DOPAMINERGIC MODULATION OF ENTORHINAL CORTEX FUNCTION

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the Department
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ABSTRACT

Dopaminergic Modulation of Entorhinal Cortex Function

Douglas A. Caruana, Ph.D.
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The neurotransmitter dopamine has been shown to play an important role in the mnemonic functions of the prefrontal cortex, but it is unclear how dopamine may affect sensory and mnemonic processing in the entorhinal cortex. Midbrain dopamine neurons project to the superficial layers of the lateral entorhinal cortex and may modulate olfactory inputs that also terminate in this area. In awake rats, increasing extracellular dopamine levels in the entorhinal cortex with a selective dopamine reuptake inhibitor facilitated basal synaptic transmission in piriform cortex inputs to layer II. Experiments in slices of the entorhinal cortex maintained *in vitro* demonstrated concentration-dependent, bidirectional effects of dopamine on synaptic responses; a low 10 μM concentration of dopamine enhanced synaptic responses and higher concentrations of 50 and 100 μM dopamine suppressed responses. The facilitation of responses was dependent on activation of D_1 receptors and the suppression was dependent on D_2 receptors. Intracellular recordings of mixed and isolated synaptic responses demonstrated that the dopaminergic suppression is mediated by a D_2 receptor-dependent reduction in glutamate release and a D_1-dependent drop in cellular input resistance. The drop in input resistance was mediated by a D_1 receptor-dependent K^+ conductance. In additional experiments, patterned stimulation of the piriform cortex that induces persistent changes in synaptic strength in the

entorhinal cortex was used to assess the effects of dopamine on mechanisms of synaptic plasticity in awake rats. Long-term potentiation and depression were successfully induced in control animals, but the same stimulation protocols failed to alter synaptic function in animals treated with a dopamine reuptake inhibitor. The effects of depleting dopamine in the entorhinal cortex on olfactory memory were also assessed using an olfactory non-match-to-sample task. Rats with 6-OHDA lesions of the entorhinal cortex made more errors and took nearly twice as long to reacquire criterion performance relative to control animals during post-surgical retraining. However, once criterion performance was re-attained, the behavior of lesioned animals was indistinguishable from controls on a version of the task involving longer delay periods. These findings point to multiple mechanisms through which exposure to different concentrations of dopamine may modulate sensory and mnemonic processing by modulating synaptic transmission within the lateral entorhinal cortex.

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CONTRIBUTION OF AUTHORS

1. **Dopamine has bidirectional effects on synaptic responses to cortical inputs in layer II of the lateral entorhinal cortex** by Caruana, Sorge, Stewart, and Chapman.

- **Douglas Caruana**: Designed the experiments, performed all stereotaxic surgeries and electrophysiological recordings, conducted the microdialysis experiment, analyzed and plotted the data, and wrote the manuscript for publication.
- **Robert Sorge**: Assisted with the microdialysis experiments and HPLC analyses.
- **Jane Stewart**: Provided laboratory resources for microdialysis experiments and offered useful comments on drafts of the manuscript.
- **Andrew Chapman**: Assisted with writing the manuscript and in designing the experiments.

2. **Dopaminergic suppression of synaptic transmission in the lateral entorhinal cortex through reduced glutamate release** by Caruana and Chapman.

- **Douglas Caruana**: Designed the experiments, performed all electrophysiological recordings, analyzed and plotted the data, and wrote the manuscript for publication.
- **Andrew Chapman**: Assisted with writing the manuscript and in designing the experiments.

3. **Dopamine reuptake inhibition blocks the induction of long-term potentiation and depression in the lateral entorhinal cortex of awake rats** by Caruana, Reed, Sliz, and Chapman.

- **Douglas Caruana**: Designed the experiments, performed all stereotaxic surgeries and electrophysiological recordings, analyzed and plotted the data, and wrote the manuscript for publication.
- **Sean Reed**: Assisted with electrophysiological recordings.
- **Diane Sliz**: Assisted with electrophysiological recordings.
- **Andrew Chapman**: Assisted with writing the manuscript and in designing the experiments.

4. **Dopamine depletion in the entorhinal cortex by 6-OHDA lesion impairs reacquisition of an olfactory non-match-to-sample task** by Caruana, Reed, Karpowicz, Shalev, and Chapman.

- **Douglas Caruana**: Designed the experiments, performed all stereotaxic surgeries, analyzed and plotted the data, and wrote the manuscript for publication.
- **Sean Reed**: Helped to collect behavioral data.
- **Lila Karpowicz**: Helped to collect behavioral data.
- **Uri Shalev**: Provided laboratory resources for locomotor experiments and helped to analyze the locomotor data.
- **Andrew Chapman**: Assisted with writing the manuscript and in designing the experiments.

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LIST OF ABBREVIATIONS

ACSF	artificial cerebrospinal fluid
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ANOVA	analysis of variance
APV	2-amino-5-phosphonovaleric acid
CA1, CA3	*Cornu Ammonis* fields of the hippocampus
Ca^{2+}	calcium
cAMP	cyclic adenosine monophosphate
CNQX	7-nitro-2,3-dioxo-1,4-dihydroquinoxaline-6-carbonitrile
DAC	digital-to-analog channel
DMSO	dimethyl sulfoxide
EEG	electroencephalographic
EPSC	excitatory postsynaptic current
EPSP	excitatory postsynaptic potential
fEPSP	field excitatory postsynaptic potential
GABA	gamma-aminobutyric acid
HPLC	high performance liquid chromatography
I_h	hyperpolarization-activated nonspecific cation current
I_{NaP}	persistent sodium current
IPSC	inhibitory postsynaptic current
IPSP	inhibitory postsynaptic potential
IRKC	inwardly-rectifying potassium current
K^+	potassium
L-DOPA	3,4-dihydroxy-L-phenylalanine
LTD	long-term depression
LTP	long-term potentiation
Na^+	sodium
N-K	Newman-Keuls
NMDA	N-methyl-D-aspartate
NMTS	non-match-to-sample
6-OHDA	6-hydroxydopamine
PKA	protein kinase A
R_{in}	input resistance
SEM	standard error of the mean
TEA	tetraethylammonium
TTX	tetrodotoxin
V_m	membrane potential

CHAPTER 1

GENERAL INTRODUCTION

Dopaminergic Modulation of Entorhinal Cortex Function

Learning and memory are fundamental processes that allow us to interact effectively with the environment. Experience tells us that placing a hand upon a hot stovetop or waiting until the last minute to study for an important examination can have undesirable consequences. Similarly, pleasant and enjoyable experiences such as the discovery of a new restaurant that serves savory food or an exciting new genre of music can be equally influential in shaping our behavior. In these examples many cognitive processes are working in tandem to help encode, modify, and integrate sensory processing with the wealth of knowledge and experience available to us from memory. We learn complex relationships between events and stimuli in the environment, bind sensory elements together into a single episode that can be recalled later, and assign significance and value to the memory. Learning and memory are the products of coordinated activity between many diffuse brain regions that, together, form a complex and interconnected information processing system (Sherry & Schacter, 1987; Squire, 2004; Squire & Zola-Morgan, 1991). Moreover, the processes of learning and memory are continuously shaped by feedback from additional brain regions that mediate motivation and emotional state.

Research into understanding brain structures and physiological mechanisms that underlie the acquisition, consolidation, and retention of new information has been intense since it was first demonstrated that removal of the medial temporal lobes produces profound anterograde amnesia. In the seminal study by Scoville and Milner (1957) it was shown that the patient H.M., who had his medial temporal lobes removed bilaterally as a treatment for chronic and

debilitating epilepsy, was unable to form any new and lasting declarative memories. The areas of the brain removed during the procedure included the hippocampal formation (dentate gyrus, Ammon's horn, and subicular complex) and parahippocampal cortices (perirhinal and entorhinal cortices and the parahippocampal gyrus; Scoville & Milner, 1957). These observations suggested that the hippocampus and related medial temporal lobe structures are essential for the acquisition and consolidation of new information. This was also consistent with the growing anatomical literature demonstrating the rich interconnectivity shared between these regions (Blackstad, 1958). It is generally accepted that the hippocampal formation is involved in the encoding, consolidation, and retrieval of declarative information, but there is growing evidence to suggest that the functional integrity of the parahippocampal cortices including the entorhinal cortex is also critical, and that these regions can play a much larger role in memory processing than has been traditionally ascribed (Leonard, Amaral, Squire, & Zola-Morgan, 1995; Squire & Zola, 1996).

The parahippocampal region shares reciprocal connections with major sensory and association cortices, and provides the hippocampus with the bulk of its cortical sensory input (Amaral & Witter, 1989; Burwell, 2000; Witter, Wouterlood, Naber, & Van Haeften, 2000b). In particular, multi-modal sensory information destined for hippocampal processing converges on the dendrites of neurons located in the superficial layers (I, II, and III) of the entorhinal cortex. Recent evidence suggests that the entorhinal cortex is essential for the integration of this multi-modal sensory information into unified neuronal representations (Chrobak & Buzsáki, 1998; Dickson, Biella, & de Curtis, 2000;

Dickson, Magistretti, Shalinsky, Hamam, & Alonso, 2000). Neurons in the superficial layers project directly to the hippocampal formation, and processed information from the hippocampus is relayed back to the entorhinal cortex (deep layers V-VI) en route to other cortical areas (Amaral & Witter, 1989; Burwell, 2000; Witter et al., 2000b). In this respect, the entorhinal cortex occupies a strategic anatomical position in the medial temporal lobe as the primary link between the hippocampal formation and the neocortex. This thesis deals with the modulation of synaptic responses within the entorhinal cortex, and how this modulation can contribute to sensory processing, and to processes involved in learning and memory. Determining the factors that affect synaptic transmission in the entorhinal cortex, and the mechanisms by which sensory information is integrated within its circuitry is critical to our understanding of how the entorhinal cortex contributes to declarative memory.

The neurotransmitter dopamine has been shown to be involved in brain mechanisms of reward, motivation, and stress (Berridge, 2007; Hyman, Malenka, & Nestler, 2006a, 2006b; Iversen & Iversen, 2007; Schultz, 2005, 2007; Wise, 2005), and dysfunctions in dopaminergic systems have been linked to the etiology of Schizophrenia and Parkinson's disease (Arnsten, 1998). Moreover, dopamine has also been shown to play a central role in working memory processes mediated by the prefrontal cortex (Goldman-Rakic, 1999; Seamans & Yang, 2004). Although the entorhinal cortex is thought to contribute to memory processing, and projections from midbrain dopamine neurons terminate in the both the superficial and deep layers, little is known about the effects of dopamine on memory processing in the entorhinal cortex. The principal goal of this thesis

was to examine the role of dopamine in modulating the synaptic and intrinsic excitability of neurons located in the superficial layers of the lateral entorhinal cortex and to assess the contributions of dopamine to olfactory memory processing by the entorhinal cortex. This was accomplished using experiments at the cellular level that examined the effects of dopamine on synaptic responses and physiological mechanisms central to memory formation, as well as experiments at the behavioral level that assessed the effects of dopamine depletion on olfactory working memory.

The following sections of this *General Introduction* will provide an overview of the anatomy and physiology of the entorhinal cortex, review the role of the entorhinal cortex in sensory and mnemonic processing, and outline the rationale for examining the possible modulatory role of dopamine on synaptic function and memory processing in the lateral entorhinal cortex.

PART 1: THE ENTORHINAL CORTEX AND MEMORY

1.1. Anatomical Perspectives

The location of the entorhinal cortex within the medial temporal lobe has often been considered to suggest that it plays an important role in processes central to declarative memory. The entorhinal cortex has been described as occupying a "unique", "pivotal", or even "strategic" position in the brain, and analogies have been made comparing it to the likes of a "funnel" or even a "gatekeeper" (Fernández & Tendolkar, 2006; Kerr, Agster, Furtak, & Burwell, 2007; Pinto, Fuentes, & Paré, 2006; Witter, Groenewegen, Lopes da Silva, & Lohman, 1989; Witter, Room, Groenewegen, & Lohman, 1986; Wyss, 1981;

Young, Otto, Fox, & Eichenbaum, 1997). As will be highlighted in the following sections, because the entorhinal cortex provides the hippocampal formation with most of its cortical sensory input (Amaral & Witter, 1989; Burwell, 2000; Witter et al., 2000b), it does indeed occupy a unique position in the mammalian brain and can be seen to function as a gatekeeper for cortical sensory inputs to the hippocampus. In addition, reciprocal connections that the entorhinal cortex shares with the hippocampus, neocortex, and other subcortical structures also emphasize the importance of the entorhinal cortex in processes central to learning and memory.

The entorhinal cortex can be classified as "transition" cortex since it is situated between typical isocortical association areas (i.e., neocortex) and the allocortical regions of the hippocampal formation (Amaral, Insausti, & Cowan, 1987; Solodkin & Van Hoesen, 1996; Witter et al., 1989; Witter et al., 2000a). Although the entorhinal cortex has 6-layers and shares features common to other neocortical areas, it differs in several fundamental ways. For instance, in most of the neocortex, the largest neurons are typically output neurons located in the deeper layers (V-VI), but in the entorhinal cortex the largest cells are those found in layer II that receive sensory inputs (Lingenhöhl & Finch, 1991; Solodkin & Van Hoesen, 1996). Moreover, in most neocortical regions the dense organization of cells produces a uniform and banded appearance, but in the anterior parts of the entorhinal cortex the principal cells appear clumped together into "cell islands" (Blackstad, 1956; Carboni & Lavelle, 2000; Steward, 1976; Wyss, 1981). A region containing few or no cells, known as the lamina dessicans, is also present in the entorhinal cortex (Akil, Edgar, Pierri, Casali, & Lewis, 2000), and it is

thought that this atypical feature is a phylogenetic remnant of a primitive molecular layer that once existed in the structure (Solodkin & Van Hoesen, 1996). Because the entorhinal cortex shares both iso- and allocortical cytoarchitectonic features, the term "schizocortex" has sometimes been used to classify it (Solodkin & Van Hoesen, 1996; Stephan, 1983).

