4	85.7 ±20.1	93.6 ±21.5	68.4 ±21.2
desc.	139.6 ±23.7	118.5 ±25.6	95.1 ±25.1	91.2 ±24.9
comb.	117.8 ±13.1	99.2 ±15.9	94.2 ±16.3	77.8 ±18.9

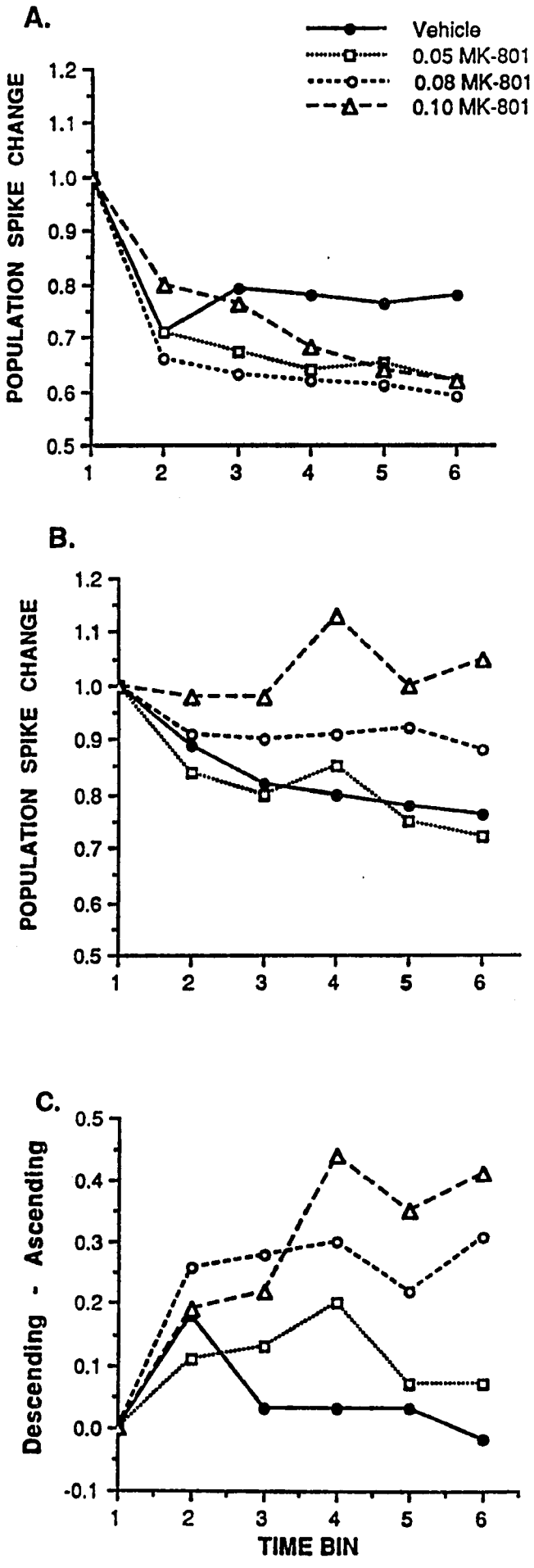
Figure 7. Normalized EPSP values (proportion of baseline EPSP) for all animals (ascending and descending dose series are combined) at each of the four doses of MK-801 (vehicle, .05 mg/kg, .08 mg/kg, and .10 mg/kg) as a function of time after environmental transfer (time presented in three minute time bins).


Vehicle
0.05 MK-801
0.08 MK-801
0.10 MK-801
EPSP CHANGE
1.5
1.4
1.3
1.2
1.1
1.0
1.0
2.0
3.0
4.0
5.0
6.0
TIME BIN

significant effect of dose series order ($F(1,20) = 2.25$, $p < .149$), such that animals receiving the ascending dose series did not differ significantly from those receiving the descending dose series. No other interaction effects were significant.

Also in agreement with previous findings (Sharp et al., 1989; Green et al., 1990), population spike magnitudes showed a gradual decrease throughout the session (main effect of time bin, $F(5,75) = 14.58$, $p < .0001$) (refer to Figure 8). Although the ANOVA for population spike area failed to detect any significant main effects of dose series order ($F(1,15) = 2.15$, $p > .16$) or drug dose ($F(3,45) = 1.65$, $p > .19$), there was a significant three-way interaction of time bin by drug dose by dose series order ($F(15,225) = 1.79$, $p < .037$). Population spike data are shown in Figure 8, in which panels A and B represent data from rats in the ascending and descending dose series, respectively. Rats receiving 0.10 mg/kg of MK-801 in the descending dose series showed no population spike depression. When population spike depression was observed, descending dose series animals showed a dose-dependent reduction of population spike depression, with the between dose differences increasing over time within a session. Animals in the ascending dose series group showed no obvious drug effects on population spike depression. In addition, the level of population spike depression observed across all doses within

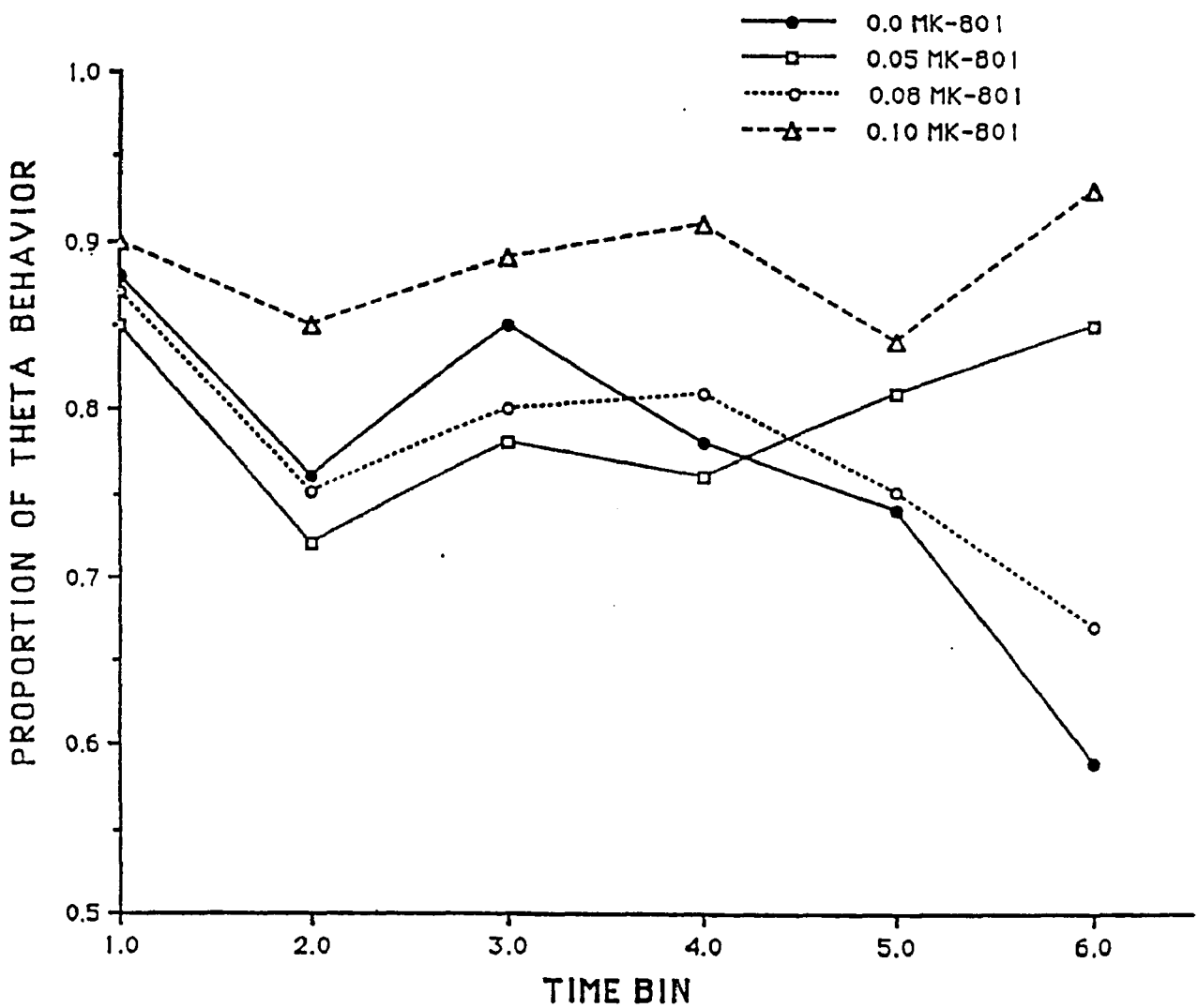
Figure 8. Normalized population spike values (proportion of baseline spike) for each of the four doses of MK-801 (vehicle, .05 mg/kg, .08 mg/kg, and .10 mg/kg) as a function of time (time presented in three minute time bins) after environmental transfer. A) animals in ascending dose series group B) animals in descending dose series group C) differences between ascending and descending population spike values across time at each dose of MK-801


A.
Vehicle
0.05 MK-801
0.08 MK-801
0.10 MK-801
POPULATION SPIKE CHANGE
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
1
2
3
4
5
6
B.
POPULATION SPIKE CHANGE
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
1
2
3
4
5
6
C.
Descending - Ascending
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
1
2
3
4
5
6
TIME BIN

the descending dose series group was less than that observed within the ascending dose series group. This observation suggests that MK-801 interfered with population spike depression selectively in animals in the descending dose series group.

To examine the effects of MK-801 on behavior, the behavioral observations originally made during data collection were collapsed into two categories, exploratory (or theta) behavior and nonexploratory behavior. The data were collapsed in this manner because STEM is suspected to be predicted from an animal's recent history of theta behavior (Sharp et al., 1989). Therefore, the behavioral measure of primary interest was the amount of theta in which the animal was engaged during any given time bin. The first category, theta behavior, consisted entirely of those behaviors which had been labelled theta, or exploratory during data collection. The second category, nonexploratory behavior, consisted of the combination of the non-theta active behaviors and still alertness. For each time bin, the proportion of theta behavior relative to total behavior was calculated, because previous research had demonstrated that theta behavior is related to STEM (Sharp et al., 1989). The ANOVA found only a significant effect of time bin ($F(5,55) = 2.66$, $p < .032$), such that theta behavior decreased as the session progressed (Figure 9). Behavior

Figure 9. Proportion of theta behavior to total behavior for all animals at each of the four doses of MK-801 (vehicle, .05 mg/kg, .08 mg/kg, and .10 mg/kg) calculated for each time bin after environmental transfer.


0.0 MK-801
0.05 MK-801
0.08 MK-801
0.10 MK-801
PROPORTION OF THETA BEHAVIOR
1.0
0.9
0.8
0.7
0.6
0.5
1.0
2.0
3.0
4.0
5.0
6.0
TIME BIN

was not significantly affected by drug dose ($F(3,33) = 2.23$, $p > .10$) or by dose series order ($F(1,11) = 0.01$, $p > .93$).

D. Discussion

Dentate gyrus evoked potentials exhibited significant population EPSP amplitude enhancement and population spike depression over the 20-minute recording session in a manner consistent with previous research (Sharp et al., 1989; Green et al., 1990). Population EPSP and spike area changes were affected differentially by MK-801, such that population EPSP enhancement was suppressed by MK-801 in all animals, while population spike depression appeared to be suppressed only in animals in the descending-dose series. Thus, NMDA receptor activation appears to be necessary for full expression of environmentally induced population EPSP enhancement.

In contrast to the relatively straightforward effects of MK-801 on population EPSP enhancement, the effects of MK-801 on population spike depression are more difficult to interpret. MK-801 affects the pattern of population spike depression over time within a recording session, but only in animals receiving a descending dose series. The fact that population spike depression is more suppressed overall in the animals that received the descending dose series (as illustrated in Figure 8B) demonstrates that animals that

have been exposed to MK-801 during the initial recording session are prevented from exhibiting dramatic population spike depressions during future sessions (including the vehicle-only session). Two possible explanations of these data are evident. Population spike area depression may be part of a relatively longer-term type of information processing about an environment that may extend to all experience in a given environment. This process would be in contrast to population EPSP enhancement, which appears to occur more as a function of each exposure to the environment. Given this notion, it could be that interference with the expression of population spike depression during initial exposure to an environment enduringly alters the way that population spike depression occurs in that environment. As an alternative explanation, rats in the ascending-dose series could have developed a tolerance to the effects of MK-801 during initial exposure to low doses. This explanation seems less likely because it fails to explain why rats in the descending-dose series never attain the level of population spike depression observed for rats in the ascending dose series, even when they are not receiving MK-801. Further research will be necessary to determine the nature of MK-801's effects on population EPSP enhancement and population spike depression, indicating that the two phenomena may be mediated via different mechanisms.

It is well known that evoked potential amplitude varies with momentary behavioral state (Winson & Abzug, 1977; Green et al., 1990; Hargreaves et al., 1990). In addition, MK-801 can affect behavior (Clineschmidt et al., 1982). Therefore, there is a possibility that the effects of MK-801 on evoked potentials could be completely explained by these effects on behavior, and their subsequent effects on physiology. Behavioral measures taken during recording sessions, however, suggest that the drug effects cannot be accounted for by drug effects on behavior alone. First, the effects of MK-801 on population spike depression cannot be accounted for by changes in behavioral state induced by MK-801 in any parsimonious way. The effect of MK-801 on population spike depression was dependent on dose series order, such that animals receiving a descending dose series showed a dramatic reduction of population spike depression, while those in the ascending series did not. As mentioned previously, there was no effect of dose series order ($p>.93$) on behavioral state, indicating that changes in behavioral state that occur as a consequence of MK-801 administration do not account for the effects of MK-801 on population spike depression. The effects of MK-801 on population EPSP enhancement also are not likely to be fully attributed to changes in behavioral state induced by MK-801. Here, dose-related effects of MK-801 on population EPSP enhancement and changes in behavioral state are different, such that

population EPSP enhancement is depressed by MK-801 administration at all doses of MK-801 given, while detectable (though not significant) behavioral effects occur only at the highest dose of MK-801. Further, the time course of the two effects is different, such that MK-801 significantly decreases population EPSP enhancement from about ten minutes after environmental transfer (third time bin), while the nonsignificant effect of MK-801 on behavior occurs predominantly 20 minutes after environmental transfer (final time bin). Based on these arguments, MK-801-induced changes in behavioral state may not wholly account for the effects of MK-801 on population EPSP enhancement. It is also important to note, however, that the behavioral categories used here were relatively crude, and more refined behavioral observations may have revealed drug effects. Thus, the possibility that motoric factors played some contributory role in the drug effects observed on the evoked potential cannot be discarded completely.