The entorhinal cortex of the rat lies on the ventrolateral surface of the posterior part of the brain (Fig. 1.1A,B) and is situated beneath the perirhinal cortex and subicular complex (Blackstad, 1956; Kerr et al., 2007; Paxinos & Watson, 1998). The cytoarchitecture of the entorhinal cortex and its interconnectivity with the hippocampus, cortex, and other subcortical regions are strikingly homologous across species including the rat (Köhler, 1985, 1986, 1988), monkey (Amaral et al., 1987; Insausti, Amaral, & Cowan, 1987a, 1987b), cat (Room & Groenewegen, 1986a, 1986b; Witter et al., 1986), guinea pig (Sørensen, 1985; Sørensen & Shipley, 1979), and mouse (Burwell, 2000). Early anatomical studies conducted by Ramón y Cajal (1902) and then later by his student Lorente de Nó (1934) clearly demonstrated that the entorhinal cortex shares rich interconnections with the hippocampus. On the basis of these anatomical connections alone, it was believed that the two structures likely performed related functions (Witter et al., 1989). But it was not until much later that the importance of the medial temporal lobe to sensory and mnemonic processing would come to be fully realized (Scoville & Milner, 1957).

Blackstad (1958) was one of the first to demonstrate that the entorhinal cortex provides the major source of afferent input to the dentate gyrus and hippocampus via the so-called perforant path. Discrete lesions confined to the

superficial layers of the entorhinal cortex resulted in the degeneration of axonal boutons in the dentate gyrus and provided the first experimental evidence that the entorhinal cortex innervates the hippocampus via the perforant path (Blackstad, 1958). The term "perforant path" was originally used by Ramón y Cajal to describe the transverse course of fibers which originate from the superficial layers of the entorhinal cortex and perforate the subiculum en route to the hippocampus (Ramón y Cajal, 1902; Swanson & Köhler, 1986). Subsequent anatomical studies using discrete lesions and histochemical and fluorescent tracers have demonstrated that perforant path fibers originating in layer II of the entorhinal cortex terminate in either the molecular layer of the dentate gyrus (Blackstad, 1958; Dolorfo & Amaral, 1998b; Hjorth-Simonsen, 1972; Hjorth-Simonsen & Jeune, 1972; Witter et al., 1989) or the CA3 region of the hippocampus (Witter & Amaral, 1991; Witter et al., 2000b). Projections from layer III target the stratum lacunosum-moleculare of area CA1 (Kerr et al., 2007; Steward, 1976; Sybirska, Davachi, & Goldman-Rakic, 2000) and the molecular layer of the subiculum (Baks-Te Bulte, Wouterlood, Vinkenoog, & Witter, 2005; Steward, 1976; van Groen, van Haren, Witter, & Groenewegen, 1986). From a strategic viewpoint, the entorhinal cortex is perfectly situated to influence processing at all levels of the hippocampus through its projections to each major cell field.

Because of the massive perforant path projections to the hippocampus, it was initially believed that the entorhinal cortex functioned mainly as a simple relay for information requiring hippocampal processing (Insausti et al., 1987a; Van Hoesen & Pandya, 1975a, 1975b; Van Hoesen, Pandya, & Butters, 1975).

However, although anatomical reports demonstrated that processed information originating from the CA3 and CA1 regions could exit the hippocampus via the fornix and reach cortical targets through relays in the thalamus, additional tracing studies showed that there was a second major output from the hippocampus *back* to the entorhinal cortex (Swanson & Cowan, 1977; Witter et al., 1989). Specifically, neurons located in both the CA1 and subicular regions of the hippocampal formation project to the deep layers (V-VI) of the entorhinal cortex (Amaral & Witter, 1989; Burwell, 2000; Köhler, 1985; Room & Groenewegen, 1986b; Sørensen & Shipley, 1979; Swanson & Cowan, 1977; van Groen et al., 1986; Witter et al., 1989; Witter et al., 2000b). These studies demonstrated that the entorhinal cortex and connectivity with the hippocampus formed a loop through which information entered the circuit via the superficial layers of the entorhinal cortex and exited the loop via the deep layers of the entorhinal cortex. This set of reciprocal connections of the entorhinal cortex with both the hippocampal formation and neocortex is consistent with an important role in sensory and mnemonic processes that are thought to involve interactions between the hippocampal formation and neocortex (e.g., consolidation; Nadel & Moscovitch, 1997; Sirota, Csicsvari, Buhl, & Buzsáki, 2003).

Interestingly, neurons in the deep layers of the entorhinal cortex send axonal collaterals back to the same superficial layer projection neurons that give rise to the perforant path input to the hippocampus (Dolorfo & Amaral, 1998a; Gloveli, Dugladze, Schmitz, & Heinemann, 2001; Köhler, 1986). Ultrastructural analyses have shown that pyramidal and horizontal cells located in layer V make excitatory synaptic contact with spines and shafts of dendrites in the superficial

layers (I to III) of the entorhinal cortex (van Haeften, Baks-te-Bulte, Goede, Wouterlood, & Witter, 2003). About half of the cells innervated were principal cells and the remaining half were local inhibitory interneurons. These findings suggest that processed information leaving the hippocampus might re-enter the circuit via deep layer activation of superficial layer projection neurons. Moreover, deep layer excitation of feedforward inhibitory circuits in the superficial layers can constrain or "gate" the excitability of principal neurons and inhibit the transfer of new information into the hippocampal formation (van Haeften et al., 2003). Indeed, stimulation of CA1 fibers that project to the deep layers of the entorhinal cortex was shown to evoke long-latency polysynaptic responses recorded at different upstream locations along the entire hippocampal circuit (Kloosterman, van Haeften, & Lopes da Silva, 2004). In this way, processed information can "reverberate" within the entorhinal-hippocampal loop while being continuously updated via new sensory information entering the system through the superficial layers of the entorhinal cortex.

As incredible as the highly organized interconnectivity between the entorhinal cortex and hippocampus is, it pales in comparison to the *massive* innervation that the entorhinal cortex receives from primary sensory and association cortices, as well as from various subcortical structures (Fig. 1.1C). Information from *every* sensory modality projects to the entorhinal cortex either directly or indirectly through relays in the perirhinal and postrhinal association cortices (Amaral & Witter, 1989; Burwell, 2000; Kerr et al., 2007; Witter et al., 2000b). Inputs converge in the superficial layers (I to III) of both the medial and lateral divisions of the entorhinal cortex before being transferred via the perforant

and temporoammonic paths to the hippocampus. Moreover, processed information that leaves the hippocampus via the CA1/subicular region is relayed from the deep layers of the entorhinal cortex back to the *entire* cortical mantle (Burwell, 2000; Kerr et al., 2007; Swanson & Köhler, 1986). It is in this way that the entorhinal cortex occupies a "strategic" position in the mammalian brain since the majority of sensory information that reaches the hippocampus must do so via the entorhinal cortex. Similarly, a large majority of information processed by the hippocampus cannot return to cortex without first being relayed through the entorhinal cortex.

The tight integration that that the entorhinal cortex shares with sensory cortices and the hippocampus suggests that it plays an important role in the mnemonic functions of the entire medial temporal lobe. The different sensory inputs that the medial and lateral divisions of the entorhinal cortex receive together with the topographic specificity of outputs to different locations in the hippocampus suggest that the medial and lateral divisions of the entorhinal cortex mediate different types of information and that these two streams are processed independently in the hippocampus. There is a striking degree of topography present in projections from the entorhinal cortex to the hippocampal formation and it is clear that there are two major divisions in the entorhinal cortex that differ both anatomically and functionally. These two sub-regions were originally termed the pars lateralis (or lateral entorhinal cortex) and pars medialis (or medial entorhinal cortex; Blackstad, 1958). The lateral division of the entorhinal cortex projects via the lateral perforant path to the most septal regions of the dentate gyrus, whereas the medial entorhinal cortex targets sites in the

temporal portions of the dentate through the medial perforant path (Hjorth-Simonsen, 1972; Hjorth-Simonsen & Jeune, 1972; Ruth, Collier, & Routtenberg, 1988). Moreover, discrete sub-regions, or bands, within both the medial and lateral divisions of the entorhinal cortex also terminate at specific locations along the septotemporal axis of the dentate gyrus (Dolorfo & Amaral, 1998b; Kerr et al., 2007; Tamamaki, 1997). These findings suggest that different sub-regions within the entorhinal cortex might process qualitatively different types of sensory information, and that this difference in sensory information processed by each division is maintained through the segregation of efferents from the medial and lateral entorhinal cortices to the hippocampus (Fig. 1.2). This idea is supported by both behavioral and electrophysiological findings demonstrating clear differences between the electroresponsiveness of projection neurons in the medial and lateral divisions of the entorhinal cortex (Alonso & Klink, 1993; Tahvildari & Alonso, 2005; Wang & Lambert, 2003) and the types of sensory information carried by medial and lateral perforant path inputs to the hippocampus (Hargreaves, Rao, Lee, & Knierim, 2005; Sewards & Sewards, 2003). Specifically, the lateral entorhinal cortex receives most of its input from the primary olfactory cortex and perirhinal cortex (Burwell, 2000; Kerr et al., 2007) and this is consistent with the idea that it is involved in the processing of olfactory information (Ferry, Ferreira, Traissard, & Majchrzak, 2006). In contrast, the medial entorhinal cortex receives inputs from multimodal association cortices and from the postrhinal cortex which receives inputs from auditory, somatosensory, and visual cortices (Burwell, 2000; Kerr et al., 2007). This is consistent with a role for the medial entorhinal cortex in spatial processing, and

with a major contribution of the entorhinal cortex to spatial processes mediated by the hippocampal formation (Fyhn, Molden, Witter, Moser, & Moser, 2004; Hafting, Fyhn, Molden, Moser, & Moser, 2005; Witter & Moser, 2006).

1.2. Behavioral Studies of Entorhinal Cortical Function

In humans, the entorhinal cortex is the first structure to show evidence of degeneration in Alzheimer's dementia and this suggests that the cognitive impairments associated with the onset of the disease result, in part, from a loss of cells in this region (Van Hoesen, Hyman, & Damasio, 1991). The degeneration of neurons in the entorhinal cortex effectively isolates the hippocampus from its cortical sensory input and this has profound consequences for sensory and mnemonic processing (Dickerson, 2007). Interestingly, the deterioration of the entorhinal cortex in Alzheimer's dementia typically occurs in a laminar fashion with neurofibrillary tangles first appearing in layer II during the initial stages of the disease and then progressing to the deeper layers as the pathology worsens (van Hoesen, Augustinack, Dierking, Redman, & Thangavel, 2000). The initial degeneration observed in layer II may underlie the early cognitive impairments associated with the disease because it is layer II neurons that give rise to the perforant path projection to the hippocampus thereby providing the hippocampal formation with the bulk of its sensory input (Blackstad, 1958; Dolorfo & Amaral, 1998b; Hjorth-Simonsen, 1972; Hjorth-Simonsen & Jeune, 1972; Witter et al., 1989). Similar memory impairments are also observed in other neurodegenerative disorders that have effects on the entorhinal cortex,

including argyrophilic grain disease and Pick's disease (Braak, Del Tredici, Bohl, Bratzke, & Braak, 2000).

The finding that bilateral removal of the medial temporal lobe in humans resulted in severe anterograde amnesia (Scoville & Milner, 1957) became associated with the widespread belief that the hippocampus was required for declarative memory formation, and much less attention was given to the potential role of the parahippocampal cortices in mnemonic processing. More recently in primates, it has been concluded that it is damage to parahippocampal cortices that was responsible for many of the memory deficits associated with medial temporal lobe damage (Leonard et al., 1995; Squire & Zola, 1996; Squire & Zola-Morgan, 1991; Zola-Morgan, Squire, & Amaral, 1989; Zola-Morgan, Squire, & Mishkin, 1982). The use of non-match-to-sample tasks, which require trial-specific visual stimuli to be remembered during a variable delay period, were instrumental in determining the contributions of the parahippocampal cortices to recognition memory impairments following bilateral ablation of these structures in monkeys. These studies identified the perirhinal cortex damage as being most clearly associated with the memory deficits (Squire & Zola-Morgan, 1991; Suzuki, 1996), but anatomical information regarding the connectivity of the entorhinal cortex with both the hippocampus and neocortex suggests that the entorhinal cortex also contributes to the mnemonic functions of the medial temporal lobe.

There is a vast experimental literature dealing with memory deficits in rodents following hippocampal (Eichenbaum, 1999; Moser & Paulsen, 2001) and entorhinal cortical (Hasselmo & Stern, 2006; Schwarcz & Witter, 2002a) ablation. Determining the individual contributions of the hippocampus and entorhinal

cortex to mnemonic processing is often difficult to interpret (see Aggleton, Vann, Oswald, & Good, 2000), however, because lesions of the entorhinal cortex were often made to assess the effects of disconnecting the hippocampal formation from cortical sensory input (e.g., Olton, Walker, & Gage, 1978). Therefore, the behavioral effects of entorhinal cortex lesions must always be interpreted cautiously, because the deficits produced by the lesion could result from either the disruption of sensory processing by the entorhinal cortex, or from a disruption of the function of other cortical areas that are dependent on the output of the entorhinal cortex. However, the results from behavioral studies that have examined the effects of entorhinal cortex damage are consistent with an important role of the entorhinal cortex in learning and memory.

Early lesion studies demonstrated that extensive damage to the entorhinal cortex induced by electrolytic, aspiration, or radiofrequency lesions resulted in pronounced impairments in spatial memory. A feature common to the tasks used to assess spatial memory in these studies was that animals were required to utilize and remember extramaze visual cues or landmarks to successfully complete the task. Lesions of the entorhinal cortex most commonly produced deficits in spatial working and reference memory on tasks such as the radial arm maze (Cho & Kesner, 1996; Hunt, Kesner, & Evans, 1994; Jarrard, Okaichi, Steward, & Goldschmidt, 1984; Johnson & Kesner, 1994; Olton et al., 1978; Olton, Walker, & Wolf, 1982; Rasmussen, Barnes, & McNaughton, 1989) and Morris water maze (Galani, Jarrard, Will, & Kelche, 1997; Hardman et al., 1997; Nagahara, Otto, & Gallagher, 1995; Schenk & Morris, 1985). The findings of these studies are consistent with the idea that damage to the entorhinal cortex

produces memory impairments similar to the impairments observed following hippocampal ablation alone.

Recent work involving excitotoxic lesions of the entorhinal cortex, which spare fibers of passage to and from the hippocampus, has shown that the entorhinal cortex may play little or no role in spatial memory processing. These studies show that excitotoxic lesions of the entorhinal cortex do not impair performance on either the radial arm maze or Morris water maze (Aggleton et al., 2000; Bannerman et al., 2001a; Bannerman et al., 2001b; Bouffard & Jarrard, 1988; Burwell, Saddoris, Bucci, & Wiig, 2004; Galani, Obis, Coutureau, Jarrard, & Cassel, 2002; Jarrard, Davidson, & Bowring, 2004; Oswald et al., 2003; Pouzet et al., 1999). Such findings contradict earlier reports and suggest that inputs originating from brain regions other than the entorhinal cortex are important contributors to spatial memory processing, and that direct damage to the entorhinal cortex does not produce significant spatial memory deficits. Anatomical studies have shown that collateral projections from the perirhinal and postrhinal cortices can bypass the entorhinal cortex completely and convey visuospatial information to the hippocampus directly (Naber, Witter, & Lopes da Silva, 2001; Naber, Witter, & Lopes da Silva, 1999). Further, subcortical projections from the thalamus can also reach the hippocampus, and thalamic inputs may also convey visual information to the hippocampal formation that might be required for successful completion of spatial memory tasks (Dolleman-Van der Weel & Witter, 2000; Wouterlood, Saldana, & Witter, 1990). More recently, however, it has been argued that the excitotoxic lesions used to damage the entorhinal cortex in these studies rarely included the most

dorsomedial extent of the entorhinal cortex and therefore *preserved* the cells required for spatial memory processing (Steffenach, Witter, Moser, & Moser, 2005). The dorsomedial entorhinal cortex receives the most dense visuospatial inputs originating from the primary visual cortex and from the postrhinal cortex (Burwell, 2000), and cells in the dorsomedial entorhinal cortex also fire reliably when an animal enters the same spatial location in an open field (Fyhn et al., 2004). Indeed, excitotoxic lesions restricted to the dorsomedial extent of the entorhinal cortex produce profound spatial memory impairments in rats and suggest that cells in this region are required for spatial memory processing (Steffenach et al., 2005).

Studies that have used electrophysiological recording methods to track the firing of neurons in the entorhinal cortex offer further support in favor of a role for the dorsomedial entorhinal cortex in spatial processing. In a groundbreaking study by Hafting and colleagues, it was shown that some of the cells in the dorsomedial entorhinal cortex are specialized place cells referred to as "grid cells" because of their pattern of firing in relation to the position of the animal in the environment (Fyhn et al., 2004; Hafting et al., 2005; Witter & Moser, 2006). The place fields of grid cells form a repeating triangular pattern which establishes a grid of the entire spatial environment when an animal roams freely in a large open arena. Grid cells appear to be informed about the spatial location of the animal through inputs from visual and parietal areas via relays from the postrhinal cortex to the medial entorhinal cortex, and from cells in the subicular complex (Caruana & Chapman, 2004; Hafting et al., 2005; Witter & Moser, 2006). Grid cells indicate the involvement of the entorhinal cortex in spatial

processes that were previously attributed mainly to the hippocampal formation (O'Keefe & Nadel, 1978).

Grid cells are located exclusively within the medial entorhinal cortex consistent with the functional segregation of the medial and lateral entorhinal cortices. Although the lateral entorhinal cortex receives inputs from structures including the perirhinal cortex, its major input is from cells in the primary olfactory cortex which is also known as the piriform cortex (Burwell, 2000; Kerr et al., 2007). This suggests that the entorhinal cortex plays a major role in olfactory sensory processing, and may also have a role in olfactory memory. It has been suggested that the cognitive processes underlying olfactory memory in rats might be similar to those used by humans and primates for object recognition memory (Eichenbaum, Fagan, & Cohen, 1986; Staubli, Fraser, Kessler, & Lynch, 1986). Learning to discriminate between sets of visual stimuli in order to successfully complete a mnemonic task typically takes longer during initial training since there are often specific rules that need to be acquired (e.g., the non-match-to-sample rule). As these rules are mastered, however, performance improves dramatically. This phenomenon was first described by Harlow (1949) as "learning to learn" and it was initially thought that this form of cognitive processing was present only in higher-order mammals. Subsequent work has shown that the rate of acquisition of olfactory discrimination learning sets in rats is similar to that of visual learning set acquisition in humans and monkeys (Slotnick & Katz, 1974) and may thus reflect similar underlying cognitive processes. Olfactory recognition memory in rodents may be a useful animal model for studying higher cognitive functions that are typically observed in

humans and monkeys (Eichenbaum et al., 1986; Staubli et al., 1986). Further, considering the importance of the parahippocampal cortices to visual object recognition memory and the dense olfactory projections that terminate in the region, the entorhinal cortex is therefore likely to play a key role in olfactory mnemonic processing.

Early lesion studies have shown that the entorhinal cortex plays an important role in olfactory memory. In a study by Staubli, Ivy, and Lynch (1984), rats were trained to discriminate between pairs of odors in order to obtain a water reward. Different odor pairs were used each day, and once animals could reliably distinguish between a rewarded odor and a distracter within a minimal number of trials they received sham lesions or electrolytic lesions of either the dorsal or lateral entorhinal cortex. Following recovery, both lesion groups could distinguish between odors at presurgery levels, but only rats with lesions to the lateral entorhinal cortex were impaired when the intertrial interval was extended to 10 min (Stäubli et al., 1984). Further, if the reward association was switched from the original odor to the distracter, then sham rats and dorsal entorhinal cortex-lesioned rats continued to show a preference for the previously-rewarded odor when retested after a 1-hour delay. In contrast, rats with lesions to the lateral entorhinal cortex showed no such bias towards the previously-rewarded odor indicating that they were unable to recall which of the two odors had been rewarded prior to the delay period. Additional experiments also showed that lesions to the entorhinal cortex do not impair memory for an olfactory discrimination learned prior to surgery (Staubli et al., 1986). Taken together, these findings indicate that the lateral entorhinal cortex plays an important role in

the acquisition and retention of new olfactory memories and that damage to this area does not result in retrograde amnesia for olfactory information acquired prior to the lesion.

An important way that animals can recognize conspecifics or receptive mates is through the aromatized chemicals they secrete. The hamster is a species that relies heavily on olfaction for many social behaviors as well as for the identification of individual conspecifics (Johnston, 1993). Hamsters have also been used extensively in studies assessing the neural correlates of social recognition (Johnston, 1993; Petrulis, Peng, & Johnston, 2000), and the entorhinal cortex has been shown to play an important role in social recognition memory. When presented with the scent of a novel male, both sham- and entorhinal cortex-lesioned female hamsters will immediately approach the source of the odor and sniff it intensely (Petrulis et al., 2000). Presentation of the same odor repeatedly every 3 min over a 12-min period causes a gradual reduction in the amount of time spent investigating the odor. This reduction indicates that the odor has become familiar to the animal. The presentation of a second male's scent reinstates investigative behavior in sham-lesioned hamsters, but not in entorhinal cortex-lesioned hamsters. This indicates that the lesioned animals could not recognize the scent of the second male as being different from the scent of the first (Petrulis et al., 2000). Thus, although female hamsters with lesions to the entorhinal cortex could recognize an odor as being familiar, they had difficulty discriminating between odors from two *different* males. These findings suggest that the entorhinal cortex plays an important role in discriminating individual conspecific scents.

The entorhinal cortex has also been shown to contribute to social recognition memory in rats. When presented with an unfamiliar juvenile, both sham- and entorhinal cortex-lesioned rats spend ≈75% of a 5-min test session investigating and sniffing the novel animal (Bannerman et al., 2002). In control animals, re-exposure to the same juvenile following a 30-min period of separation does not reinstate investigative behavior, and this is taken to indicate that the sham-lesioned animals recognize the juvenile as being familiar. In contrast, lesioned rats do not recognize the same juvenile after 30-min of separation and spend a significant amount of time during the test session re-investigating and sniffing the juvenile male. Thus, the entorhinal cortex is important not only for discriminating between different conspecifics based on olfactory cues (Petrulis et al., 2000), but also for remembering the scent of a new conspecific for delay periods lasting at least 30 min.

Interestingly, lesions to the lateral entorhinal cortex have also been shown to *enhance* memory for conditioned olfactory aversions. If the ingestion of a scented, but tasteless, liquid is paired with immediate toxicosis, then normal rats will quickly learn to avoid any liquid with that particular scent for a brief period of time (Ferry, Oberling, Jarrard, & Di Scala, 1996). The memory for this conditioned olfactory aversion persists for at least 30 min in intact animals, but is enhanced to last for up to 2 hours in rats with lesions to the lateral entorhinal cortex (Ferry et al., 2006; Ferry et al., 1996). These findings argue against the entorhinal cortex as being the storage site for conditioned olfactory aversions, but instead suggest that the entorhinal cortex normally influences the activity of other structures that mediate the storage of the memory trace. It has been shown that

the infusion of a $GABA_A$ agonist into the basolateral amygdala in rats with lesions to the lateral entorhinal cortex can restore the duration of the conditioned olfactory aversion from 2 hours back to the typical 30 min (Ferry, Wirth, & Di Scala, 1999). These findings have been taken to suggest that excitatory projections from the lateral entorhinal cortex innervate GABAergic interneurons that regulate local neuronal networks involved in the storage of the conditioned olfactory aversion in the basolateral amygdala (Ferry et al., 1999).

There is also considerable evidence to suggest that the entorhinal cortex plays a prominent role in olfactory working memory. Similar to results obtained for spatial working memory, rats with lesions to the entorhinal cortex are unable to use trial-specific olfactory cues to help them remember which arms they had visited when tested on an olfactory version of the radial arm maze task (Staubli, Le, & Lynch, 1995). Lesions to the entorhinal cortex do not impair acquisition of an olfactory non-match-to-sample task nor impair memory for odors during short (≤3 sec) intertrial intervals when performing the task (Otto & Eichenbaum, 1992). However, when the delay period between trials is 30 sec or longer, entorhinal cortex-lesioned rats show significant impairments in olfactory working memory (Otto & Eichenbaum, 1992). But perhaps the clearest evidence linking the entorhinal cortex to olfactory working memory is the finding that unit activity in the lateral entorhinal cortex is time-locked to behavior when animals are engaging in an olfactory non-match-to-sample task (Ramus & Eichenbaum, 2000; Young et al., 1997). Reliable changes in neural activity were linked to the period when rats sampled stimulus odors, approached the reward, initiated a trial, or waited for the delay period of the trial to elapse. Cells that responded to stimulus odors were

shown to be odor-specific and also coded information about whether the stimulus odor was a match or a non-match to the odor presented during the previous trial (Young et al., 1997). Further, the finding that the firing of lateral entorhinal cells increased during the delay period of the task suggests that these cells were actively maintaining olfactory information in working memory. These findings indicate that neurons in the lateral entorhinal cortex are not only involved in coding specific information about olfactory sensory stimuli, but also in retaining this information during variable-duration delay periods (Young et al., 1997).

1.3. Synaptic Plasticity in the Entorhinal Cortex

Perhaps the most widely-studied cellular models of memory storage in the mammalian brain are long-term synaptic potentiation (LTP) and long-term synaptic depression (LTD) (Abraham & Williams, 2003; Bliss & Lomo, 1973; Kemp & Bashir, 2001; Lisman, 2003; Lynch, 2004; Malenka, 1994; Massey & Bashir, 2007; Morris, Davis, & Butcher, 1990). Persistent increases in the efficacy of synaptic connections are typically induced by intense presynaptic stimulation that results in LTP (Malenka & Nicoll, 1999). In contrast, synaptic connections can be weakened by less intense presynaptic stimulation that results in LTD (Kemp & Bashir, 2001). These bidirectional changes in the strength of synaptic connections can last from hours to days and are appealing candidates as substrates for information storage in the brain (Kandel & Pittenger, 1999). Together, LTP and LTD provide a mechanism through which new memory traces can be formed in the brain in a manner that is dependent on increased synaptic

activity. A mechanism similar to LTP was envisioned by Hebb (1949) to mediate the formation of "cell assemblies" that represented new memories.