The results of this experiment are consistent with the idea that NMDA receptors play a role in STEM, just as they do in LTP. Hence, STEM may share at least one common mechanism with dentate LTP in freely behaving rats.

Chapter 5. Experiment 3: The Effects of MK-801 on EPSP to Spike Relationships During Long-Term Potentiation in Freely Behaving Rats

A. Introduction

Previous studies have demonstrated that LTP results in a post-stimulation leftward shift of the E-S relationship, such that lower EPSP values lead to greater spike magnitudes (Bliss & Lomo, 1973; Abraham et al., 1984; Kairiss et al., 1987). Experiment 3 was performed to determine if a leftward shift of the E-S relationship would be observed after LTP induction in freely behaving rats. In addition, it examined the effects of MK-801 on the observed shifts to see if MK-801 interfered with the shifts in the E-S relationship just as it interferes with the EPSP and spike enhancements observed with LTP. This experiment is described in Croll & Bostock (1991).

B. Methods

Procedure

Rats were randomly assigned to four experimental conditions in a 2 (stimulation type) x 2 (drug dose) factorial design. For stimulation type, half of the rats

received 15 high frequency (400 Hz) trains as described for Experiment 1, and half of the rats received 15 single pulses serving as control stimuli corresponding to the fifteen trains of stimuli. Within each stimulation group, half of the rats received .00 mg/kg MK-801 (vehicle), and the other half received .10 mg/kg MK-801 30 minutes prior to the recording session. After the 30 minute delay, rats were placed in an opaque carrying box and transported to the recording chamber.

Baseline recordings were taken from each animal for 20 minutes to provide a pre-stimulation baseline as well as to allow the evoked responses of the animals to stabilize. After the 20 minute baseline, two ascending and two descending series of stimulus voltages were delivered through the stimulating electrodes to each perforant path (order of delivery was ascending series-descending series-ascending series-descending series). Evoked responses were measured from each stimulus pulse. Immediately after the four voltage series, the animals received either the high-frequency stimulation or the control stimulation. Immediately after the high-frequency or control stimulation, each animal was given another set of two ascending and two descending voltage series. Animals received one final set of two ascending and two descending series at 40 minutes post-stimulation to assess the persistence of any induced E-S relationship shifts. Each stimulus voltage series

consisted of 11 different voltage values delivered at .16 Hz separated by equal voltage steps. The stimulus voltages ranged from 1 to 12 volts for different animals, and voltage steps were either .5 volt or 1 volt steps. The amperage corresponding to each voltage is unavailable. For each channel, the 11 step range in which the most refined measure of EPSP and spike changes could be measured was selected. Specifically, an attempt was made to select a range in which the initial voltage value resulted in no spike, and the maximum voltage value represented the maximum evoked potential obtainable.

Data Analysis

EPSP and spike values from one selected stimulus intensity were measured for the time periods immediately following LTP induction and 40 minutes after LTP induction, and were normalized against the same stimulus intensity values taken during baseline recording. The stimulus intensity value was selected to be comparable to the stimulus intensity values used during Experiment 1. These values were used to determine whether LTP had been successfully induced by high frequency stimulation.

In order to analyze the EPSP to spike relationships for each group at each time point, linear regressions were computed for the linear portion of the EPSP to spike

function. The linear portion of each function was determined by graphing each EPSP against its corresponding spike and selecting points only from the voltage range in which the function had a linear shape. The regression formula for each function was used to calculate the EPSP value that would be expected to predict spike values equal to 25%, 50%, and 75% of the maximum spike value elicited during the first stimulus intensity series. This method allowed for assessment of the direction of the shift of predicted EPSPs. In addition, it allowed for measurement of whether or not the shift was parallel. If the predicted EPSP values for a given spike decreases over time, then the EPSP to spike relationship shifts to the left. If the magnitude of movement to the left is different for each of the three spike values, the shift is not parallel.

For each time point measured following stimulation, ANOVAS were performed on expected EPSP values for MK-801 vs. vehicle LTP groups, MK-801 vs. vehicle control groups, vehicle LTP vs. vehicle control groups, and MK-801 vs. vehicle control groups.

C. Results

As seen in Table 3, baseline EPSP and population spike values failed to differ significantly for vehicle versus MK-801 groups. Both EPSP ($F(2,46)=3.69$, $p<.04$) and population

Table 3. Baseline EPSP and spike values (mean ± SEM) for vehicle versus MK-801 animals in Experiment 2. MK-801 had no significant effect on baseline EPSP values ($t(25)=.570$, $p>.58$) or baseline spike values ($t(25)=1.518$, $p>.14$)

Group	baseline EPSP	baseline spike
Vehicle	222.91 ± 60.85	204.96 ± 43.81
MK-801	269.35 ± 58.86	302.38 ± 50.66

spike values ($F(2,46)=15.41$, $p<.0001$) increased significantly over time, indicating that LTP was successfully induced (refer to Figure 10). MK-801 significantly reduced (cut in half) the potentiation normally observed after high frequency stimulation for spike values ($F(1,23)=9.07$, $p<.007$). MK-801 tended to decrease EPSP potentiation as was observed in Experiment 1, however, this effect was not statistically significant ($F(1,23)=.80$, $p>.37$) (refer to Figure 11).

There was a significant interaction of stimulation by time for both EPSP ($F(2,46)=3.34$, $p<.05$) and spike values ($F(2,46)=9.41$, $p<.0005$) such that hippocampi that received high frequency stimulation showed a potentiation across the 40 minute time period, whereas control hippocampi did not. No other effects or interactions were significant for the EPSP values. All interactions were significant for the spike values (drug x stimulation: $F(1,23)=5.89$, $p<.03$; drug x time: $F(2,46)=7.74$, $p<.002$; stimulation x time: $F(2,46)=9.41$, $p<.0005$; drug x stimulation x time: $F(2,46)=5.30$, $p<.009$) suggesting that MK-801 significantly interfered with spike potentiation over time.

Figures 12 and 13 present changes in the E-S relationship following LTP induction. The E-S relationship measured at the initial time point is represented by the vertical line drawn at 1.0. All later E-S relationships were graphed in reference to the initial relationship.

Figure 10. Normalized population spike values (proportion of baseline spike) for each group (vehicle-high frequeny stimulation, vehicle-control stimulation, MK-801-high frequency stimulation, MK-801-control stimulation) as a function of time after stimulation.

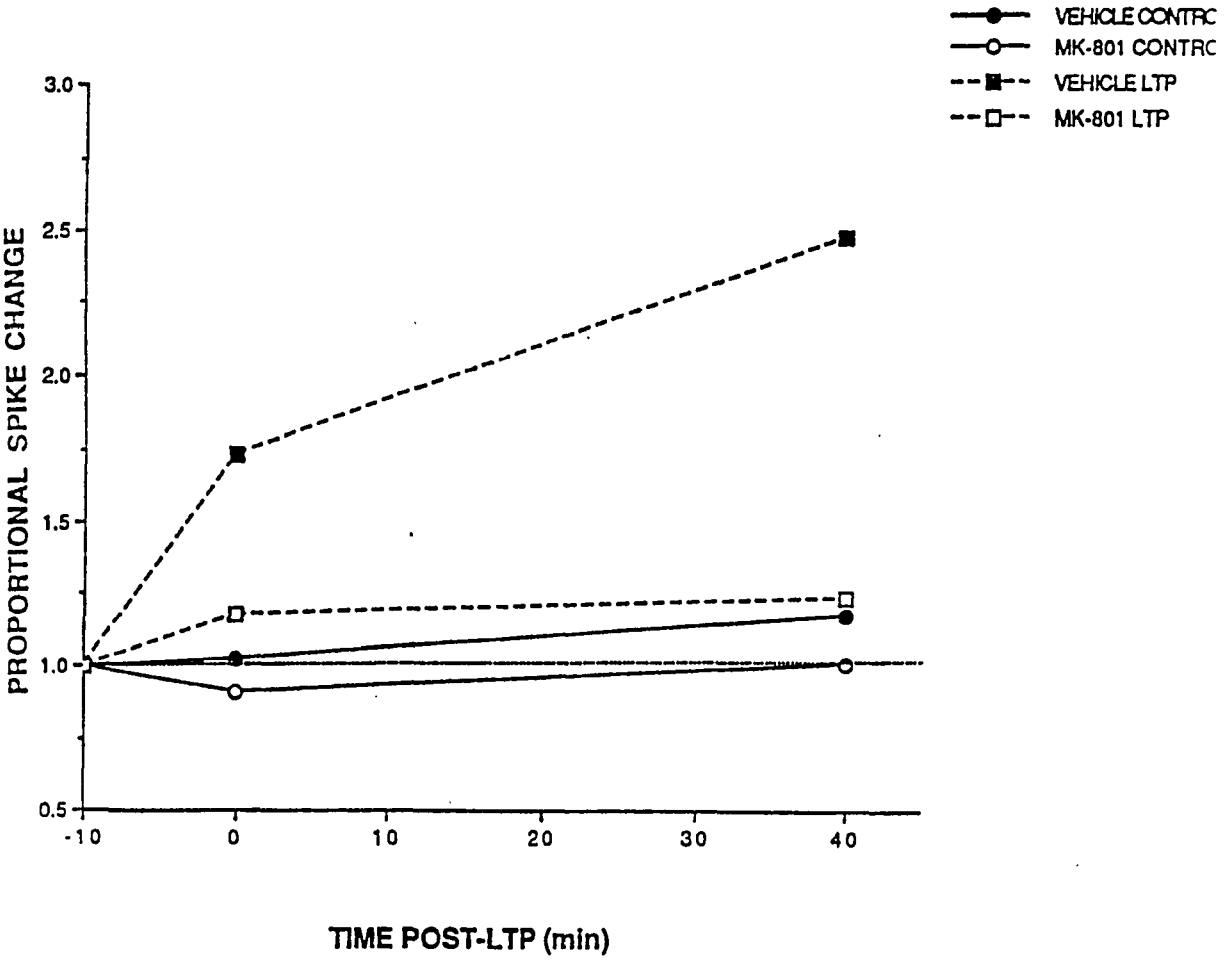

VEHICLE CONTRC
MK-801 CONTRC
VEHICLE LTP
MK-801 LTP
PROPORTIONAL SPIKE CHANGE
3.0
2.5
2.0
1.5
1.0
0.5
-10
0
10
20
30
40
TIME POST-LTP (min)

Figure 11. Normalized EPSP values (proportion of baseline EPSP) for each group (vehicle-high frequeny stimulation, vehicle-control stimulation, MK-801-high frequency stimulation, MK-801-control stimulation) as a function of time after stimulation.

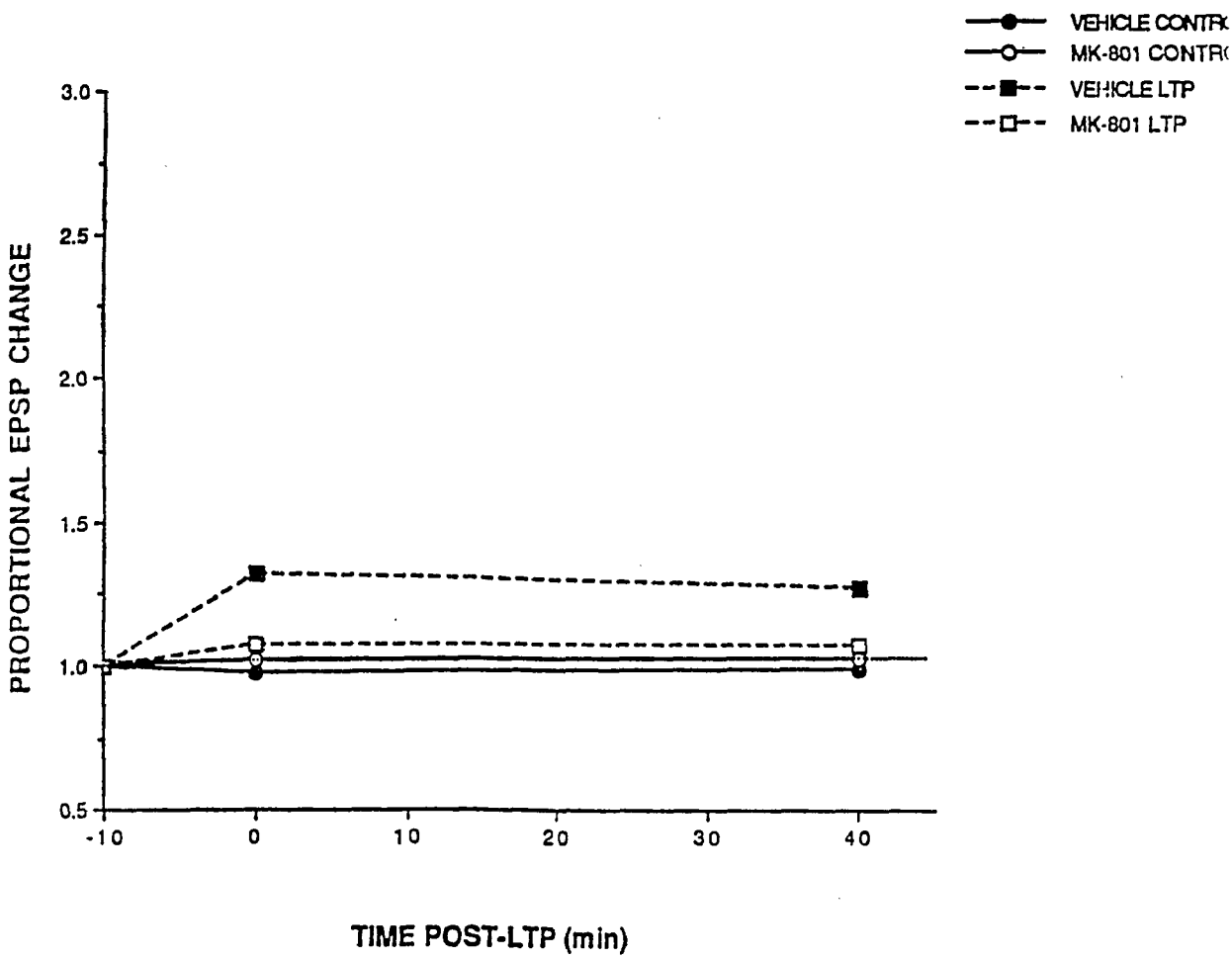

VEHICLE CONTR
MK-801 CONTR
VEHICLE LTP
MK-801 LTP
PROPORTIONAL EPSP CHANGE
3.0
2.5
2.0
1.5
1.0
0.5
-10
0
10
20
30
40
TIME POST-LTP (min)

Figure 12. EPSP to spike relationships for each group (vehicle-high frequeny stimulation, vehicle-control stimulation, MK-801-high frequency stimulation, MK-801-control stimulation) measured immediately after stimulation.