One of the earliest studies of synaptic plasticity in the entorhinal cortex was conducted by Alonso and colleagues (1990) in which it was shown that theta-patterned stimulation of olfactory inputs to layer II of the entorhinal cortex could cause a lasting increase in synaptic strength. This basic finding was later confirmed by Chapman and Racine (1997b) in field potential recordings of awake rats. They also demonstrated that the amount of LTP induced in the entorhinal cortex could be enhanced by theta-patterned stimulation of the medial septum which provides cholinergic input to the entorhinal cortex. This suggests that neuromodulatory projections to the entorhinal cortex play a significant role in modulating the degree to which synaptic plasticity can be induced. More recently, LTD in the entorhinal cortex has been investigated and has been shown to be induced following low-frequency stimulation of the piriform cortex in behaving animals (Bouras & Chapman, 2003) and also following stimulation of layer I in entorhinal cortex slices (Deng & Lei, 2006; Kourrich & Chapman, 2003). Mechanisms of LTD are thought to be important for shaping the content of cell assemblies, and for removing irrelevant associations from these memory traces (Kemp & Bashir, 2001).

The capacity of the entorhinal cortex for lasting changes in synaptic strength is consistent with the other evidence presented here that the entorhinal cortex is an essential component of the medial temporal lobe which likely contributes in important ways to the processing of sensory information and the formation of new declarative memories. Changes in the strength of synaptic

connections in cortical inputs to the entorhinal cortex are likely an important mechanism involved in modulating sensory processing in the entorhinal cortex, and could also contribute to the mnemonic functions of the entorhinal cortex. In addition, neuromodulatory inputs to the entorhinal cortex are likely to play a critical role in shaping ongoing information processing, and may also contribute in important ways to the acquisition of new memories.

The entorhinal cortex receives substantial innervation from the serotonergic, cholinergic, noradrenergic, and dopaminergic diffuse neuromodulatory systems (Bjorklund & Dunnett, 2007; Eckenstein, Baughman, & Quinn, 1988; Fallon & Loughlin, 1987; Gaykema, Luiten, Nyakas, & Traber, 1990; Köhler, Chan-Palay, Haglund, & Steinbusch, 1980a; Köhler, Chan-Palay, & Steinbusch, 1981; Loy & Moore, 1979; Oades & Halliday, 1987). Increased cholinergic transmission has been shown to have a powerful suppressive effect on basal synaptic strength in cortical inputs to layer II (Hamam, Sinai, Poirier, & Chapman, 2006), and serotonin and norepinephrine have both been shown to elicit powerful modulatory effects on basal synaptic transmission in layer II (Pralong & Magistretti, 1994, 1995; Schmitz, Gloveli, Empson, Draguhn, & Heinemann, 1998; Schmitz, Gloveli, Empson, & Heinemann, 1999). However, the role of dopamine in the entorhinal cortex is unclear. Ascending midbrain dopaminergic projections originating from the ventral tegmental area and substantia nigra innervate both the medial and lateral divisions of the entorhinal cortex (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987; Oades & Halliday, 1987), but the role that dopamine plays in processes central to learning and memory in the entorhinal cortex remains to be elucidated.

Early studies have shown that application of dopamine to slices of entorhinal cortex causes a potent suppression of synaptic transmission (Pralong & Jones, 1993; Rosenkranz & Johnston, 2006; Stenkamp, Heinemann, & Schmitz, 1998), but the mechanisms of this effect and its significance for information processing is not clear. Further, although LTP and LTD have been demonstrated in the entorhinal cortex, the effects of dopamine on the mechanisms underlying the induction and/or maintenance of LTP and LTD in sensory inputs to the entorhinal cortex have not been investigated. The experimental chapters of this thesis deal with addressing these questions, and an overview of the dopaminergic system and its possible relationship to entorhinal functioning will therefore now be given.

PART II. DOPAMINE AND THE ENTORHINAL CORTEX

2.1. The Mesocortical Dopamine System

The mesocortical dopamine system is a branch of the diffuse neuromodulatory dopaminergic system that has been shown to contribute to a diverse range of appetitive behaviors, motivation, addiction, and stress (Berridge, 2007; Hyman et al., 2006a, 2006b; Iversen & Iversen, 2007; Schultz, 2005, 2007; Wise, 2005). The dopaminergic neuromodulatory system has been implicated in the etiology of Schizophrenia and Parkinson's disease (Arnsten, 1998) and also contributes to processes that regulate learning and memory (Goldman-Rakic, 1999; Seamans & Yang, 2004). Although cell bodies for dopamine-containing neurons originate from nine distinct cell groups throughout the midbrain and olfactory bulb (designated A8 through A16; Bjorklund & Dunnett, 2007), the ones

most heavily implicated in cognitive, motor, and mnemonic function are the A8 cells of the retrorubral field, the A9 cells of the substantia nigra, and the A10 cell group of the ventral tegmental area. These cells project to the striatum and nucleus accumbens, as well as to the prefrontal cortex (Oades & Halliday, 1987; Swanson, 1982), and there is also a major branch of the mesocortical dopamine system that projects to both the medial and lateral divisions of the entorhinal cortex (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987; Oades & Halliday, 1987).

Early anatomical reports in the rat demonstrated that the most robust dopaminergic projections to cortex terminated in the deep layers of the frontal cortices and in the superficial layers of the lateral entorhinal cortex (Lindvall, Björklund, Moore, & Stenevi, 1974). Interestingly, the dopaminergic fibers projecting to the lateral entorhinal cortex formed numerous dense clusters which spanned both layers II and III and surrounded principal cell islands located in these layers (Collier & Routtenberg, 1977; Fluxe et al., 1974; Hökfelt, Ljungdahl, Fuxe, & Johansson, 1974; Lindvall et al., 1974). The occurrence of distinct terminal clusters receded in more caudal locations of the entorhinal cortex, and although dopamine-positive fibers were indeed present in the medial entorhinal cortex, the density was considerably less (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987) and distributed in a more homogeneous manner (Collier & Routtenberg, 1977; Fallon, Koziell, & Moore, 1978). Moreover, labeled fibers were also observed in the deeper layers (V and VI) of both the medial and lateral divisions of the entorhinal cortex, but the density was less than in the superficial layers (Fallon et al., 1978).

Selective lesions to the ventral tegmental area (the A10 cell group) significantly deplete dopamine levels in the entorhinal cortex ipsilateral to the lesion (Fallon et al., 1978) and this suggests that the ventral tegmental area is a major source of dopaminergic innervation to the entorhinal cortex (Fig. 1.3). Interestingly, electron microscopic images of degenerating dopamine terminals in the entorhinal cortex following similar lesions demonstrated that dopamine fibers projecting to the lateral division make synaptic contacts onto dendrites in layer II (Collier & Routtenberg, 1977). In other anatomical experiments conducted around the same time it was shown that injections of the retrograde tracer horseradish peroxidase into the lateral entorhinal cortex labeled neurons in the ventral tegmental area and this provided corroborating evidence in support of the lesion data (Beckstead, 1978; Beckstead, Domesick, & Nauta, 1979). Subsequent tracing experiments also demonstrated that dopaminergic neurons in the substantia nigra contributed to the terminal clusters in the lateral entorhinal cortex (Haglund, Köhler, Ross, & Kelder, 1979) and that the projections originated from cells located in the most caudal regions (Loughlin & Fallon, 1984). In more recent experiments, the projections to the entorhinal cortex have been shown to originate primarily from the A10 cell field of the ventral tegmental area as well as from the A8 retrorubral field of the substantia nigra (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987; Oades & Halliday, 1987). A similar pattern of dopaminergic innervation of the entorhinal cortex has also been observed in the monkey (Akil & Lewis, 1993) and human (Akil & Lewis, 1994).

Shortly after the cloning of both D_1- and D_2-like dopamine receptors, numerous pharmacological agents were developed with the specificity to

recognize and bind to different subtypes of dopamine receptors. Radioactive isotopes of these agents have been used to map the distribution of dopamine receptors throughout the brain including the entorhinal cortex. In early experiments, radioactive ligand binding to both D_1-like (Diop, Gottberg, Brière, Grondin, & Reader, 1988; Reader, Brière, Gottberg, Diop, & Grondin, 1988; Savasta, Dubois, & Scatton, 1986) and D_2-like (Dewar, Montreuil, Grondin, & Reader, 1989; van der Weide, Camps, Horn, & Palacios, 1987) dopamine receptors was demonstrated in the medial and lateral divisions of the entorhinal cortex with D_2 receptor binding being localized primarily to the superficial layers (Köhler, Hall, & Gawell, 1986; Köhler & Radesäter, 1986; Richfield, Young, & Penney, 1986) and at a much lower density than D_1 receptor binding (Diop et al., 1988; Reader et al., 1988). These findings confirm that both types of dopamine receptors are present in the entorhinal cortex and that there is a higher density of D_1 receptors. Moreover, mRNA for both D_1 and D_2 receptors has also been identified in the entorhinal cortex (Weiner & Brann, 1989; Weiner et al., 1991).

Radiolabeling and *in situ* hybridization techniques have been used to determine the precise laminar distribution of dopamine receptor mRNA in the entorhinal cortex. The results of these studies demonstrated that D_2 receptors are concentrated primarily in layers I and III (Goldsmith & Joyce, 1996; Köhler, Ericson, Högberg, Halldin, & Chan-Palay, 1991a; Köhler, Ericson, & Radesäter, 1991b) whereas D_1 receptors are located mainly in layer II (Q. Huang et al., 1992; Weiner et al., 1991) and in layers V and VI (Köhler et al., 1991b; Richfield, Young, & Penney, 1989). More recent experiments using selective ligands for subtypes of D_2-like receptors have demonstrated significant D_4 receptor binding

in the entorhinal cortex (Defagot, Malchiodi, Villar, & Antonelli, 1997; Primus et al., 1997), however the laminar distribution of D_4 receptors has yet to be determined. The differential pattern of dopamine receptors expressed across layers in the entorhinal cortex suggests that dopamine might selectively modulate sensory inputs to different layers via actions on different dopamine receptor subtypes.

2.2. Working Memory Requires Dopamine

Although a role for dopamine in modulating memory processing in the entorhinal cortex has yet to be clearly established, a great deal is known about the effects of dopamine on processes central to working memory and executive function in the prefrontal cortex. For many years it has been known that successful performance on working memory tasks depends on the functional integrity of the prefrontal cortex. The term "working memory" itself has undergone numerous revisions during the past 50 years, but generally refers to the temporary (i.e., short-term) storage of online information necessary for problem solving and comprehension (Goldman-Rakic, 1999). A second important tenet regarding working memory is that it is an active process that requires rehearsal or "feedback" mechanisms for its proper functioning (Funahashi & Kubota, 1994).

The most common tasks for assessing working memory in animals involves a delayed response or a delayed alternation between responses (Funahashi & Kubota, 1994; Goldman-Rakic, 1999). Although there are several variations of either paradigm, the most important feature common to each is the

fact that trial-specific information needs to be retained in working memory during a variable delay period for successful completion of the task. Some mental representation, whether it is the position of a crosshair on a screen or whether the previous trial was a "go" or "no-go" trial, must be actively retained prior to making a response (Funahashi & Kubota, 1994; Goldman-Rakic, 1999).

Early lesion studies demonstrated a clear role for the prefrontal cortex in working memory processing. Profound deficits on delayed response and delayed alternation tasks have been consistently shown following focal lesions to the prefrontal cortex in both rats (Sakurai & Sugimoto, 1985) and primates (Passingham, 1975). Imaging studies in humans have also demonstrated activation in the prefrontal cortex during working memory tasks such as the Wisconsin card sorting task (Freedman & Oscar-Berman, 1986). During the 1970's, the involvement of the dopaminergic system in working memory was examined using a selective neurotoxin to deplete dopamine levels in the prefrontal cortex in monkeys (Brozoski, Brown, Rosvold, & Goldman, 1979). Rhesus monkeys trained to criterion performance on a delayed alternation task received bilateral infusions of the selective catecholaminergic toxin 6-OHDA into the dorsolateral prefrontal cortex. Following surgery, dopamine levels in the prefrontal cortex were depleted to approximately 56% of control levels, but serotonin levels remained unaffected. The significant drop in the amount of dopamine was accompanied by a corresponding decrease in delayed alternation performance (Brozoski et al., 1979) suggesting that dopaminergic innervation of the prefrontal cortex is required for optimal working memory processing. Interestingly, the effects of regional dopamine depletion could be partly reversed

by administration of either L-DOPA or apomorphine and this indicated that the impairment in performance was likely due to a specific loss of dopamine within the prefrontal cortex. The general conclusion that dopamine in the prefrontal cortex is required for normal working memory performance is further strengthened by similar observations that 6-OHDA infused directly into the A10 cell field of the ventral tegmental area can also produce similar deficits in delayed alternation performance in rats (Simon, Scatton, & Moal, 1980).

Some of the most compelling behavioral evidence for the role of prefrontal cortex dopamine in working memory comes from pharmacological manipulations of dopamine receptors in the prefrontal cortex. Drugs specific to D_1-like dopamine receptors have been used to show that D_1 receptor antagonism can significantly attenuate performance on an occulomotor delayed response task in primates (Sawaguchi & Goldman-Rakic, 1991). In subsequent experiments, it was further demonstrated that spatial location was represented topographically in the prefrontal cortex since D_1 receptor antagonism had differential effects on delayed response performance depending on infusion site and on the spatial position of the cue stimulus (Sawaguchi & Goldman-Rakic, 1994). Local administration of D_2 receptor antagonists, in contrast, was found to have no effect on performance in this task (Sawaguchi & Goldman-Rakic, 1994). In addition to the evidence of memory impairments associated with D_1 receptor antagonists, administration of D_1 receptor *agonists* can reverse the cognitive impairments produced by dopamine depletion and improve working memory in young monkeys (Arnsten, Cai, Murphy, & Goldman-Rakic, 1994). High doses of D_1 receptor agonists lead to deficits in working memory similar to D_1 receptor

blockers, however, and this suggests that the effects of dopamine on working memory function in the prefrontal cortex can be described by an inverted U-shaped function (Arnsten, 1998; Phillips, Ahn, & Floresco, 2004).

Similar behavioral impairments on working memory tasks following D_1 receptor antagonism have also been observed in the rat (Seamans, Floresco, & Phillips, 1998), and selective blockade of the D_1-mediated adenylate cyclase-cAMP-PKA intracellular signaling pathway can produce similar cognitive deficits (Aujla & Beninger, 2002). These findings suggest that activation of D_1 receptors is critical for working memory, and that the effects of D_1 receptors on working memory are mediated in part by activation of PKA. Thus, mnemonic processing in the rat prefrontal cortex also appears to be dependent upon the degree to which D_1 receptors are activated (Arnsten, 1998; Seamans & Yang, 2004).

Fluctuations in dopaminergic tone have been shown to occur in the prefrontal cortex when animals perform working memory tasks. Recent work using *in vivo* microdialysis has revealed that extracellular dopamine levels in the dorsolateral prefrontal cortex increase significantly (between 15 to 28% of basal levels) when monkeys perform a delayed alternation task, but not when they perform a sensory-guided control task (Watanabe, Kodama, & Hikosaka, 1997). Furthermore, dopamine levels in the premotor area rise during both the working memory task and the sensory-guided control task suggesting that dopamine may play a role in the initiation of movement via its actions on premotor cortical circuitry (Watanabe et al., 1997). A similar finding has been observed in the medial prefrontal cortex of rats performing a working memory task in the radial arm maze (Phillips et al., 2004).

Perhaps one of the most important electrophysiological findings in memory research has been the discovery that the firing properties of individual prefrontal cortical neurons are often "time-locked" to specific behavioral events (Goldman-Rakic, 1999). In primates, it has been shown that single units recorded from the prefrontal cortex *in vivo* significantly increase their firing rates during the delay-period of working memory tasks (Constantinidis & Steinmetz, 1996; Funahashi, Bruce, & Goldman-Rakic, 1989; Fuster, 1973; Fuster & Alexander, 1971; Kubota & Niki, 1971). Consequently, it has been proposed that this increase in persistent activity represents the cellular basis of working memory processes and the active maintenance of trial-specific "online" information required to perform the task (Goldman-Rakic, 1999). Subsequent research has demonstrated that these responses in prefrontal cortical neurons can be spatially tuned and can show increased delay-period firing for stimuli presented in a specific spatial location relative to the target, as well as decreased firing for stimuli presented in the opposite spatial location (Funahashi et al., 1989; Goldman-Rakic, 1999). These data suggest that prefrontal cortical neurons possess intrinsic "memory fields" that may be related, not only to spatial location, but also to other stimuli such as objects and faces as well. Further, the excitatory, inhibitory, and neuromodulatory inputs acting on prefrontal cortical circuitry are thought to contribute significantly to the formation and maintenance of these fields (Goldman-Rakic, 1999). However, determining the nature of the cellular physiology and neuronal circuitry that contributes to this persistent activity during the delay period has proven to be a sizable challenge, and the mechanisms are still poorly understood.

Considering the dense dopaminergic innervation of the prefrontal cortex and the cognitive impairments associated with manipulation of prefrontal dopamine, it is not surprising that dopamine has been shown to play a powerful role in the modulation of memory fields of prefrontal cortex neurons. The development of techniques to apply small quantities of drugs to a restricted brain area while simultaneously recording unit activity (iontophoresis) has facilitated our understanding of dopamine's role in prefrontal cortical functioning tremendously. There are however, conflicting reports regarding the effects of dopamine on the excitability of prefrontal cortical neurons.

One set of studies has shown that iontophoretic application of dopamine or even electrical stimulation of the ventral tegmental area can inhibit spontaneous activity in the prefrontal cortex (Bunney & Aghajanian, 1976; Ferron, Thierry, Le Douarin, & Glowinski, 1984; Pirot et al., 1992; Sesack & Bunney, 1989). This is supported by the finding that iontophoretic application of SCH39166, a selective D_1 receptor antagonist, can enhance the memory fields of neurons recorded in the dorsolateral prefrontal cortex of primates (Williams & Goldman-Rakic, 1995). This suggests that the normal function of dopamine in the prefrontal cortex is to inhibit network activity during working memory tasks (Williams & Goldman-Rakic, 1995), and also challenges earlier behavioral reports that local application of D_1 antagonists attenuate working memory performance (Sawaguchi & Goldman-Rakic, 1991, 1994; Seamans et al., 1998). In addition, it was also shown that higher concentrations of iontophoretically applied SCH39166 can completely abolish neuronal activity in the prefrontal cortex, and this is therefore consistent with earlier behavioral data. These

findings suggest that an optimal level of D_1 receptor occupancy is required for normal working memory functioning in the prefrontal cortex.

In contrast, a large number of other studies have shown that dopamine can have a facilitatory effect on spontaneous prefrontal cortical activity (Sawaguchi, Matsumura, & Kubota, 1990), as well as on firing rates recorded from dorsolateral prefrontal cortical units in monkeys performing a delayed response task (Sawaguchi, Matsumura, & Kubota, 1988; Sawaguchi et al., 1990). The dopamine-induced increase in both delay- and go-period firing was blocked by the D_1 receptor antagonist fluphenazine, but not by the D_2 receptor antagonist sulpiride (Sawaguchi et al., 1988, 1990), thereby suggesting a D_1 receptor-mediated mechanism of action. It is possible however, that the high iontophoretic current used to apply dopamine (50 nA as opposed to <30 nA in previous reports) together with the use of a slightly different delayed response paradigm (one requiring hand movements as opposed to eye saccades) could account for the differences.

In addition to the behavioral and electrophysiological evidence summarized in *Part I* of this introduction that is consistent with an important role of the entorhinal cortex in learning and memory, there is also evidence to suggest that the entorhinal cortex contributes to working memory functions. Neurons in the lateral entorhinal cortex show activity during an olfactory non-match-to-sample task that is time-locked to the different phases of the task, and the activity of many entorhinal cells increases during the delay period (Young et al., 1997). This is consistent with the idea that these cells help represent working memory for the sample odor that must be held on-line during the delay period.

There is additional intracellular evidence that also suggests that the entorhinal cortex contains circuitry to support working memory. When they are depolarized, layer V neurons in the medial entorhinal cortex show persistent firing activity that might underlie working memory processing by maintaining activity in local networks of entorhinal cortex neurons (Egorov, Hamam, Fransen, Hasselmo, & Alonso, 2002; Fransen, Tahvildari, Egorov, Hasselmo, & Alonso, 2006). It is the persistence of the firing activity, in the absence of maintained excitatory inputs, that suggest that this type of activity could support working memory during delay intervals. Layer III neurons in the lateral entorhinal cortex also show acetylcholine-dependent persistent firing in response to depolarizing inputs which might also represent a neuronal substrate for working memory in the entorhinal cortex (Tahvildari, Fransen, Alonso, & Hasselmo, 2007). Taken together, these findings suggest that both the deep output layers and superficial input layers of the entorhinal cortex contain cells with firing properties consistent with those thought to support working memory.

2.3. Dopaminergic Modulation of Synaptic Transmission in the Entorhinal Cortex

An understanding of how dopamine may affect sensory processing and memory formation should begin with an assessment of how dopamine affects basal glutamatergic synaptic transmission of input pathways to the entorhinal cortex. The idea that dopamine might affect the propagation of information to the hippocampus is not new and was first proposed in a review over 20 years ago by Oades and Haliday (1987). However, although the entorhinal cortex is a critical

step in the transfer of sensory information to the hippocampal formation, there have only been 3 published reports that have examined the effects of dopamine on the intrinsic and synaptic excitability of cells in the entorhinal cortex. In the first report, the effects of bath-applied dopamine on glutamate-mediated synaptic transmission was examined in stellate cells in slices of entorhinal cortex maintained *in vitro* (Pralong & Jones, 1993). The addition of high-concentrations of dopamine (100 μM or greater) to the bathing medium hyperpolarized membrane potential in the majority of cells recorded and lowered the resistance of the membrane to direct current injection. These changes in intrinsic excitability induced by dopamine were also accompanied by a suppression of excitatory synaptic transmission: high concentrations of dopamine, acting primarily through D_1 receptors, significantly attenuated the amplitude of pharmacologically isolated AMPA- and NMDA-mediated EPSPs (Pralong & Jones, 1993). Although an increase in local inhibition could have contributed to the suppression, it was shown that isolated $GABA_A$ and $GABA_B$ IPSPs were unaffected by dopamine. The authors suggested therefore that the potent inhibitory effects of dopamine on synaptic transmission likely resulted from either a reduction in the probability of transmitter release or from a postsynaptic change in intrinsic conductances that mediate input resistance (Pralong & Jones, 1993).

Similar inhibitory effects of dopamine have also been observed in layer III of the medial entorhinal cortex. In these experiments, bath-application of high concentrations of dopamine suppressed the amplitude of extracellulary recorded field potentials evoked in layer III by stimulation of layer I inputs (Stenkamp et al., 1998). It was also shown that co-application of either the D_1 receptor antagonist

SCH23390 or the D_2 receptor antagonist sulpiride with dopamine could partially attenuate the suppression of synaptic responses. Moreover, high concentrations of dopamine increased the amount of paired-pulse facilitation observed suggesting that dopamine suppressed synaptic responses by reducing the amount of neurotransmitter released from glutamatergic terminals in the entorhinal cortex (Stenkamp et al., 1998). The results of this study and that of Pralong and Jones (1993) suggest that the physiological role of dopamine in the superficial layers of the entorhinal cortex may be to dampen the salience of sensory inputs, and to reduce activity-dependent synaptic plasticity that may be associated with lasting changes in sensory processing or memory formation. Such a mechanism might be useful if overactivity of cortical projections that converge on the superficial layers of the entorhinal cortex were to overstimulate the entorhinal cortex during physiologically relevant situations that are normally associated with activation of the dopamine system (i.e., stress) and disrupt sensory processing or memory formation.

In the third and most recent published report on the effects of dopamine on synaptic transmission and neuronal excitability in the entorhinal cortex, it was shown that bath-application of 10 μM dopamine has potent suppressive effects on the excitability of layer V neurons in slices of lateral entorhinal cortex (Rosenkranz & Johnston, 2006). Dopamine significantly reduced the number of action potentials elicited in response to depolarizing current injection, hyperpolarized the membrane potential of layer V neurons, and also reduced the apparent input resistance of the cells. All of these effects were attributed to a dopamine-induced enhancement of the hyperpolarization-activated cation current

I_h since blockade of H-channels with selective antagonists prevented the changes in intrinsic excitability induced by dopamine. Although dopamine did not directly suppress individual evoked EPSPs in layer V neurons, it did reduce temporal summation of EPSPs by enhancing the I_h current (Rosenkranz & Johnston, 2006).

The number of published papers on the modulatory effects of dopamine on synaptic transmission in the entorhinal cortex is extremely small given the potential importance of the dopaminergic input to the entorhinal cortex (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987; Oades & Halliday, 1987). This dense innervation of the entorhinal cortex by midbrain dopamine neurons suggests that dopamine might play an important role in modulating the strength or salience of synaptic inputs to the entorhinal cortex or the extent to which lasting changes in synaptic strength that could contribute to short- or long-term memory processing might be induced. A substantial portion of this thesis, therefore, was aimed at determining the effects of dopamine on the strength of synaptic responses in piriform cortex inputs to layer II cells of the lateral entorhinal cortex which also receive robust dopaminergic inputs. Experiments were conducted *in vivo*, using systemic administration of a selective dopamine reuptake inhibitor (Chapter 2), and in *in vitro* recordings from acute brain slices containing the entorhinal cortex (Chapters 2 and 3).

In order to assess the possible contribution of dopamine to lasting forms of synaptic plasticity that may underlie memory formation in the entorhinal cortex, this thesis also assessed the modulatory effects of dopamine on LTP and LTD induction. Not only is dopamine required for maintaining the delay-period activity

of neurons during a working memory task in the prefrontal cortex (Constantinidis & Steinmetz, 1996; Funahashi et al., 1989; Fuster, 1973; Fuster & Alexander, 1971; Kubota & Niki, 1971), it also plays an important modulatory role in the induction and maintenance of lasting forms of synaptic plasticity (Jay, 2003). Stimulation of the ventral tegmental area (Gurden, Tassin, & Jay, 1999) or infusion of a D_1 receptor agonist directly into the prefrontal cortex (Gurden, Takita, & Jay, 2000) can enhance the amount of LTP induced at synaptic inputs from the hippocampus to the prefrontal cortex. In slices of prefrontal cortex, bath application of dopamine that is paired with tetanic stimulation will reliably induce LTD that is dependent on D_2 receptor activation (Otani, Blond, Desce, & Crépel, 1998; Otani, Daniel, Roisin, & Crepel, 2003). Interestingly, if slices are pretreated with dopamine 30-min prior to tetanization, LTP is induced instead of LTD (Matsuda, Marzo, & Otani, 2006). These findings suggest that dopamine likely promotes both LTP and LTD via activation of different dopamine receptors. However, there has been no assessment of how dopamine might affect processes of LTP and LTD in the entorhinal cortex. The data presented in this thesis suggest that increasing dopamine with a reuptake inhibitor in awake animals may actually suppress both LTP and LTD in the lateral entorhinal cortex, and may therefore play an important role in limiting the degree of synaptic plasticity induced by afferent sensory drive (Chapter 4).