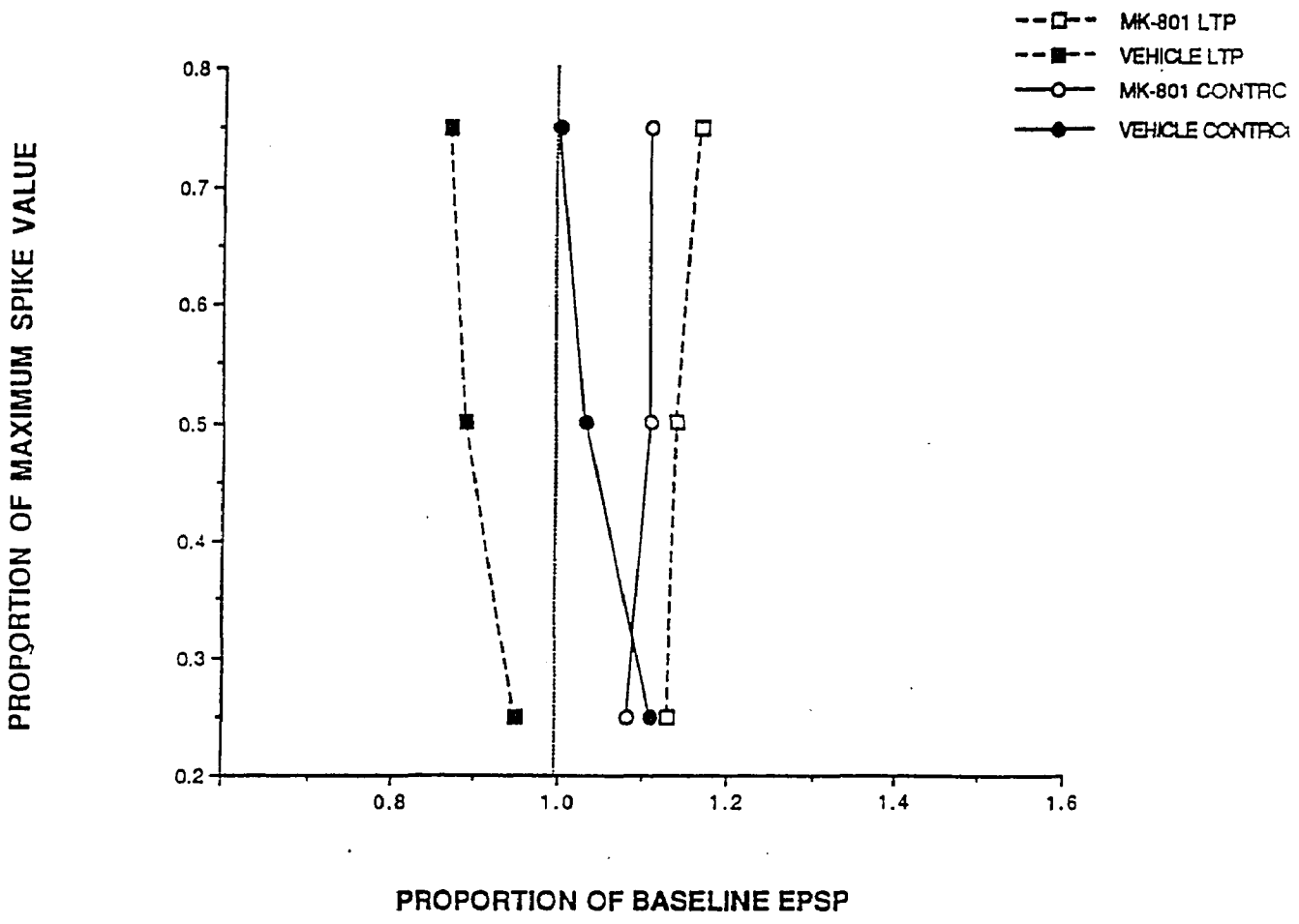
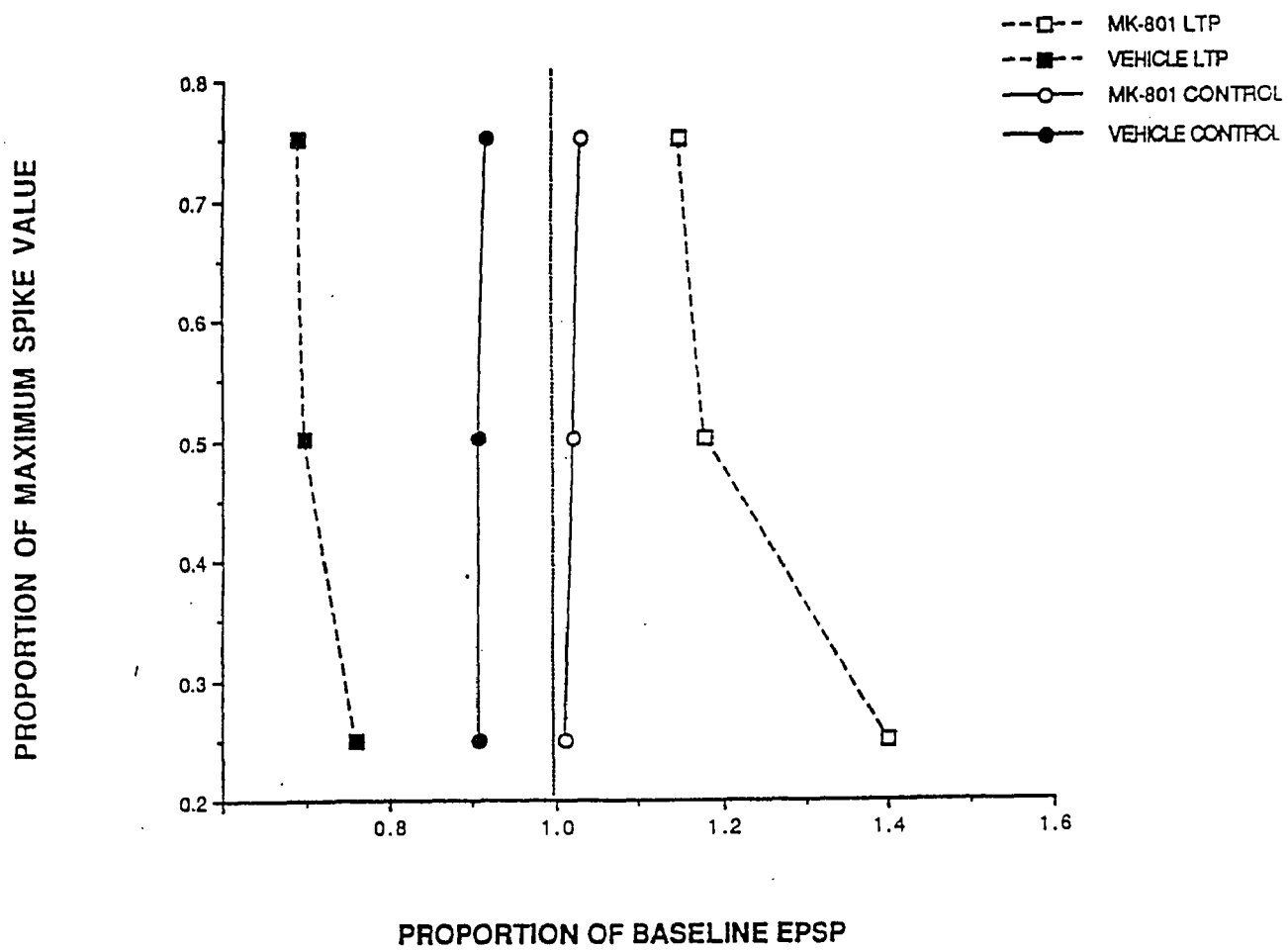

MK-801 LTP
VEHICLE LTP
MK-801 CONTRC
VEHICLE CONTRC
PROPORTION OF MAXIMUM SPIKE VALUE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.8
1.0
1.2
1.4
1.6
PROPORTION OF BASELINE EPSP

Figure 13. EPSP to spike relationships for each group (vehicle-high frequeny stimulation, vehicle-control stimulation, MK-801-high frequency stimulation, MK-801-control stimulation) measured 40 minutes after stimulation.


MK-801 LTP
VEHICLE LTP
MK-801 CONTROL
VEHICLE CONTROL
PROPORTION OF MAXIMUM SPIKE VALUE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.8
1.0
1.2
1.4
1.6
PROPORTION OF BASELINE EPSP

Therefore, lines to the left of the normalized initial relationship represent increases in synaptic efficacy such that smaller EPSPs are adequate to generate the same size population spike values. Lines to the right of the initial baseline represent decreases in synaptic efficacy such that larger EPSPs are necessary for the generation of the same size population spikes. For the time point measured immediately following LTP induction (Figure 12), there were no significantE-S relationship shifts observed (for comparison of vehicle groups, $F(1,12)=1.59$, $p>.23$), and only a trend for a significant effect of MK-801 (for MK-801 versus vehicle high frequency stimulation groups $F(1,12)=3.28$, $p>.09$). For the time point measured 40 minutes post-stimulation (Figure 13), a significant shift of the E-S relationship to the left was observed for vehicle injected animals receiving high frequency stimulation ($F(1,12)=10.24$, $p<.008$), indicating that high frequency stimulation increased synaptic efficacy. MK-801 completely blocked the leftward shift ($F(1,12)=7.29$, $p<.02$) and tended to reverse it to a rightward shift. All of the observed shifts were fairly parallel. Control animals failed to show significant changes in their EPSP to spike relationships.

D. Discussion

Consistent with previous findings (Bliss & Lomo, 1973; Abraham et al., 1984; Kairiss et al., 1987), we observed a significant shift of the E-S relationship to the left following induction of LTP, indicating an increase in synaptic efficacy. This shift developed over time, since it was very small immediately after LTP induction, but continued to shift over the forty minutes following high frequency stimulation. In addition, we noted that 0.10 mg/kg MK-801 completely blocked the shift of the E-S relationship. This result was not unexpected, since MK-801 interfered with the potentiation observed during LTP as well. Interestingly, Figure 13 seems to illustrate a shift of the E-S relationship to the right in animals receiving high frequency stimulation after MK-801 administration, such that EPSPs produced spikes less effectively. Although this shift was not significantly different from that observed in control animals ($p<.10$), it appears to be worthy of consideration. It is possible that this decreased synaptic efficacy appeared because some part of the hippocampal circuitry was still being potentiated, even if the medial perforant path to dentate granule cell NMDA receptor dependent synapse was not. The potentiated circuit is probably inhibitory in nature, because a larger EPSP was necessary to elicit a population spike. Hence, granule cell

firing was being inhibited rather than potentiated. A good candidate for this potentiation is the feedforward inhibitory circuit in the dentate gyrus. Evoked responses in this circuit can be potentiated by stimulation (Wilson, Levy & Steward, 1981; Buzsaki & Eidelberg, 1982). In addition, this circuit uses GABA as a neurotransmitter, rather than the excitatory amino acids (Kairiss et al., 1987). Therefore, it would not be blocked by NMDA receptor blockade. Whether this inhibitory mechanism is associated with the normal phenomenology of LTP, or is only free to exert influence on dentate physiology under NMDA receptor blockade will need to be determined by future research.

Chapter 6. Experiment 4: The Effects of MK-801 on EPSP to Spike Relationships During Short-Term Exploratory Modulation

A. Introduction

Experiments 1 and 2 determined that both LTP and STEM depend on NMDA receptor activation for their expression in freely behaving rats. Experiment 3 confirmed that leftward shifts of the E-S relationship occur in LTP of freely behaving rats, and demonstrated the NMDA receptor-dependence of the shift. E-S relationship shifts during STEM have not been studied. Experiment 4 used stimulus intensity series to determine if E-S relationship changes occur during STEM. In addition, it evaluated the effects of NMDA receptor antagonism on any observed shifts in the E-S relationship. The results of this experiment allow for further comparisons between the changes in synaptic plasticity associated with LTP, and those associated with STEM.
This experiment is described in Croll & Bostock (1991).

B. Methods

Procedures

Rats were injected subcutaneously with MK-801 at a dose of 0.10 mg/kg or with a similar volume of vehicle. Thirty

minutes after the injection, each animal was placed in an opaque carrying box and rapidly transported to the recording room. Rats were randomly assigned to two experimental conditions (vehicle or MK-801) in a single factor between groups design. As described for Experiment 3, two ascending and two descending series of stimulus voltages were delivered immediately after the rats entered the recording chamber. Only values from the ascending series were included in analyses so that changes could be assessed across brief time periods. After 15 minutes, each animal was given another set of two ascending and two descending voltage series. Each series consisted of 11 different voltage values delivered as described for Experiment 3.

Data Analysis

To evaluate changes in the EPSP and spike, and the ability of MK-801 to block any changes, EPSP and spike values from one selected stimulus intensity value were used (selected as described for Experiment 3). These values was compared immediately following the start of the session, three minutes into the session, and for the last three minutes of the session. These later values were normalized against the same stimulus intensity values taken during the first ascending series of recording.

To evaluate shifts in the E-S relationship for each group at each time point, linear regressions were computed and analyzed as described for Experiment 3.

C. Results

As seen in Table 4, baseline EPSP and population spike values failed to differ in the vehicle versus MK-801 groups. Figure 14 represents proportional changes in EPSP values from the baseline value measured at the eighth stimulus intensity value of the drug versus vehicle groups. EPSP values increased significantly over time from the value measured at the start of recording to the value at the end of the session ($F(2,36)=28.27$, $p<.0001$). In addition, EPSPs show significantly less potentiation after MK-801 administration ($F(1,18)=10.16$, $p>.005$). There was also a significant interaction of group by time for EPSP values ($F(2,36)=8.79$, $p<.0008$) such that animals not receiving MK-801 showed an enhancement over time, whereas MK-801 animals did not. The most dramatic EPSP enhancement occurred within the first three minutes of the session, with less of an enhancement evident by the end of the twenty minute session.

As seen in Figure 15, population spike values did not change significantly over time ($F(2,36)=.24$, $p>.79$), and failed to differ for vehicle versus MK-801 animals ($F(1,18)=1.84$, $p>.19$). The interaction between group and

Table 4. Baseline EPSP and spike values (mean ± SEM) for vehicle versus MK-801 animals in Experiment 3.

Group	baseline EPSP	baseline spike
Vehicle	212.55 ± 74.54	252.56 ± 80.17
MK-801	234.68 ± 50.30	360.15 ± 58.06

Note: MK-801 had no significant effect on baseline EPSP values ($t(18)=.27$, $p>.50$) or on baseline spike values ($t(18)=1.18$, $p>.20$)

Figure 14. Normalized EPSP values (proportion of baseline EPSP) for vehicle and MK-801 groups as a function of time after environmental transfer.

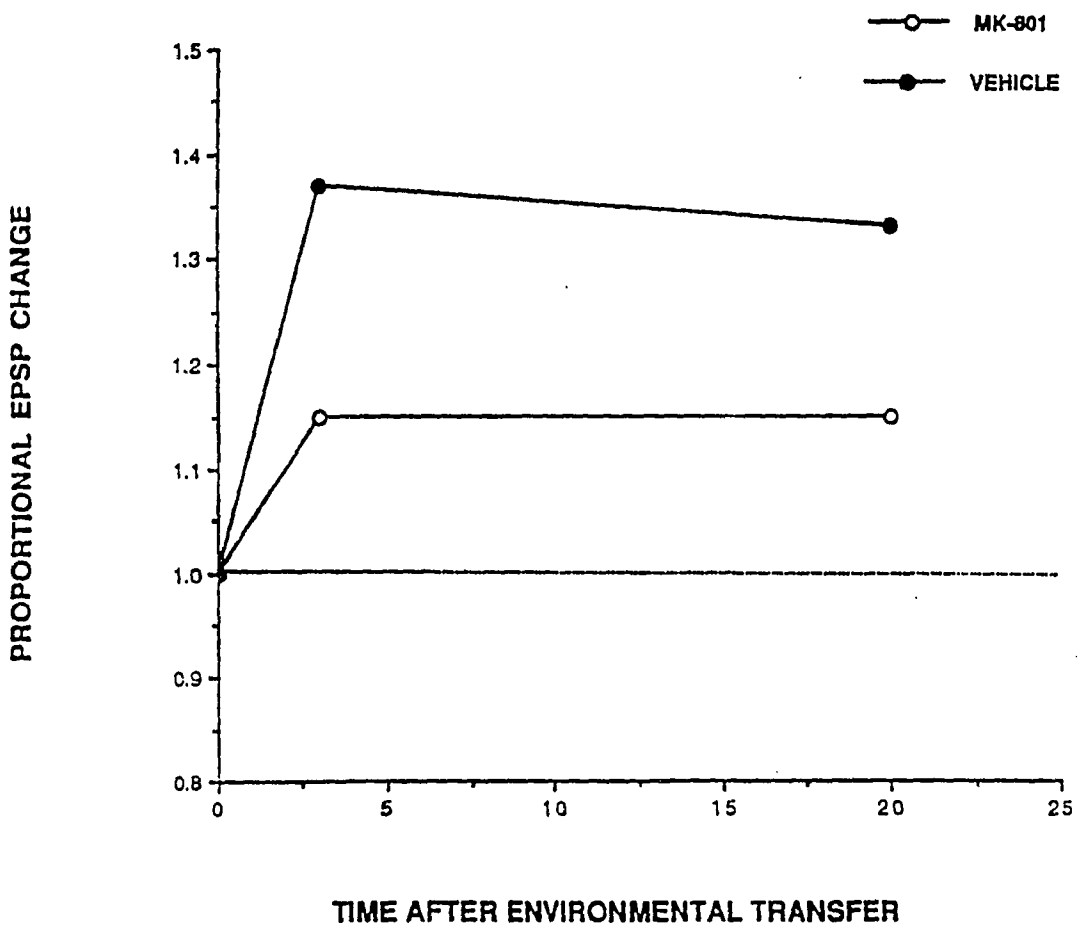
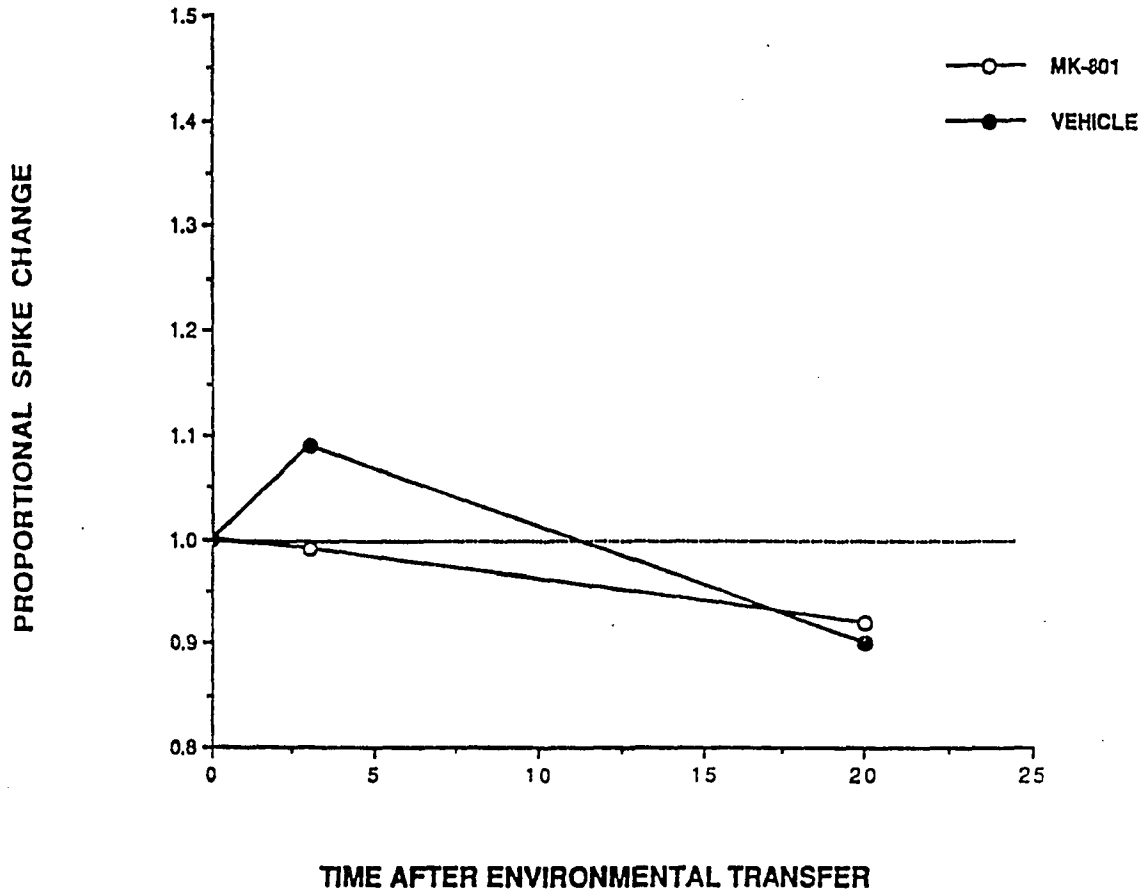

MK-801
VEHICLE
PROPORTIONAL EPSP CHANGE
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0
5
10
15
20
25
TIME AFTER ENVIRONMENTAL TRANSFER

Figure 15. Normalized population spike values (proportion of baseline spike) for vehicle and MK-801 groups as a function of time after environmental transfer.


PROPORTIONAL SPIKE CHANGE
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0
5
10
15
20
25
MK-801
VEHICLE
TIME AFTER ENVIRONMENTAL TRANSFER

time was also not significant ($F(2,36)=1.56$, $p>.22$).

Figures 16 and 17 illustrate E-S relationships based on the regressions for determining the EPSP expected for 25%, 50%, and 75% of the maximum spike value. Initial EPSP values are represented by the 1.0 normalized baseline as in Experiment 2. Figure 16 represents E-S relationships measured after three minutes in the recording chamber and Figure 17 represents E-S relationships measured after twenty minutes in the recording chamber. A significant shift to the right of the E-S relationship occurs over time during STEM ($F(2,36)=13.68$, $p<.0001$). That is, significantly higher EPSP values are required to produce population spikes of the same magnitude as the session progresses. Animals given MK-801 prior to recording show significantly less E-S relationship shift than controls ($F(1,18)=5.60$, $p<.03$). The shifts appear not to be parallel for both groups, such that the shift is more dramatic for lower population spike values, and hence at lower stimulus intensities. This apparently non-parallel shifting was not statistically significant, but did show a trend towards significance ($F(2,36)=2.85$, $p<.08$). Finally, there was a significant time by group interaction ($F(2,36)=4.08$, $p<.03$), such that MK-801 and vehicle rats do not show identical patterns of shifting across time. No other interactions were significant.

Figure 16. EPSP to spike relationship for vehicle and MK-801 groups three minutes after transfer.

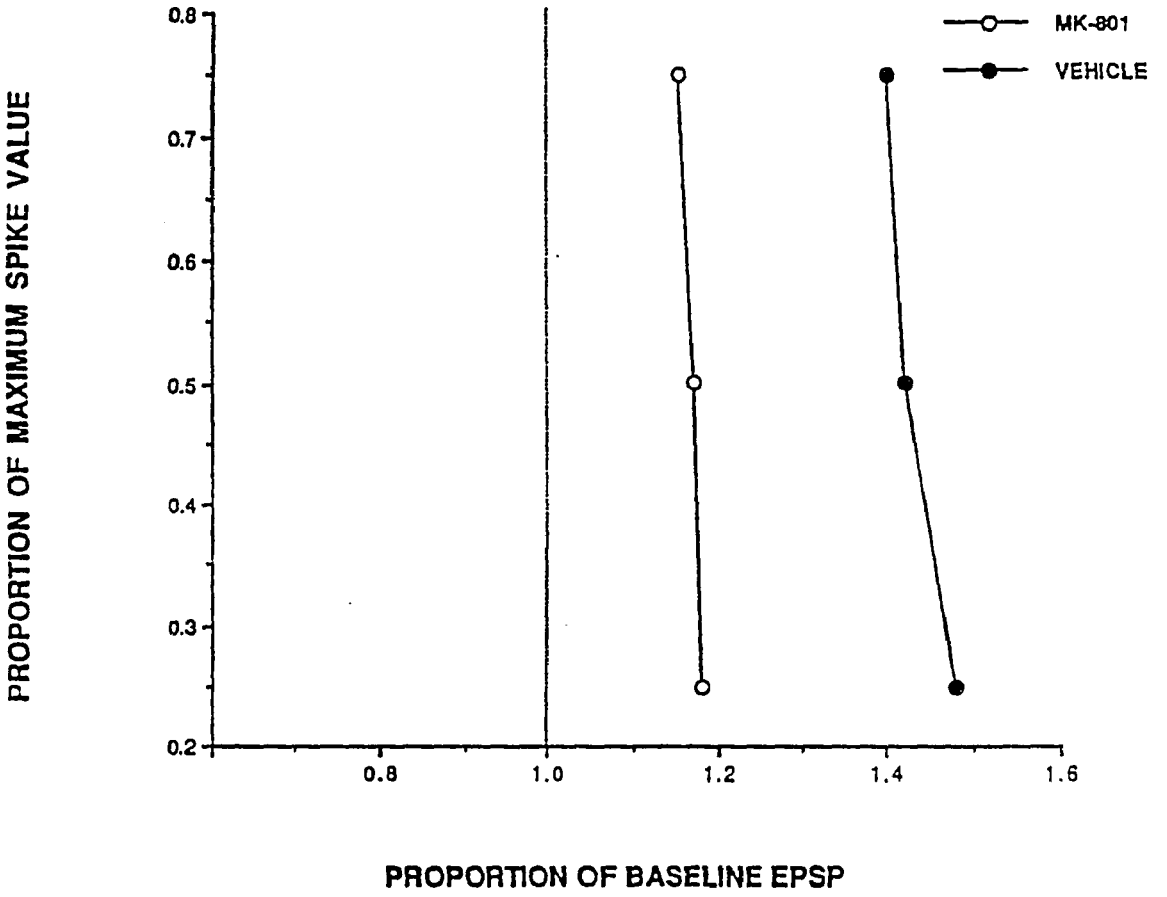
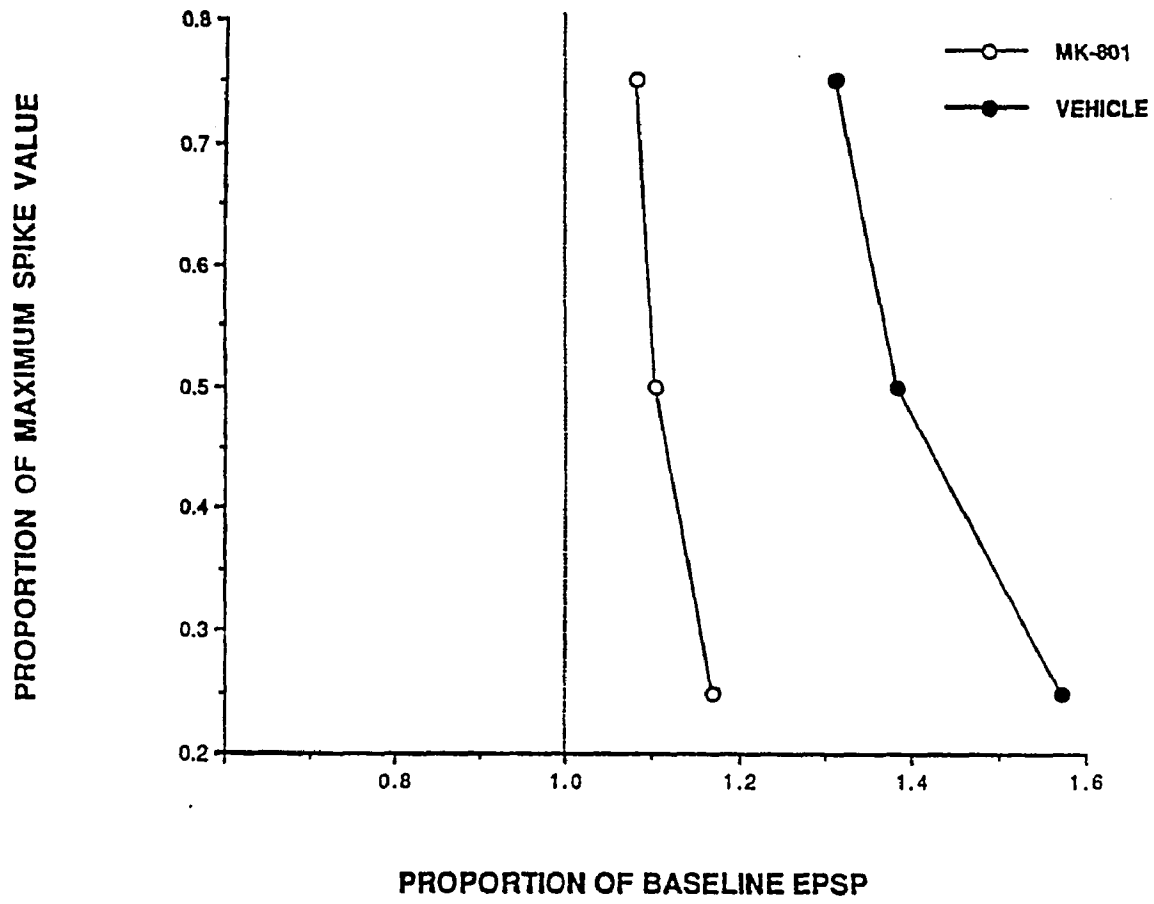