The lateral entorhinal cortex has been shown to be involved in social recognition memory (Bannerman et al., 2002; Petrulis, Alvarez, & Eichenbaum, 2005; Petrulis et al., 2000), conditioned odor aversion (Ferry et al., 2006; Ferry et al., 1996; Ferry et al., 1999), and olfactory working memory (McGaughy, Koene,

Eichenbaum, & Hasselmo, 2005; Otto & Eichenbaum, 1992; Staubli et al., 1995; Young et al., 1997), but it is unknown what the contributions of the mesocortical dopamine system might be to these forms of sensory and mnemonic processing. Considering the dense olfactory projections to the entorhinal cortex, the behavioral data suggesting a role for the entorhinal cortex in olfactory processing, and the fact that many of the tasks used to assess olfactory memory in the entorhinal cortex are "appetitive by design" (i.e., requiring animals to be food or water restricted), there is reason to suspect that dopamine inputs to the entorhinal cortex may strongly influence processing during an olfactory memory task. The final experiments in this thesis examined the role of the mesocortical dopamine system on olfactory working memory mediated by the entorhinal cortex. Rats were trained on an olfactory-non-match-to sample task (Dudchenko, Wood, & Eichenbaum, 2000; McGaughy et al., 2005) and then received bilateral 6-hydroxydopamine lesions of the medial and lateral divisions of the entorhinal cortex. The effects of dopamine depletion in the entorhinal cortex on olfactory working memory were then assessed by testing rats on a delayed version of the same olfactory non-match-to-sample task (Chapter 5).

2.4. Summary of Experimental Chapters

There are four main experimental chapters in this thesis. The first experiments used field potential recording techniques to determine the effects of increased dopamine on synaptic inputs to the entorhinal cortex from the piriform cortex in awake rats. Results showed that synaptic responses were enhanced by dopamine suggesting that the salience of sensory inputs to the entorhinal

cortex may be facilitated by dopaminergic inputs to this region. Interestingly, the effects of dopamine on basal synaptic transmission were shown to be dose-dependent and bidirectional in subsequent studies in acute brain slices *in vitro*. Different concentrations of dopamine were bath-applied and the receptor dependence of the effects was assessed by co-applying different dopamine receptor antagonists. Low concentrations of dopamine facilitated synaptic responses through a mechanism dependent on D_1 receptor activation. In contrast, D_2 receptor activation by high concentrations of dopamine suppressed synaptic responses. These findings indicate that changes in dopaminergic tone in the lateral entorhinal cortex can alter basal synaptic transmission via actions on different dopamine receptor subtypes. In this way, dopaminergic inputs to the entorhinal cortex are likely to enhance or suppress the salience of sensory information that is transferred to the hippocampal formation as well as affect the encoding of olfactory information by entorhinal cortex networks.

The second experimental chapter examines the cellular mechanisms underlying the potent suppression of synaptic responses induced by high concentrations of dopamine using whole-cell current clamp recordings of neurons in layer II of the lateral entorhinal cortex *in vitro*. The concentration-dependent and bidirectional effects of dopamine demonstrated in Chapter 2 were replicated and results further demonstrated that the suppression of synaptic responses was dependent on a D_2 receptor-mediated reduction in glutamate release. This is further supported by the observation that both the isolated AMPA- and NMDA-mediated components of the excitatory synaptic response were reduced by dopamine. Moreover, high concentrations of dopamine also affected the intrinsic

excitability of layer II neurons by causing a reduction in apparent input resistance, a hyperpolarization of membrane potential, and a reduction in the number of action potentials elicited in response to suprathreshold current injection. The drop in input resistance also likely contributed to the suppression of EPSPs and was shown to be dependent on activation of a D_1-dependent K^+ conductance since co-application of either a D_1 receptor antagonist or K^+ channel blocker could prevent the drop in input resistance. These findings indicate that high concentrations of dopamine can act through a variety of mechanisms to suppress synaptic responses in the lateral entorhinal cortex and suggests that dopamine may dampen sensory processing in the entorhinal cortex under certain behavioral conditions.

Experiments conducted in the third chapter assessed the effects of dopamine on the induction of lasting forms of synaptic plasticity in the lateral entorhinal cortex of awake rats. Patterned stimulation to induce either long-term potentiation or long-term depression was delivered to olfactory inputs to the lateral entorhinal cortex in rats pretreated with either saline or the selective dopamine reuptake inhibitor GBR12909. Both LTP and LTD were successfully induced in control animals and were stable for several hours following induction. In contrast, the induction of LTP and LTD in olfactory inputs to the entorhinal cortex were blocked in animals pretreated with GBR12909. These findings suggest that dopamine plays a potent inhibitory role in the induction of enduring forms of synaptic plasticity, and may serve to protect the entorhinal cortex from undue changes in synaptic strength during periods of intense sensory processing.

In the fourth and final experimental chapter, the behavioral consequences of dopamine depletion in the entorhinal cortex on olfactory working memory are examined. Food-restricted rats were trained to dig in cups filled with scented sand and to discriminate between different odors to obtain a buried food reward. Upon reaching criterion performance on the olfactory non-match-to-sample-task (NMTS) rats received either sham lesions or 6-OHDA lesions of the medial and lateral entorhinal cortex. Following recovery, all rats were retrained on the same NMTS task, but only the dopamine-depleted animals showed significant impairments during this period. Rats that received 6-OHDA lesions took significantly longer and made significantly more errors during the first 3 days of retraining when compared to the behavior of sham-operated controls. Although dopamine-depleted rats eventually re-attained criterion levels of performance, it took nearly twice as long as sham animals to reach this level. Interestingly, once 6-OHDA-lesioned rats reached criterion performance their behavior was indistinguishable from sham-operated controls. Both groups could successfully perform a delayed version of the NMTS task when the delay interval was ≤15 min and made similar errors at delay intervals ≥30 min. These findings suggest that dopamine-depletion in the entorhinal cortex affects either attentional mechanisms required for successful re-learning of simple NMTS performance or memory for the NMTS rule acquired prior to the lesion.

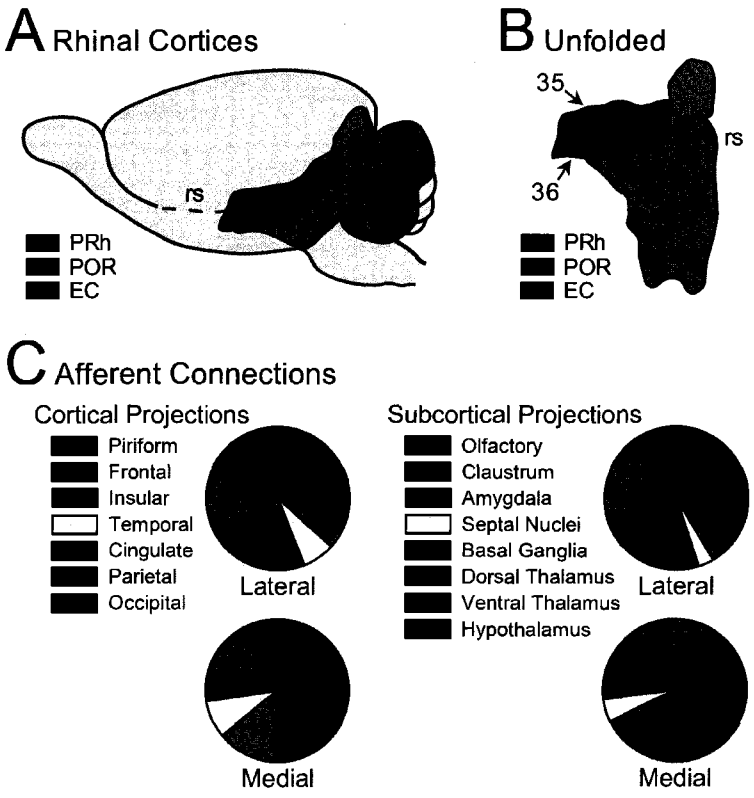

A Rhinal Cortices
rs
PRh
POR
EC
B Unfolded
35
36
rs
PRh
POR
EC
C Afferent Connections
Cortical Projections
Piriform
Frontal
Insular
Temporal
Cingulate
Parietal
Occipital
Lateral
Medial
Subcortical Projections
Olfactory
Claustrum
Amygdala
Septal Nuclei
Basal Ganglia
Dorsal Thalamus
Ventral Thalamus
Hypothalamus
Lateral
Medial

Figure 1.1. The entorhinal cortex receives sensory projections from multiple cortical and subcortical areas. **A**. A schematic diagram indicating the location of the rhinal cortices on the lateral surface of the rat brain (PRh: perirhinal cortex; POR: postrhinal cortex; EC: entorhinal cortex; rs: rhinal sulcus). **B**. An unfolded view of the rhinal cortices showing areas 35 and 36 of the perirhinal cortex and the medial (med) and lateral (lat) divisions of the entorhinal cortex. Illustrations in A and B have been adapted from Burwell (2000). **C**. Pie charts illustrate the relative strength of cortical and subcortical inputs to the medial and lateral divisions of the entorhinal cortex (inputs from the perirhinal and postrhinal cortices to the entorhinal cortex are not shown). Data in C have been adapted from Kerr et al. (2007). Note the large olfactory projection from the piriform cortex to the lateral entorhinal cortex (shown in blue).

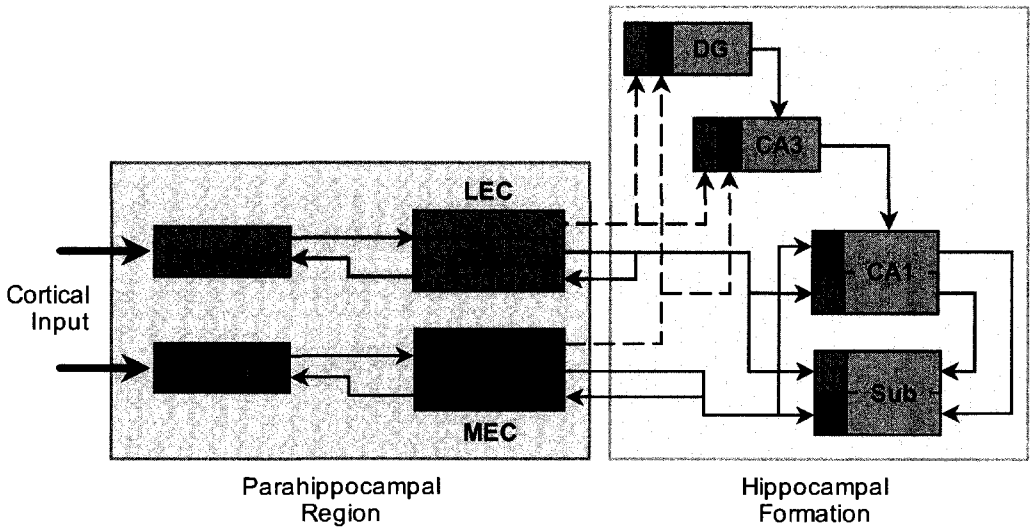

DG
CA3
LEC
CA1
Cortical
Input
Sub
MEC
Parahippocampal
Region
Hippocampal
Formation

Figure 1.2. The reciprocal connections between the parahippocampal region and hippocampal formation illustrate an anatomical and functional separation between the medial and lateral entorhinal areas. The lateral entorhinal cortex (LEC; blue) receives strong projections from the piriform cortex (not shown) and perirhinal cortex, and the medial entorhinal cortex (MEC; red) receives strong inputs from the postrhinal cortex. This segregation of sensory information is maintained through separate medial and lateral perforant path projections from the entorhinal cortex to the dentate gyrus and CA3 regions (dotted lines). Temporoammonic path projections from layer III of the medial and lateral entorhinal cortices to area CA1 and the subiculum (Sub) are also spatially segregated. The subdivisions of the hippocampal formation, where the two streams converge, are shown in purple. Arrowheads indicate the direction of information flow through the circuit. Adapted from Witter et al. (2000a).

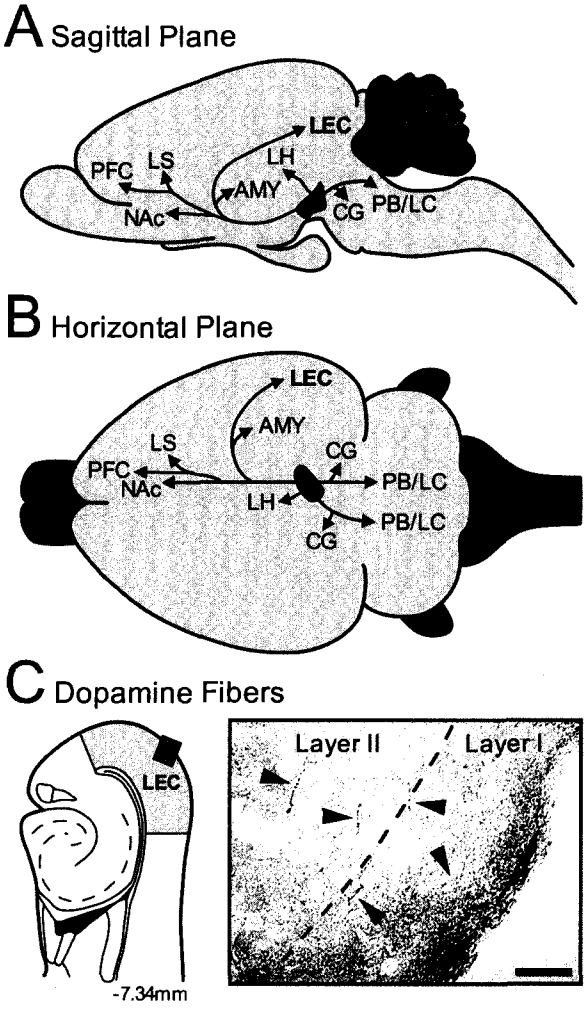

A Sagittal Plane
LEC
LH
PFC
LS
AMY
CG
PB/LC
NAc
B Horizontal Plane
LEC
AMY
LS
CG
PFC
NAc
PB/LC
LH
PB/LC
CG
C Dopamine Fibers
LEC
-7.34mm
Layer II
Layer I

Figure 1.3. The major source of dopaminergic innervation to the lateral entorhinal cortex originates from the A10 cells of the ventral tegmental area. Schematic diagrams shown in **A** and **B** highlight the cortical and subcortical targets of the ventral tegmental area in both saggital (A) and horizontal (B) planes (AMY: amygdala; CG: central gray; LEC: lateral entorhinal cortex; LH: lateral habenula; LS: lateral septum; NAc: nucleus accumbens; PB/LC: parabrachial nucleus/locus coerulus; PFC: prefrontal cortex). Illustrations in A and B are adapted from Swanson (1982). The photomicrograph in **C** shows tyrosine hydroxylase immunoreactive fibers in layers I and II of the lateral entorhinal cortex (marked by arrows; D.A. Caruana unpublished observations) at the level indicated by the red box on the corresponding section from the atlas of Paxinos and Watson (1998). Calibration bar = 100 μm.