PROPORTION OF MAXIMUM SPIKE VALUE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.8
1.0
1.2
1.4
1.6
MK-801
VEHICLE
PROPORTION OF BASELINE EPSP

Figure 17. EPSP to spike relationship shifts for vehicle and MK-801 groups 20 minutes after environmental transfer.


PROPORTION OF MAXIMUM SPIKE VALUE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.8
1.0
1.2
1.4
1.6
MK-801
VEHICLE
PROPORTION OF BASELINE EPSP

D. Discussion

As previously reported (Sharp et al., 1989; Green et al., 1990), a rapid and significant increase in EPSP magnitude developed immediately upon placing animals in a different environment. The spike depression observed concomitantly with the EPSP enhancement is less consistently observed (Sharp et al., 1989; Green et al., 1990), and in the present experiment was not significant. MK-801 administration significantly interfered with the magnitude of the EPSP enhancement, consistent with the findings from Experiment 2.

A significant shift of the E-S relationship to the right was observed during STEM. This shift of the E-S relationship indicates that after STEM induction, a greater magnitude EPSP is necessary to induce the same size population spike in the granule cells. This decrease in synaptic efficacy developed rapidly, since it was significant as early as three minutes into the recording session. In addition, 0.10 mg/kg MK-801 significantly interfered with the observed decrease in synaptic efficacy.

The finding of a decrease in synaptic efficacy was observed during STEM is not surprising. As STEM develops, larger EPSP values are measured, concomitant with declining population spike values. This physiological profile

suggests that field EPSPs are becoming less effective at inducing granule cell firing. The finding of the shift to the right of the E-S relationship confirms this suggestion. Decreases in synaptic efficacy reflected by shifts in the E-S relationship during STEM indicate that at least some aspects of STEM are post-synaptically mediated (as suggested for LTP by Bliss & Lomo, 1973). That is, if the same amount of post-synaptic excitability elicits fewer action potentials, post-synaptic mechanisms are strongly implicated. It is therefore likely that environmental stimulation results in changes in the post-synaptic spike-generating mechanism during STEM. Because MK-801 blocked not only EPSP potentiation, but also the decrease in synaptic efficacy, it is likely that MK-801 is interfering with STEM induction at least partially through a post-synaptic mechanism. This idea is further strengthened by the fact that MK-801 interferes significantly with the EPSP potentiation, without dramatic effects on the spike values.

Chapter 7. Experiment 5: The Effects of Environmental Complexity on EPSP and Spike Magnitude in Short-Term Exploratory Modulation

A. Introduction

To fully understand natural changes in dentate physiology related to environmental changes, it would be valuable to determine whether STEM interacts with other forms of environmentally-induced dentate gyrus plasticity. STEM has been studied only for animals transferred into simple environments. Because exposure to complex environments results in enhancements of both EPSP and population spike values, it would be valuable to determine if STEM would still occur if animals were transferred into complex environments. Experiment 5 measured changes in dentate evoked potentials when animals were transferred into complex environments across several days. The results of this experiment allow conclusions to be drawn about whether STEM occurs in both simple and complex environments. In addition, this experiment adds information about the parameters necessary to induce spike and EPSP enhancements after complex environmental exposure. Finally, the experiment tested both control and novel complex environment groups so that a distinction can be made between changes resulting from complexity alone, and those resulting from

the novelty of the complex stimuli. This experiment has been presented in Croll & Bostock (1992).

B. Methods

Procedure

Rats were rapidly transported to the recording room (as described for Experiment 2) daily for seven consecutive days, and evoked potentials were recorded for 20 minutes each day. The experiment was conducted as a four-condition between-groups design. The four conditions were the four types of cage environments into which rats were transferred. Rats were either transferred into a dimly lit room with minimal sensory stimulation, a normally lit room with minimal sensory stimulation, a complex sensory environment (including toys in the cage, olfactory cues, and auditory cues) remaining constant through the seven days, or a complex sensory environment in which the stimuli change each day. Data collection was performed as described for Experiment 2, except that behavioral observations were made by a trained observer watching the animal on a monitor from another room. In addition, the behavioral categories were expanded from those described in Experiment 2 to include two categories of theta behavior. These categories were passive theta, which included sniffing and head movements, and

locomotion, which included active exploration such as turning, rearing, and walking.

Data Analysis

The recording sessions were divided into six time bins of approximately three minutes each to examine changes within each recording session as described for Experiment 2. Initial EPSP and population spike values for the first time bin in days two through seven were also normalized against the EPSP and population spike values measured during the first day so that changes in evoked potentials could be assessed across days in addition to within each session.

C. Results

Measurable EPSPs were obtained for four hippocampi in the dim group, five hippocampi in the simple group, seven hippocampi in the complex same-stimuli group, and six hippocampi in the complex novel-stimuli group. Measurable population spikes were obtained for five hippocampi in the dim group, five hippocampi in the simple group, seven hippocampi in the complex-same group, and six hippocampi in the complex-novel group.

Significant EPSP enhancements ($F(5,510) = 91.241$, $p<.001$) and spike depressions ($F(5,798) = 33.771$, $p<.001$)

Table 5. Normalized EPSPs (proportion of baseline EPSP) at the final time bin (20 minutes after environmental transfer) for rats transferred into each of four environmental conditions (dim, simple, complex-same, and complex-novel).

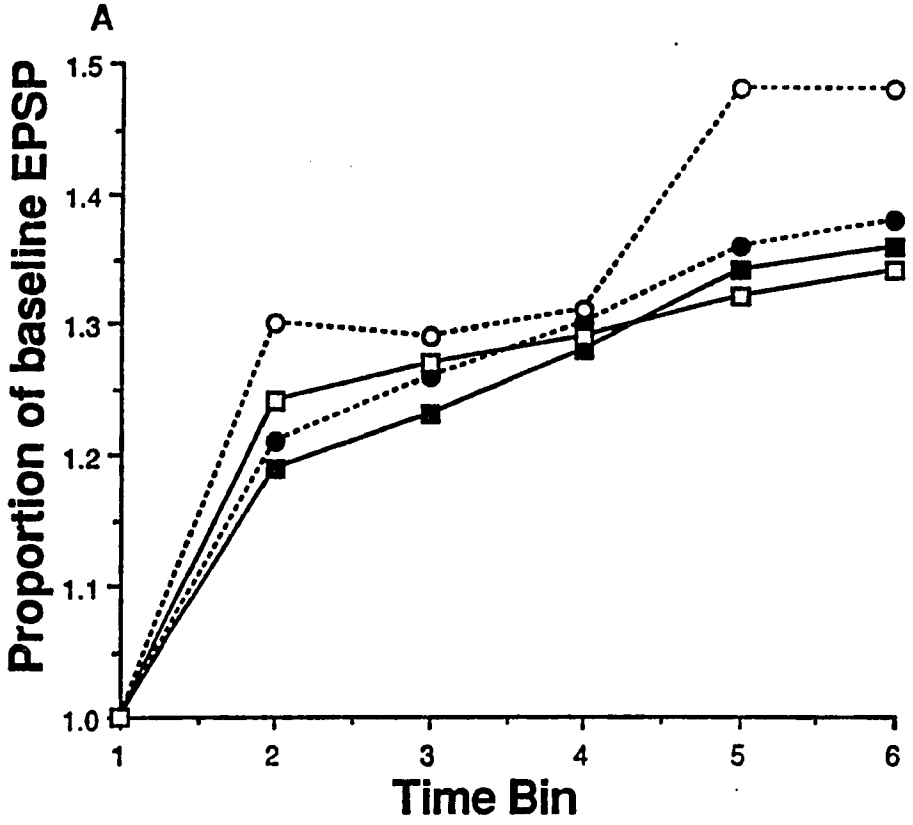
Day	Dim	Simple	Same	Novel
1	1.38 ± .20	1.48 ± .12	1.36 ± .05	1.34 ± .05
2	1.15 ± .08	1.54 ± .09	1.42 ± .11	1.45 ± .14
3	1.18 ± .11	1.47 ± .07	1.46 ± .14	1.20 ± .05
4	1.20 ± .10	1.40 ± .09	1.34 ± .09	1.26 ± .12
5	1.06 ± .14	1.26 ± .07	1.22 ± .11	1.19 ± .08
6	1.09 ± .05	1.37 ± .07	1.31 ± .12	1.28 ± .12
7	1.04 ± .08	1.30 ± .04	1.25 ± .06	1.17 ± .03

Note: Data are expressed as mean ± SEM

associated with STEM developed across the 20 minute session for all groups during all seven days. The mean EPSP enhancement on the first day was 1.39 for all groups combined during the last time bin, and the mean population spike depression was .75. Although EPSP enhancements occurred for all seven days, their magnitude declined significantly across days ($F(6,510) = 7.554$, $p<.001$) (see Figure 18). By the seventh day, the mean EPSP enhancement for all groups combined was only 1.19. In contrast, population spike values did not change significantly across days ($F(6, 798) = 1.803$, $p<.10$) (see Figure 19). Although not significant, the decrease in spike values across days exhibited a trend toward significance ($p<.10$). The mean population spike depression across groups for the seventh day was .63.

Significant differences among environmental complexity groups were found both for EPSP ($F(3,510)=12.327$, $p<.001$) (see Table 5) and population spike ($F(3,798)=6.174$, $p<.001$) (see Table 6) values. For EPSP values, the dim group showed less EPSP enhancement overall than any of the other groups. Unexpectedly, it was the simple and complex-same groups which showed the most overall EPSP enhancement, while the complex novel group showed a moderate amount of overall enhancement. Although all interactions for EPSP values failed to achieve significance, EPSP values (see Figure 18 and Table 5) for the four groups appeared to change

Figure 18. Day 1 (A) and Day 7 (B) normalized EPSP values (porportion of baseline EPSP) for all four environmental complexity groups as a function of time bin after environmental transfer.


A
Proportion of baseline EPSP
1.5
1.4
1.3
1.2
1.1
1.0
1
2
3
4
5
6
Time Bin

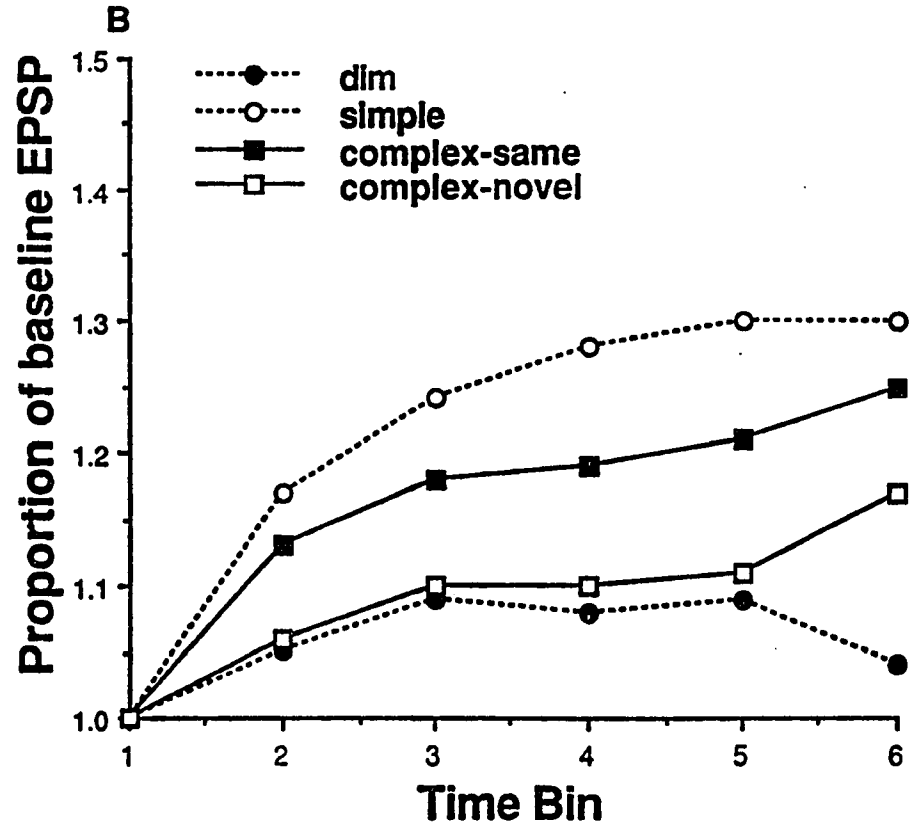

B
dim
simple
complex-same
complex-novel
Proportion of baseline EPSP
1.5
1.4
1.3
1.2
1.1
1.0
1
2
3
4
5
6
Time Bin

Table 6. Average normalized spikes (proportion of baseline spike) at the final time bin (20 minutes after environmental transfer) for rats transferred into each of four environmental conditions (dim, simple, complex-same, and complex-novel).

Day	Dim	Simple	Same	Novel
1	.87 ± .10	.76 ± .12	.82 ± .17	.55 ± .13
2	.64 ± .11	.72 ± .13	.76 ± .07	.73 ± .24
3	.64 ± .12	.74 ± .06	.80 ± .12	.56 ± .11
4	.76 ± .10	.65 ± .08	.90 ± .07	.67 ± .07
5	.66 ± .14	.73 ± .06	.67 ± .11	.65 ± .10
6	.78 ± .13	.64 ± .09	.61 ± .10	.68 ± .07
7	.71 ± .11	.51 ± .10	.65 ± .10	.64 ± .11

Note: Data are expressed as mean ± SEM

differentially across days (the group by day effect had a $p>.11$). During the first day, all groups showed some EPSP enhancement, although the dim group showed the least change across time bins. The dim group immediately fell to low levels of enhancements (by day 2), whereas the simple and complex-same groups showed only modest declines in EPSP enhancements across days. Finally, the complex-novel group showed a fairly substantial decline across days, but it started later than that observed for the dim group (around day 3 or 4), and never declined as dramatically.

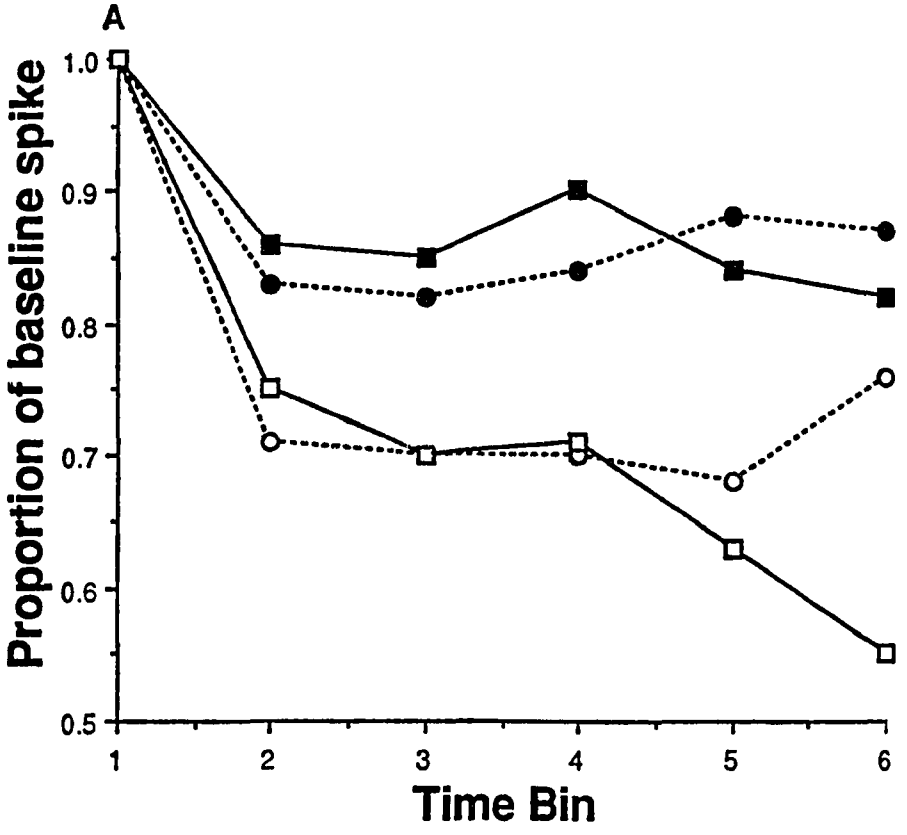
The differences detected among groups for population spike depression were different from those observed for EPSP enhancement (see Figure 19 and Table 6). As observed for EPSP enhancements, the magnitude of spike depressions was less for the dim group than for the other three groups. Also as observed for EPSP enhancements, the profile of change for the complex-novel group differed from that of the other two groups, simple and complex-same, which showed similar profiles. The differences in spike depression between the complex-novel and other groups was of a different nature than that for EPSP values, however. Specifically, the complex-novel group showed an unvarying, robust spike depression throughout the experiment. The simple and complex-same groups both achieved robust spike depressions by the seventh day, but unlike the complex-novel group, these groups gradually depressed throughout the seven

days. This differential pattern of spike depressions throughout the seven days of the experiment for the four groups is reflected in a significant group by day interaction effect ($F(18,798)=1.838$, $p<.02$). No other interaction effects were significant.

Because the differences among groups might reflect differences in actual baseline EPSP and spike values, as opposed to differences in enhancement or depression from the same baseline value, analyses of variance were performed to test thebaseline EPSP and spike values for the four groups across the seven days of the experiment. No significant differences between groups were detected in baseline EPSP ($F(3, 126)=2.035$, $p>.11$) or spike ($F(3,133)=1.249$, $p>.29$) values. In addition, no significant differences were detected across days for baseline EPSP ($F(6,126)=.209$, $p>.97$) or spike ($F(6,133)=.173$, $p>.98$) values. The baseline EPSP and spike values for all four groups across all seven days are represented in Figures 20 and 21. Therefore, it is not likely that baseline EPSP and spike values influenced the results obtained for differences between groups and days in EPSP enhancements and spike depressions.

Behavioral data were again analyzed as proportion of time spent in theta behavior. An additional analysis of proportion of time spent in active theta, or locomotor behavior, was undertaken to provide information about the relationship between locomotion and STEM. Therefore, total

Figure 19. Day 1 (A) and Day 7 (B) normalized population spike values (proportion of baseline spike) for all four environmental complexity groups as a function of time bin after environmental transfer.


A
Proportion of baseline spike
1.0
0.9
0.8
0.7
0.6
0.5
1
2
3
4
5
6
Time Bin

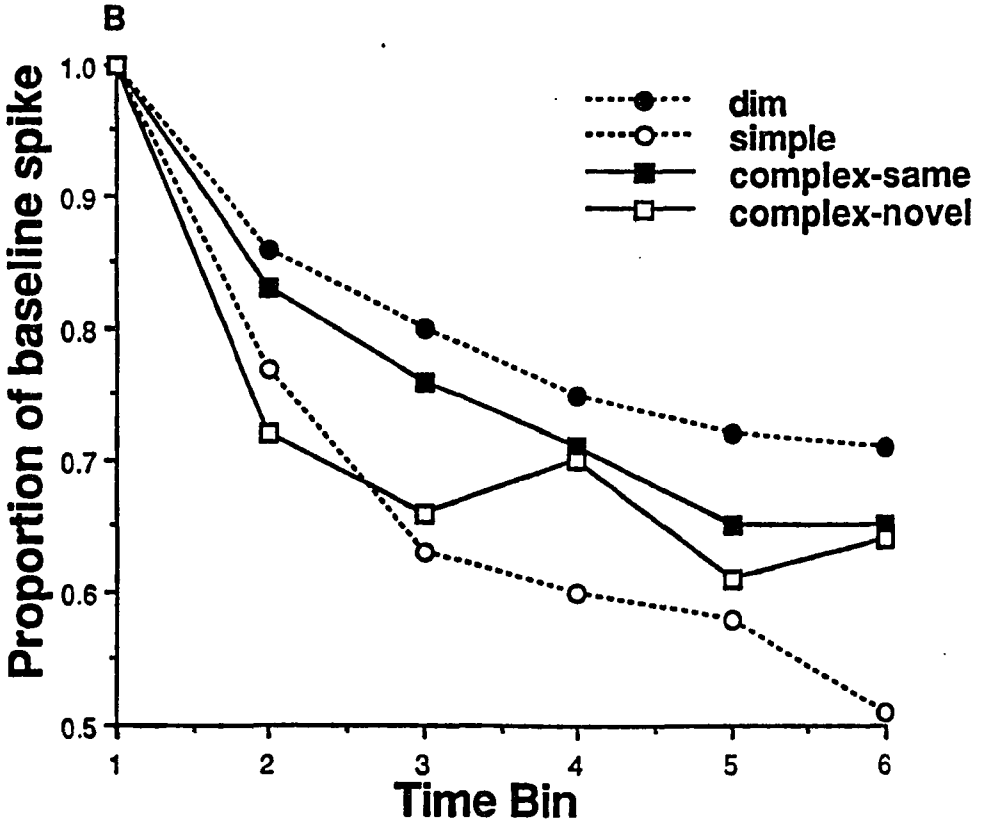

B
Proportion of baseline spike
1.0
0.9
0.8
0.7
0.6
0.5
1
2
3
4
5
6
Time Bin
dim
simple
complex-same
complex-novel

Figure 20. Baseline EPSP values for all four environmental complexity groups as a function of day. EPSP values are normalized against day 1 values.

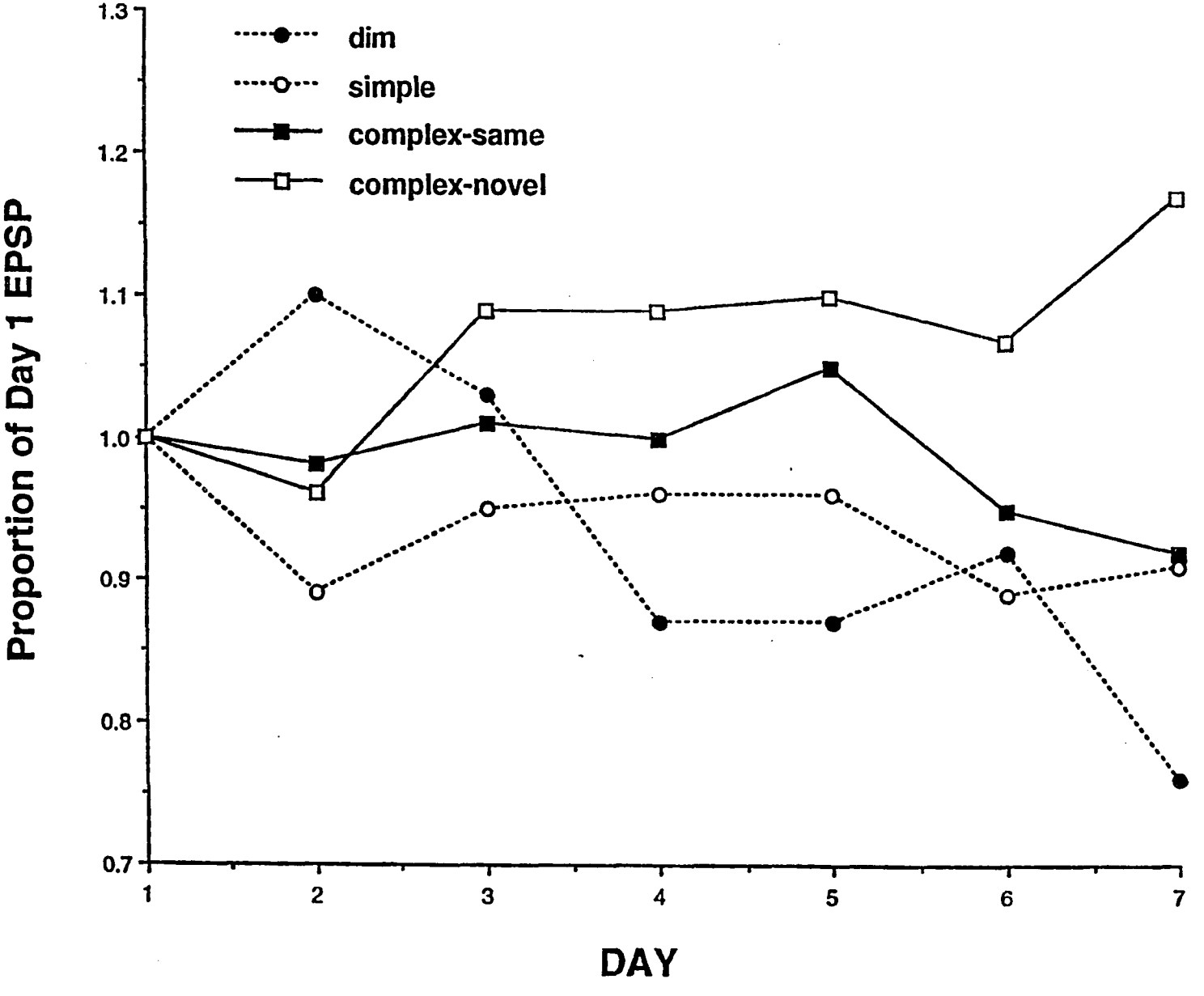
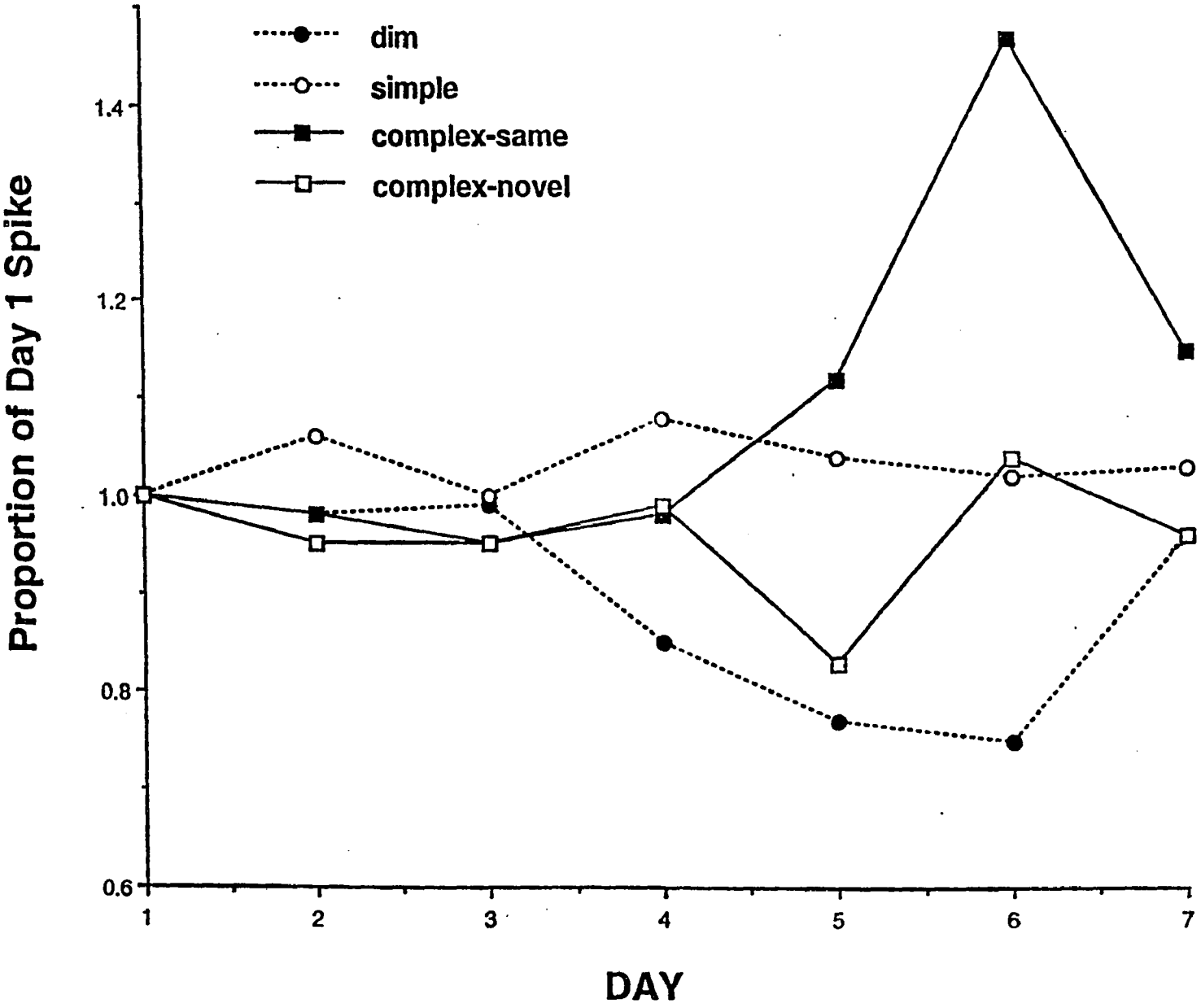