CHAPTER 2

DOPAMINE HAS BIDIRECTIONAL EFFECTS ON SYNAPTIC RESPONSES TO CORTICAL INPUTS IN LAYER II OF THE LATERAL ENTORHINAL CORTEX

Douglas A. Caruana, Robert E. Sorge, Jane Stewart, and C. Andrew Chapman

ABSTRACT

Dopaminergic modulation of neuronal function has been extensively studied in the prefrontal cortex, but much less is known about its effects on glutamate-mediated synaptic transmission in the entorhinal cortex. The mesocortical dopamine system innervates the superficial layers of the lateral entorhinal cortex and may therefore modulate sensory inputs to this area. In awake rats, systemic administration of the dopamine reuptake inhibitor GBR12909 (10 mg/kg; i.p.) enhanced extracellular dopamine levels in the entorhinal cortex and significantly facilitated fEPSPs in layer II evoked by piriform cortex stimulation. An analysis of the receptor subtypes involved in the facilitation of evoked fEPSPs was conducted using horizontal slices of lateral entorhinal cortex *in vitro*. The effects of 15-min bath-application of dopamine on synaptic responses were bidirectional and concentration-dependent. Synaptic responses were enhanced by 10 μM dopamine and suppressed by concentrations of 50 and 100 μM. The D_1 receptor antagonist SCH23390 (50 μM) blocked the significant facilitation of synaptic responses induced by 10 μM dopamine, and the D_2 receptor antagonist sulpiride (50 μM) prevented the suppression of fEPSPs observed with higher concentrations of dopamine. We propose here that dopamine release in the lateral entorhinal cortex, acting through D_1 receptors, can lead to an enhancement of the salience of sensory representations carried to this region from adjacent sensory cortices.

The entorhinal cortex is a major structure of the medial temporal lobe which plays a central role in sensory processing and declarative memory formation (Lavenex & Amaral, 2000; Schwarcz & Witter, 2002b; Squire, Stark, & Clark, 2004; Squire & Zola, 1996). The superficial layers of the entorhinal cortex receive projections from primary sensory and association cortices and provide the hippocampus with the majority of its cortical sensory input (Amaral & Witter, 1995; Bouras & Chapman, 2003; Burwell, 2000; Caruana & Chapman, 2004; Chapman & Racine, 1997a; Lavenex & Amaral, 2000). This great convergence of sensory information within the entorhinal cortex suggests that it contributes heavily to multimodal sensory integration, and to functions of the hippocampal formation that depend on highly processed sensory input.

The midbrain dopamine system may help promote cognitive performance when animals are engaged in appetitive behaviors linked to natural rewards or when responding to aversive stimuli (Seamans & Yang, 2004). Dopaminergic inputs to the prefrontal cortex are thought to contribute to selection of adaptive behavioral responses, in part, by enhancing working memory (Fuster, 1973; Goldman-Rakic, 1999). Regional depletion of dopamine (Brozoski et al., 1979) and disruption of dopamine receptor function in the prefrontal cortex (Sawaguchi & Goldman-Rakic, 1991; Seamans et al., 1998) can impair working memory on tasks that require a delayed response to obtain a reward (Goldman-Rakic, 1999). The entorhinal cortex receives one of the largest cortical projections of the midbrain dopamine system (Baulac, Verney, & Berger, 1986; Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987; Oades & Halliday, 1987), but little has

been done to investigate the functional role of this projection or its physiological characteristics.

Dopaminergic modulation of glutamate-mediated synaptic responses in the entorhinal cortex has been assessed in few published reports. In the medial entorhinal cortex, dopamine suppresses synaptic transmission in layers II, III, and V *in vitro* (Pralong & Jones, 1993; Stenkamp et al., 1998). Dopaminergic projections to the lateral entorhinal cortex are much more dense than those to the medial entorhinal cortex (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987), and the two divisions differ markedly in electroresponsiveness of principal cells (Alonso & Klink, 1993; Tahvildari & Alonso, 2005; Wang & Lambert, 2003) and connectivity with cortical inputs (Hargreaves et al., 2005; Sewards & Sewards, 2003). Although dopamine does not directly suppress EPSPs in layer V neurons of lateral entorhinal cortex, it does reduce temporal summation of EPSPs by enhancing the I_h current (Rosenkranz & Johnston, 2006). Dopaminergic modulation of inputs to layer II of the lateral entorhinal cortex, however, has not been assessed either *in vivo* or *in vitro*.

To determine how dopamine modulates the responsiveness of the lateral entorhinal cortex to cortical sensory inputs, field excitatory postsynaptic potentials evoked by stimulation of the piriform (primary olfactory) cortex were recorded before and after systemic administration of the dopamine reuptake inhibitor GBR12909 in awake rats. Elevations in extracellular dopamine induced by GBR12909 were confirmed using *in vivo* microdialysis. Bath application of receptor blockers *in vitro* was then used to determine the contributions of specific

dopamine receptors to the dose-dependent facilitation and inhibition of glutamate-mediated synaptic transmission.

MATERIALS AND METHODS

Microdialysis and High Performance Liquid Chromatography

Surgery. Male Long-Evans hooded rats (9 to 11 weeks old; 300 to 320 g) were anesthetized with a 5% isoflurane and 95% oxygen mixture and placed in a stereotaxic apparatus with bregma and lambda leveled. A stainless-steel guide cannula (Plastics One, 20 gauge) was lowered to a position ≈2.7 mm above the ventral surface of the right lateral entorhinal cortex (P, 6.7 mm; L, 5.2 mm; V, 6.0 mm relative to bregma). Dialysis probes were constructed to protrude 2.6 mm beyond the tip of the guide cannula. Three stainless-steel jewelers screws were secured to the scull and the screws and cannula were embedded in dental cement. An obturator (Plastics One, 24 gauge) was inserted into the guide cannula. Buprenorphine (0.02 mg/kg, s.c.) was used as a post-surgical analgesic. Animals were housed individually and tested after a ≥10 day-recovery period during the lights-off phase of a 12-hour light-dark schedule.

Apparatus and Microdialysis Probes. During microdialysis sampling, animals were housed in a 42 x 39 x 34 cm Plexiglas chamber with a stainless-steel grid floor. Each chamber was housed within a 65 x 65 x 75 cm sound-attenuating wooden cubicle. Two pairs of photo-cells were positioned 2.5 cm above the floor approximately 10 cm apart and the number of beam-breaks made by animals during 20 min sampling periods was recorded. Food was removed from chambers before dialysate sampling, but drinking water was always

available. Light exposure was minimized during testing to prevent degradation of catecholamines collected.

Dialysis probes were constructed from a 2.8 to 3.0 mm length of semi-permeable dialysis membrane (Fisher Scientific, 240 µm OD, 13000 MW cutoff) closed at one end and attached to a 21 mm long, segment of 26 gauge stainless steel tubing. A 40-50 cm-long piece of PE-20 tubing (Fisher Scientific) connected the steel tube to a liquid swivel above the testing chamber that was connected to a microinfusion pump (Harvard Apparatus, Model 22). Small diameter fused silica tubing within the dialysis probe served as the return for dialysate fluid. One end of the silica tubing rested 0.5 mm from the probe tip and the other exited the PE-20 tubing 5 cm above the stainless steel tube for collection. The entire assembly was enclosed in a light-gauge steel spring casing.

Microdialysis Sampling and HPLC Analysis. Probes were inserted into guide cannulae one day prior to microdialysis testing. To prevent occlusion of probes, artificial cerebrospinal fluid (ACSF) consisting of, in mM, 145 Na^+, 2.7 K^+, 1.2 Ca^{2+}, 1 Mg^{2+}, 150 Cl^-, 0.2 ascorbate, and 2 Na_2HPO_4 (pH = 7.4 ±0.1) was perfused overnight at a constant rate of 0.7 µl/min. All chemicals were obtained from Sigma. Dialysate sampling and activity monitoring began the next morning with dialysate samples (≈14 µl/sample) and measures of locomotion (number of photo-cell beam-breaks) collected at 20 min intervals. Dialysate samples were analyzed immediately using high-performance liquid chromatography (HPLC) with electrochemical detection. A stable baseline of dopamine was established with a criterion of less than 10% variation over three consecutive samples. Rats

(n = 10) were then injected with either saline (0.9%; 1 ml/kg, i.p.) or the selective dopamine reuptake inhibitor GBR12909 (10 mg/kg, i.p.; Nakachi et al., 1995) and dialysate samples and activity measures were collected at 20-min intervals for 120 min. Differences in post-injection locomotion and dopamine levels following either saline or GBR12909 were analyzed using mixed design ANOVAs. GBR12909 was prepared daily by dilution in distilled water.

For HPLC analysis, a 10-µl volume was extracted from each sample and loaded onto a C-18 reverse-phase column (5 µm, 15 cm) through a manual injection port (Rheodyne, Model 7125, 20 µl loop), and the redox current for dopamine was measured with a dual-channel coulometric detector (ESA Biosciences, Coulochem III with a Model 5011 analytical cell). The mobile phase (20% acetonitrile, 0.076 M SDS, 0.1 M EDTA, 0.058 M $NaPO_4$, and 0.27 M citric acid; pH = 3.35) was circulated through the system at a rate of 1.1 ml/min by a Waters 515 HPLC pump and the peak for dopamine was quantified by EZChrom Chromatography Data System (Scientific Software Inc.).

Histology. Animals were deeply anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and were perfused intracardially with 0.9% saline followed by 10% formalin. Brains were stored in 10% formalin and transferred to a 30% sucrose solution one day prior to sectioning with a cryostat and coronal sections (40 µm thick) were stained with formal-thionin. Tissue obtained from animals with chronic electrodes (below) was processed in the same manner.

Synaptic Responses In Vivo

Surgery. Male Long-Evans hooded rats (9 to 11 weeks old; 300 to 350 g) were treated with atropine methylnitrite (0.1 mg/kg, i.p.), anesthetized with sodium pentobarbital (65 mg/kg, i.p.), and placed in a stereotaxic apparatus. A bipolar Teflon-coated stimulating electrode (tip separation of 1.0 mm) made from stainless-steel wire (125 µm exposed tips) was lowered into the right piriform cortex (P, 3.6 mm; L, 6.5 mm; V, 9.0 mm relative to bregma), and a bipolar recording electrode (tip separation of 0.6 mm) was lowered into the superficial layers of the lateral entorhinal cortex (P, 6.5 mm; L, 6.5 mm; V, 7.5 to 8.5 mm). Coordinates for the recording electrode were chosen based on the distribution of dopaminergic afferents originating from the ventral tegmental area (A10) and retrorubral area of the substantia nigra (A8) to layers II and III of the anterioventral portion of the lateral entorhinal cortex (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987; Oades & Halliday, 1987). The vertical placement of the stimulating electrode was adjusted to minimize current thresholds, and the position of the recording electrode was adjusted to maximize the amplitude of evoked field excitatory postsynaptic potentials (fEPSPs). A stainless-steel jeweler's screw in the contralateral frontal bone served as a reference electrode, and a second screw in the left occipital bone served as ground. Electrode leads were connected to gold-plated Amphenol pins and mounted in a plastic 9-pin connector. The assembly was embedded in dental cement and anchored to the skull with jeweler's screws. Buprenorphine (0.02 mg/kg, s.c.) was administered after surgery. Animals were housed individually and tested during the lights-on phase of a 12-hour light-dark schedule.

Stimulation and Recording. Electrical stimuli were generated with a pulse generator (AMPI, Master 8 or A-M Systems, Model 2100) or computer digital-to-analog channel (50 kHz), and 0.1 ms biphasic constant current square-wave pulses were delivered to the piriform cortex via a stimulus isolation unit (A-M Systems, Model 2200). Evoked field potentials were analog filtered (0.1 Hz to 5 kHz passband), amplified (A-M Systems, Model 1700), and digitized at 10 or 20 kHz (12 bit) for storage on computer hard disk using the software package Experimenter's Workbench (Datawave Tech.).

Animals were placed in a 40 x 40 x 60 cm Plexiglas chamber surrounded by a Faraday cage, and recordings were obtained after animals had habituated and were in a quiet, resting state. Stability of responses was assessed using input/output tests conducted every 2 days over a 5 day baseline period. During each input/output test, 10 responses to stimulation of the piriform cortex were recorded and averaged at each of 10 intensities (100 to 1000 μA) using a 10 sec inter-trial interval. Peak amplitudes of evoked field potentials were measured relative to the prestimulus baseline.