dim
simple
complex-same
complex-novel
Proportion of Day 1 EPSP
1.3
1.2
1.1
1.0
0.9
0.8
0.7
1
2
3
4
5
6
7
DAY

Figure 21. Baseline population spike values for all four environmental complexity groups as a function of day. Population spike values are normalized against day 1 values.

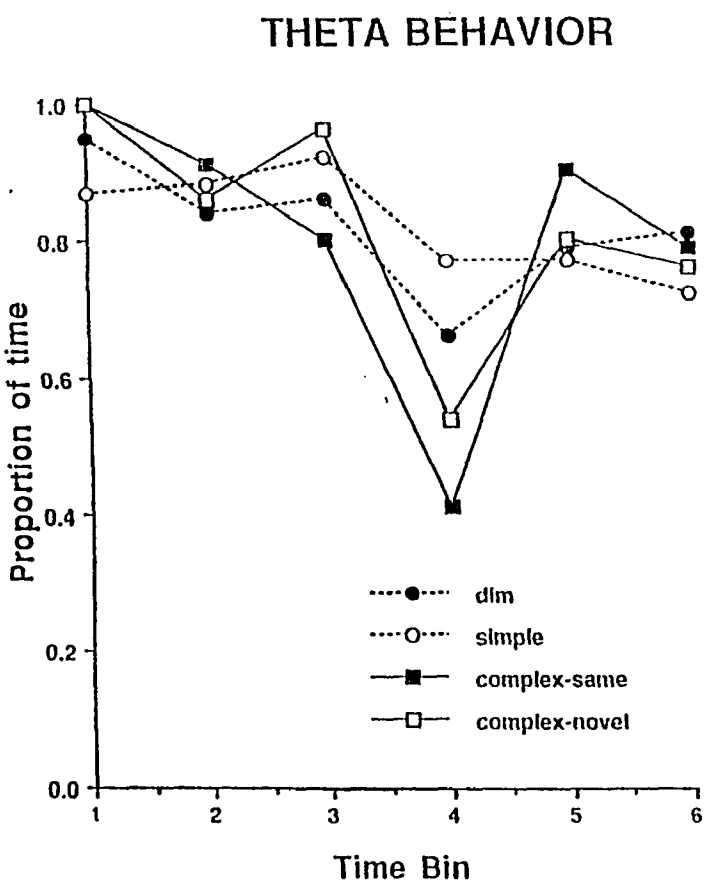

dim
simple
complex-same
complex-novel
Proportion of Day 1 Spike
1.4
1.2
1.0
0.8
0.6
1
2
3
4
5
6
7
DAY

theta behavior measured both locomotor behavior and more passive exploratory behaviors such as sniffing, whereas the analysis of locomotor behavior included only those theta behaviors in which the animal was actively moving around the cage. Figures 22 and 23 show proportion of time spent in theta (Figures 22A and 23A) and locomotor (Figures 22B and 23B) behavior for days 1 (Figure 22) and 7 (Figure 23). The data reveal no obvious relationship between theta behavior and the evoked potentials measured in this experiment. The slope and pattern of the functions for locomotor behavior are more similar to those of EPSP enhancement and spike depression.

D. Discussion

The results of this experiment suggest that STEM retains its integrity regardless of the type of environment into which a rat is transferred. The EPSP enhancement and concomitant population spike depression first reported by Sharp et al. (1989) occurs for transfers into both simple and complex environments, and into both familiar and novel environments. Although STEM was consistently observed within the seven daily transfers in this experiment, the magnitude of the EPSP enhancement did decrease with repeated exposures. Because the decreases in the EPSP enhancement did not asymptote during the seven days of recording, it is difficult to determine whether STEM would eventually

Figure 22. Day 1 behavior for all four environmental complexity groups as a function of time bin after environmental transfer. A) proportion of theta behavior B) proportion of locomotor behavior


THETA BEHAVIOR
Proportion of time
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
Time Bin
dim
simple
complex-same
complex-novel

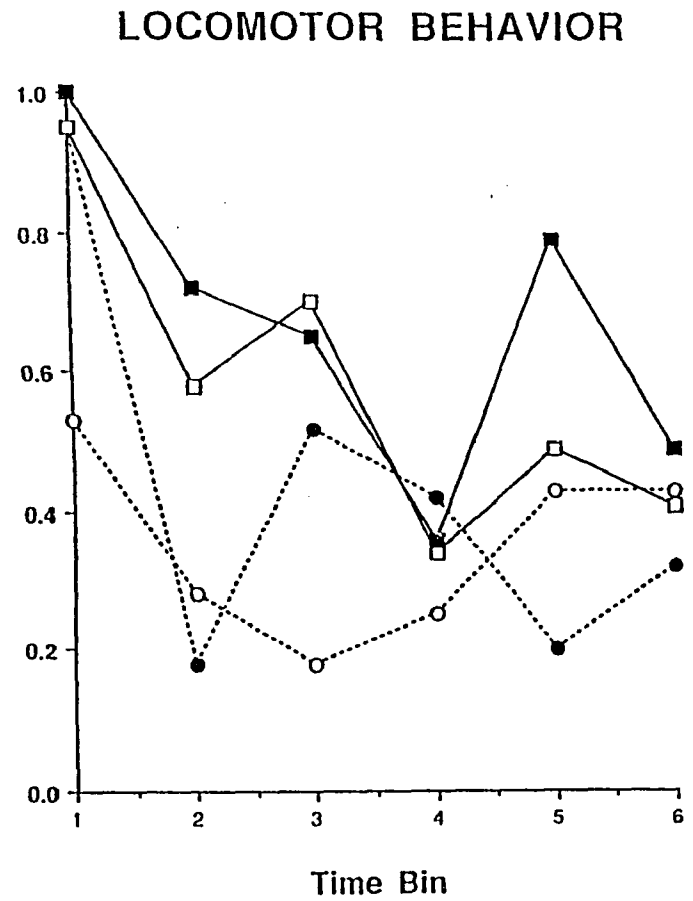
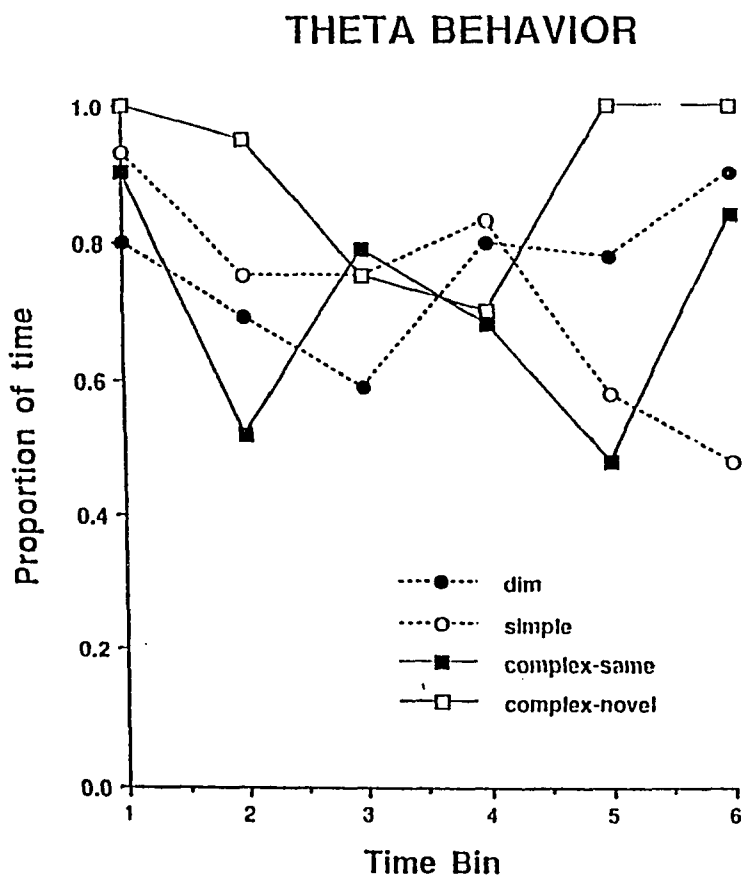
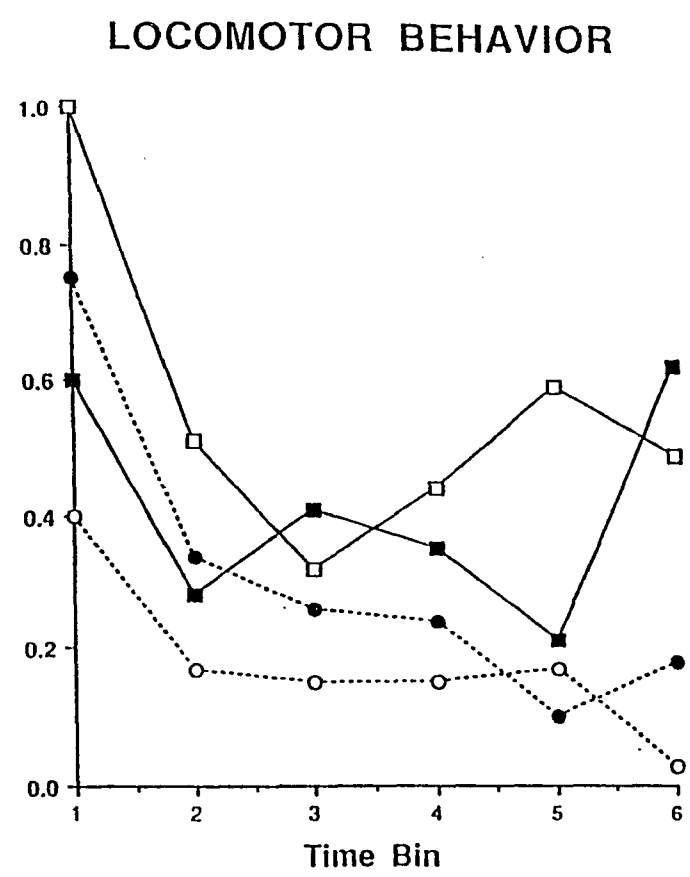

LOCOMOTOR BEHAVIOR
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
Time Bin

Figure 23. Day 7 behavior for all four environmental complexity groups as a function of time bin after environmental transfer. A) proportion of theta behavior B) proportion of locomotor behavior


THETA BEHAVIOR
Proportion of time
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
Time Bin
dim
simple
complex-same
complex-novel


LOCOMOTOR BEHAVIOR
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
Time Bin

disappear with additional exposures. Further experiments measuring STEM for more than seven consecutive days may answer this question.

It is difficult to draw conclusions about the effects of varying amounts of environmental stimulation on STEM based on the data from this experiment. Although the animals in the dim group, which were exposed to the least amount of stimulation, appear to show less STEM than the other animals, the relationship between amount of complexity and STEM is clearly not linear. For EPSP enhancement, the most complex group, the complex-novel animals, show less enhancement overall than the simple and complex-same groups. For spike depression, the relationship between complexity and STEM may be more linear, because the complex-novel animals develop their maximal spike depression more quickly than either the simple or complex-same groups.

The similarity between the profiles observed for simple and complex-same animals is puzzling. This similarity suggests that STEM is not sensitive to the difference in complexity between these two groups. In fact, the complex-same group is no less complex than the complex-novel group. This fact suggests that the differences obtained between the complex-same and complex-novel group are not due to complexity, but rather to novelty. The most parsimonious explanation of the group differences in STEM, especially of the EPSP enhancement, is that some minimal amount of

stimulation is necessary to induce robust STEM, but that STEM is fairly insensitive to complexity after that point. This explanation would account for the weak STEM observed in the dim group, and the similar profile of STEM observed in the simple and complex-same groups. Furthermore, the explanation must be expanded to state that there is something different about novelty, compared to complexity, which alters the profile of EPSP enhancements. That uniqueness could be due to enhanced exploration, processing of more information, or something as simple as neophobia or stress. It is unlikely that exposure to the complex environments itself altered the physiology significantly across days, because of the finding that baseline EPSP and spike values are the same across groups and days.

Although it may be possible from these data to propose that STEM is sensitive to the amount of stimulation provided, it is not likely that STEM becomes "more like" LTP with increased amounts of stimulation. STEM is characterized by spike depression and LTP by spike potentiation. The finding that, if anything, increased stimulation leads to more spike depression suggests that STEM is not a low stimulation equivalent of LTP. STEM appears to have its own profile which maintains its integrity with varying amounts of environmental stimulation.

There appears to be no consistent relationship between theta behavior and STEM in this experiment. If anything, the results presented here suggest that the profile of changes observed across time, groups, and days for locomotor behavior is more slightly more reminiscent of the changes observed for STEM than for all theta behavior. This finding suggests that STEM could be driven either by exploration, or by locomotion itself. Green et al. (1990) tested animals on a treadmill and found no relationship between locomotion and STEM. It is therefore more likely that STEM is driven specifically by active exploration. Whether this proposed dependency of STEM on locomotion reflects a need for more focused, active exploration, or a need for navigation through space will have to be examined in future experiments.

Chapter 8. General Discussion

A. Summary

The experiments presented in this dissertation sought to better characterize STEM by comparing it to the much better understood form of hippocampal dentate gyrus physiological plasticity, dentate LTP. Specifically, the aims of the dissertation were to: 1) determine if STEM shares common mechanisms of induction with LTP by studying the dependence of STEM on activation of the glutamate N-methyl-d-aspartate (NMDA) receptor subtype, 2) determine if STEM, like LTP, results in changes in synaptic efficacy, 3) determine if STEM will be activated in any type of environment, regardless of its complexity. The results of the five experiments performed to address these issues suggest that although STEM does share some properties with LTP, it also differs from LTP in several ways. Like LTP, STEM appears to be NMDA receptor-dependent, and is characterized by a change in synaptic efficacy which is also NMDA receptor-dependent. Unlike the change in synaptic efficacy observed in LTP, the synaptic efficacy change measured during STEM (at least as exhibited by a large population of granule cells), appears to represent a decrease in synaptic efficacy. That is, for LTP a given EPSP will be associated with larger population spikes after

LTP induction, suggesting an increased synaptic efficacy. For STEM, on the other hand, EPSPs will be associated with smaller spikes as the phenomenon develops, suggesting an overall decreased synaptic efficacy for the population of cells. In addition, MK-801 merely reduces the magnitude of the change in synaptic efficacy during STEM, but actually reverses the direction of the change after LTP induction. Finally, the decrease in synaptic efficacy observed during STEM has a rapid time course such that it develops during the first few minutes, whereas the alterations in synaptic efficacy observed during LTP have a more gradual time course and are not significant immediately after high frequency stimulation. Finally, STEM cannot be converted to LTP by increasing the amount of stimulation. All of these differences between STEM and LTP suggest that STEM is a unique phenomenon and may not be a natural form of LTP.

B. Relationship to Learning

Although STEM and LTP appear to be distinct, they may represent different steps in information processing in the hippocampus. It is important that we have interfered with LTP, STEM, and their associated processes in freely behaving rats at low doses of MK-801 because it allows more comparisons to be made between the mechanisms of hippocampal plasticity and those of learning. Because higher doses of

MK-801 result in severe motor impairments in behaving rats (Clineschmidt et al., 1982), learning cannot be properly assessed at these doses. Experiment 2 demonstrated that the doses used in these experiments do not significantly alter rats' behavior. When animals are tested for acquisition of learned tasks under doses of MK-801 that do not interfere significantly with the animal's ability to perform tasks, they still demonstrate significant learning impairments (Robinson et al., 1989). Because MK-801 interferes with learning at low doses, if it was not found to interfere with LTP or STEM induction, the case for either phenomenon as a learning mechanism would have been weakened. The dose of MK-801 that was tested in all of the NMDA receptor-dependency experiments (Experiments 1 to 4), 0.10 mg/kg, interferes with learning (Robinson et al., 1989). The demonstration here that MK-801 at this dose also reduces the magnitude of LTP and STEM provides evidence that either or both could be involved in changes in the hippocampal circuitry associated with learning.

C. Synaptic Efficacy

To better understand what role each of these phenomena, LTP and STEM, plays in hippocampal plasticity, it is necessary to examine their differences as well as their similarities. The most notable difference discovered is the

opposite direction of the changes in synaptic efficacy for STEM versus LTP. There are several possible explanations of LTP and STEM's NMDA-dependent alterations in synaptic efficacy being opposite in direction. The first possibility is that both alterations are dependent on the same NMDA receptor population. This possibility would be true if the NMDA receptor did not directly elicit EPSPs or IPSPs, but rather had a permissive effect for plasticity in the spike-generating mechanism. It is unclear what mechanism would be involved in this permissive effect, but it is likely that it would involve a calcium-dependent process because NMDA receptor activation results in increased intracellular calcium levels. There is, however, no empirical evidence for a permissive effect on spike threshold plasticity. Another possibility, if both depend on the same NMDA receptor population, is that the activation of the NMDA receptor results in EPSPs with high frequency stimulation and IPSPs with the low frequency stimulation assumed to result from environmental stimulation. There is little evidence that activation of the same NMDA receptor population can result in both EPSPs and IPSPs. It is possible, however, that different conformations of the same NMDA receptors may have different post-synaptic effects which could lead to different effects on the spike threshold.

D. Neuromodulatory Systems

A second possibility is that the NMDA receptors are influenced by different modulatory neurotransmitter systems for STEM versus LTP, and that these differential influences result in different effects on the E-S relationship. As discussed in section 1C, the hippocampus is rich in receptors for many types of neurotransmitters and neuropeptides which could potentially be neuromodulatory for dentate plasticity. These include (among others) glucocorticoids, norepinephrine, serotonin, enkephalin, neuropeptide Y, and somatostatin.

The case for glucocorticoids is particularly compelling because they are released during stressful events. It is likely that transferring an animal to a different environment causes some amount of stress, and would therefore increase circulating levels of glucocorticoids. Glucocorticoids have been shown to affect long-term potentiation (Pavlides, Watanabe & McEwen, 1991; Diamond, Bennett, Meltzer, Fleshner & Rose, 1991; Filipini, Gijsbers, Birmingham, Kraulis & Dubrovsky, 1991), and could therefore potentially affect STEM as well. The results of Experiment 5 are consistent with this possibility. Because neophobia, or fear of novelty, results in stress for rats, increased familiarity should decrease stress. The finding in Experiment 5 that repeated exposures to an environment

decrease the EPSP enhancement might suggest that stress contributed to the EPSP enhancement. If that is the case, however, the opposite might be true of the spike depression because the only group which experienced repeated novelty, the complex-novel group, was the only group which showed increased spike depression throughout the repeated exposures. This finding would suggest either that stress decreases spike values, or that spike depression is independent of stress.

E. Dual NMDA Receptor Population Model

A final possibility is that there are two separate NMDA receptor populations responsible for STEM versus LTP. There could be, for example, two different NMDA synapses from the perforant path to the dentate gyrus, one that synapses directly onto the granule cells, and one that synapses onto inhibitory interneurons. If the granule cell synapse had a higher threshold for activation than the interneuron synapse, it would be unlikely to be activated during STEM, and would instead be activated preferentially after large amounts of stimulation such as those used to induce LTP. The synapse onto the interneurons, on the other hand, would be activated during STEM. Activation of these synapses would result in enhanced activity at the GABAergic inhibitory input from the interneurons onto the proximal

dendrites and somata of granule cells, hence reducing the likelihood of granule cell firing.

In this proposed model, high frequency stimulation would activate both the inhibitory and excitatory inputs. If the direct excitatory input were more potent than the inhibitory inputs, post-synaptic EPSPs would be powerful enough to counteract the spike-inhibiting influences of the IPSPs, and an increase in synaptic efficacy such as that observed in LTP would result. During STEM, the inhibitory input would be activated more strongly than the excitatory input, leading to a decrease in synaptic efficacy.

This proposed dual circuitry accounts for the opposite shifts observed in LTP and STEM, and provides an explanation of why LTP, STEM, and their associated changes in synaptic efficacy are reduced by MK-801. However, it cannot account for the decreased synaptic efficacy observed in LTP animals which received MK-801 unless another input synapses onto the GABAergic inhibitory neurons. This input must be a non-NMDA input which results in increased inhibition of granule cell firing. In the presence of NMDA receptor antagonists, only this inhibitory non-NMDA circuit could be activated. Since inhibitory circuits in the hippocampus can be potentiated (Wilson et al., 1981; Buzsaki & Eidelberg, 1982), the high frequency stimulation could potentiate this non-NMDA circuit, resulting in the observed decrease in synaptic efficacy in LTP animals receiving MK-801.

One further possibility is that LTP's NMDA receptor-dependency is based in the hippocampus, whereas STEM's is based in another area. Because STEM, by its very nature, can only be studied in intact animals, the hippocampus cannot be studied in isolation. The peripheral administration of MK-801 in these experiments could have potentially influenced populations of cells in other brain areas. Particularly good candidates for this effect would be cortical areas which directly or indirectly project to the hippocampus. Perhaps the most likely of these areas is the entorhinal cortex, which is not only rich in NMDA receptors, but also projects directly to the hippocampus. If MK-801 does affect NMDA receptor populations projecting to the hippocampus, it is possible that the MK-801's interference with STEM could be caused by sensory or motor factors which could prevent an animal from taking in information from the environment, or from processing the sensory information appropriately.

F. Support for Model

This final model seems to provide the most parsimonious explanation of the findings reported in this dissertation based on previous evidence. For example, there is prior evidence for an influence of the GABAergic inhibitory interneurons on alterations in synaptic efficacy following

stimulation measured by shifts in the E-S relationship (Kairiss et al., 1987). In addition, there is good evidence that at least some portion of the NMDA receptor population is only activated after high frequency stimulation. That is, blockade of the NMDA receptors does not appear to interfere with normal synaptic transmission in the dentate gyrus (Wigstrom et al., 1986; Errington et al., 1987; Abraham & Mason, 1988), although it clearly interferes with the development of LTP in the dentate gyrus (Wigstrom et al., 1986; Morris et al., 1986; Errington et al., 1987; Gilbert & Mack, 1990). Therefore, a model that suggests that the major NMDA synapse onto the granule cells is activated preferentially at high levels of stimulation is consistent with these findings. The most elusive element of this model is the problem of which neurotransmitter system is responsible for the NMDA receptor-independent activation of inhibitory interneurons after high frequency stimulation. One possible candidate is the noradrenergic input to the dentate gyrus, since LTP can be induced by norepinephrine (Neuman & Harley, 1983). Further research will be necessary to determine which neurotransmitter is involved.

The finding in Experiment 5 that STEM does show some dependency on the amount of stimulation supports the proposed model. Specifically, the spike depression, which is the best measure of degree of activation of inhibitory circuitry, is the component of STEM most related to amount

of environmental stimulation. It is possible that increased amounts of stimulation enhance the activation of inhibitory circuitry. LTP's activation of excitatory pathways could either be due to levels of stimulation far beyond those that could be provided through environmental manipulations, or could simply be an activation that does not occur in that form naturally. An alternate possibility is that spike depression depends on information processing (Sharp et al., 1989; Green et al., 1990), while EPSP enhancement depends on elevated brain temperature (Moser et al., 1993). That is, the inhibitory circuitry could be contributing to the production of a more refined pattern of firing, reflecting a more efficient means of coding information. The concomitant EPSP enhancement could simply be an artifact of increased brain temperature which results from the animal's exploratory movements, as demonstrated in experiments using passive warming or exercise to enhance EPSPs (Moser et al., 1983). This explanation is consistent with the data collected about STEM to date, and has the added advantage of providing a simple explanation for the EPSP-spike dissociation consistently observed in these experiments. In either case, this model supports the view that STEM and LTP are unique in terms of which synapses in the intrinsic hippocampal circuitry they are likely to be activating.

G. Future Directions

STEM and dentate LTP are both characterized by NMDA receptor-dependent changes in synaptic efficacy. However, the data presented in this dissertation clearly indicate that there are differences between the mechanisms involved in the two phenomena. In addition, a recent experiment demonstrated that the effects of serial induction of the two phenomena on EPSP values is additive, rather than interactive (McNaughton, Erickson, Barnes & Stevenson, 1991). Therefore, although studies of STEM may provide clues about LTP's potential as a learning mechanism, it is more likely that studies of STEM will instead reveal the intricacies of the circuitry involved in natural plasticity in the dentate gyrus of the hippocampus. More studies need to be undertaken to further characterize subtle changes in hippocampal physiology which accompany exploratory and learning-related behaviors. These investigations should include studies to: 1. more completely characterize the neurochemistry of subtle physiological changes, 2. elucidate the nature of the EPSP-spike dissociation in these phenomena, with a particular emphasis on studies of possible heterosynaptic effects which could differentially influence EPSPs and spikes and 3. parametrically manipulate environmental information and the animal's behavioral repertoire to define precisely what behavioral and

environmental parameters are necessary to induce these subtle forms of physiological plasticity.

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