Paired-pulse tests were used to assess whether dopamine enhances synaptic responses through a pre- or postsynaptic mechanism. These tests are often used to evaluate changes in presynaptic neurotransmitter release probability (Zucker, 1989; Zucker & Regehr, 2002). During these tests, pairs of stimulation pulses, separated by interpulse intervals of 10, 30, 100 and 1000 ms, were delivered to the piriform cortex using pulse intensities that evoked responses ≈75% of the largest response. Ten responses were averaged at each interpulse interval. Responses evoked by the second of two pulses were

expressed as a percentage of responses to the first stimulation pulses. Because the second artefact can occur near the peak of the first response, ratios for the 10 ms interval were calculated relative to the average response to the first pulses at the other intervals.

To verify that vehicle injections alone have no effect, immediately following the last baseline input/output test, animals received an injection of physiological saline (0.9%; 1 ml/kg, i.p.; n = 11), followed by an input/output test 20 min later. A paired-pulse test was conducted, and animals were then injected with the dopamine reuptake inhibitor GBR12909 (10 mg/kg, i.p.) and a final set of tests was recorded 20 min later. Input/output data and paired-pulse tests were analyzed using repeated measures ANOVAs and Newman-Keuls tests that compared results following the final baseline, post-saline, post-GBR12909, and 24 hour follow-up tests at each stimulation intensity or each inter-pulse interval.

Synaptic Responses In Vitro

Slice Preparation. Slices were obtained from male Long-Evans hooded rats (3.5 to 6 weeks old) as described previously (Chapman, Perez, & Lacaille, 1998). Briefly, animals were anesthetized with halothane and brains were rapidly removed and cooled (4°C) in oxygenated ACSF. ACSF consisted of, in mM, 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose. Horizontal slices (400 µm) were cut using a vibratome (WPI, Vibroslice) and placed on a nylon net in a gas-fluid interface recording chamber (Fine Science Tools) in which oxygenated ACSF was perfused at a rate of 1.0 ml/min. Slices were maintained at 22 to 24°C and their upper surfaces were exposed to a

humidified 95% O_2, 5% CO_2 atmosphere. There was a recovery period of at least one hour prior to recordings.

Stimulation and Recording. For recordings of fEPSPs, glass micropipettes made using a Sutter Model P97 electrode puller were filled with 2 M NaCl (4 to 8 MΩ) and positioned with the aid of a dissecting microscope (Leica, MS5) into the lateral division of the entorhinal cortex along the layer I-II border 75 to 200 µm below the surface of the slice. A bipolar stimulating electrode made from two Tungsten electrodes (FHC, 0.8 MΩ) was positioned to span the layer I-II border, approximately 1.0 to 2.0 mm rostral to the recording electrode. Constant current pulses (0.1 ms) were delivered using a stimulus generator (WPI, Model A300) and a stimulus isolation unit (WPI, Model A360). Evoked field potentials were filtered (DC-3 kHz) and amplified with an AxoClamp 2B amplifier (Axon Instr.) in bridge mode, and responses were digitized at 20 kHz (Axon Instr., Digidata 1322A) for storage on computer hard-disk using the software package Clampex 8.1 (Axon Instr.).

Responses to test-pulses were monitored every 20 sec using an intensity adjusted to evoke fEPSPs with an amplitude of about 60 to 70% of maximal (typically <100 µA). This intensity was determined by delivering pulses ranging from 25 to 200 µA. Testing was conducted on slices with stable fEPSPs that showed less than ±5% drift during a 10 min baseline period. Following baseline in normal ACSF, 50 µM of the antioxidant sodium metabisulfite was bath-applied alone (n = 6) or together with 10 (n = 6), 50 (n = 8), or 100 (n = 6) µM dopamine for 15 min. Dopamine can oxidize rapidly and sodium metabisulfite effectively slows this process (Stenkamp et al., 1998; C. R. Yang & Seamans, 1996). Room

lighting was also dimmed to reduce degradation of dopamine. Responses were recorded for an additional 40 min during washout with normal ACSF. Dopamine HCl was prepared fresh daily just prior to bath application and sodium metabisulfite was stored as a concentrated stock solution at –20ºC until needed. The peak amplitude of fEPSPs was measured using the program Clampfit (Axon Instr.). Data were standardized to the mean of baseline responses for plotting. The mean amplitude of fEPSPs obtained during the last 5 min of baseline, the 5 min period during the peak effects of dopamine, and the final 5 min of washout were compared using repeated measures ANOVAs and Newman-Keuls tests.

The contributions of dopamine receptor subtypes to changes in fEPSPs were assessed using dopamine receptor antagonists added to the perfusate prior to different concentrations of dopamine. Agonists used previously in layer III of the entorhinal cortex have required unusually high concentrations and we therefore focused on use of receptor blockers in these initial studies (Pralong & Jones, 1993; Stenkamp et al., 1998). Following the baseline period, 50 µM of the D_1 receptor antagonist SCH23390 or 50 µM of the D_2 receptor antagonist sulpiride were bath-applied for 15 min to ensure that antagonist application alone had no effects on evoked synaptic responses. Similar doses of these drugs attenuate dopamine-induced reductions in evoked synaptic responses in the medial entorhinal cortex (Pralong & Jones, 1993; Stenkamp et al., 1998). Application of antagonists was continued for 15 min in the presence of 10, 50, or 100 µM of dopamine, and field responses were recorded for a 40 min washout period in normal ACSF. Sodium metabisulfite (50 µM) was co-applied during all drug applications. SCH23390 was diluted in distilled water and stored at –20°C

until needed. Sulpiride was prepared fresh daily as a concentrated stock solution by dilution in 6% DMSO and ACSF followed by further titration with 0.1 N HCl. The effects of receptor blockade on dopamine-induced changes in fEPSPs were assessed by performing a series of planned repeated measures ANOVAs that compared mean responses obtained over 5 min periods during antagonist application alone and co-application of dopamine with antagonists.

RESULTS

In Vivo Microdialysis

Histological analysis confirmed that dialysis probes were on target in the lateral entorhinal cortex (Fig. 2.1A). In most cases (8 of 10), probe placements included portions of the ventral hippocampus, and two probes were located in sites that bordered on the amygdalopiriform transition area. All probe tips were located in layer I with the exception of one case that was positioned ≈300 µm below the cortical surface in upper layer III.

Measures of basal levels of extracellular dopamine in the lateral entorhinal cortex typically stabilized within ≈120 min of baseline sampling to a mean concentration of 0.40 ±0.06 pg/10 µl of dialysate (0.21 ±0.03 nM). Systemic administration of the dopamine reuptake inhibitor GBR12909 significantly enhanced dopamine levels (Fig. 2.1B$_1$; $F_{1,14} = 4.7$, $P < 0.05$). Dopamine concentrations increased to 254.9 ±73.2% of baseline levels 20 min following GBR12909 administration and peaked 80 min post-injection at 305.9 ±79.9% (0.45 ±0.08 and 0.51 ±0.10 nM respectively). Dopamine remained elevated for

at least 2 hours after GBR12909 administration. In contrast, dopamine levels were stable following saline injections.

Locomotor activity during baseline dialysis sampling resulted in an average of only 13.1 ±4.9 beam-breaks every 20 min. The number of beam-breaks increased significantly following injections of GBR12909 and peaked at 201.1 ±21.4 breaks after one hour (Fig. 2.1B_2; $F_{1,14} = 60.9$, $P < 0.001$). Locomotor activity in treated rats remained elevated throughout testing. There was a small increase in activity following saline injections but activity returned to baseline levels within one hour.

Synaptic Responses In Vivo

Histology showed stimulating electrodes on target in the piriform cortex, and recording electrodes positioned in the superficial layers (I to III) of the lateral entorhinal cortex, with two sites located in layer IV (Fig. 2.2A,B). Field potentials in the medial entorhinal cortex evoked by piriform cortex stimulation result from activation in layers I and II (Chapman & Racine, 1997a). The lateral entorhinal cortex also receives monosynaptic afferents from the piriform cortex (Burwell, 2000; Köhler, 1988) which evoke synaptic responses in layer II (Biella & de Curtis, 2000; Boeijinga & Van Groen, 1984). The major component of field potential responses evoked here was a negative deflection with onset and peak latencies of 5.4 ±0.6 and 12.3 ±0.7 ms and a peak amplitude of 0.96 ±0.17 mV (e.g. Fig. 2.2C). In some cases (6 of 11) the major synaptic component was followed by a late-positive deflection (e.g. Fig. 2.3A) but this component was unaffected by GBR12909.

Systemic GBR12909 Administration. Systemic administration of the dopamine reuptake inhibitor GBR12909 increased the amplitude of evoked synaptic responses in the lateral entorhinal cortex ($F_{3,30} = 3.29$, $P < 0.05$; Newman-Keuls, $P < 0.05$; $n = 11$). Saline injections did not affect synaptic responses, but GBR12909 facilitated responses to 119.6 ±8.2% of control levels at the highest stimulation intensity (Fig. 2.2C,D) and responses returned to baseline levels when examined 24 hours later (not shown). These results indicate that facilitating dopaminergic transmission in awake rats enhances glutamate-mediated responses in piriform cortex inputs to the lateral entorhinal cortex.

Dopamine could enhance glutamate-mediated synaptic responses through a variety of mechanisms including an increase in neurotransmitter release, an increase in receptor-mediated currents, changes in intrinsic excitability, or a reduction in local inhibitory tone. To help determine how dopamine may modulate glutamatergic transmission, pairs of stimulation pulses were delivered using interpulse intervals of 10, 30, 100 and 1000 ms ($n = 11$). If transmitter release following a single pulse is increased by GBR12909, a reduced amount of transmitter should be available for release in response to the second pulse and facilitation should be reduced. Strong paired-pulse facilitation was observed at the 30 ms interpulse interval (see also Bouras & Chapman, 2003; Chapman & Racine, 1997a). Systemic administration of GBR12909 enhanced the amplitude of synaptic responses at all interpulse intervals tested, but the paired-pulse ratio was not reduced (Fig. 2.3, 30 ms interpulse interval; 159.8 ±21.0% following

saline versus 164.0 ±19.1% following GBR12909). This suggests that dopamine does not likely enhance fEPSPs by increasing glutamate release.

Synaptic Responses In Vitro

The *in vitro* slice preparation was used to examine the receptors involved in the dopamine-mediated enhancement of glutamatergic synaptic responses. Stimulation of layers I and II evoked field potential responses in upper layer II of the lateral entorhinal cortex similar to responses recorded *in vitro* from the superficial layers of the medial division (Alonso et al., 1990; Kourrich & Chapman, 2003; Stenkamp et al., 1998; Yun, Mook-Jung, & Jung, 2002). A short latency presynaptic fiber volley preceded the major component of the fEPSP and was not affected by any treatment. The synaptic response had mean onset and peak latencies of 3.6 ±0.1 and 7.5 ±0.2 ms, and a mean amplitude of 0.92 ±0.11 mV (e.g. Fig. 2.4A_1). The antioxidant sodium metabisulfite (50 µM) was co-applied with all drugs, and had no effect on synaptic responses when applied alone (Fig. 2.4A_3; n = 6).

10 µM Dopamine. Similar to results obtained in awake rats, bath application of 10 µM dopamine for 15 min significantly facilitated synaptic responses in the lateral entorhinal cortex (Fig. 2.4$A_{1,2}$; n = 6; $F_{3,22} = 28.09$, $P < 0.001$; N-K, $P < 0.05$). The effects of dopamine began after about 7 min as the concentration of dopamine increased in the recording chamber, and the amplitude of synaptic responses was facilitated maximally to 119.3 ±3.9% of baseline levels about 10 min into washout. Responses returned to baseline levels within 30 min. Application of the D_1 receptor antagonist SCH23390 (50

μM) alone had no significant effect on synaptic responses, but attenuated the dopamine-induced increase in fEPSPs when co-applied with dopamine (Fig. 2.4B_1; n = 5). Synaptic responses increased to only 106.0 ±2.3% of control levels during co-application of SCH23390 and dopamine.

Bath-application of the D_2 receptor antagonist sulpiride (50 μM) had no significant effect on baseline synaptic responses and did not significantly affect the peak facilitation induced by dopamine (Fig. 2.4B_2; n = 5). Responses increased significantly to 113.0 ±1.8% of control levels during co-application of sulpiride and dopamine ($F_{1,4}$ = 46.44, P < 0.01). Moreover, duration of the facilitation induced by 10 μM dopamine was similar in the presence and absence of sulpiride, and lasted about 28 min in both cases. The GBR12909-induced increase in fEPSPs is therefore likely mediated largely by D_1 receptors.

50 and 100 μM Dopamine. Higher concentrations of dopamine inhibited glutamate-mediated synaptic transmission in the lateral entorhinal cortex. Bath application of either 50 μM (n = 8) or 100 μM (n = 6) dopamine caused a significant, dose-dependent reduction in the amplitude of synaptic responses (Figs. 2.5A and 2.6A; $F_{3,22}$ = 28.09, P < 0.001; N-K 50 μM, P < 0.01; 100 μM, P < 0.001). Peak effects of dopamine were seen after about 6 min, and synaptic responses were reduced to a minimum of 77.3 ±3.7 and 57.2 ±6.1% of baseline levels by 50 and 100 μM of dopamine respectively. Responses returned to baseline levels within about 25 min, and fEPSPs rebounded to amplitudes greater than baseline at the end of the recording period (117.6 ±6.2 and 123.3 ±6.1% of baseline for 50 and 100 μM respectively; $F_{3,22}$ = 6.33, P < 0.01; N-K 50

μM, $P < 0.05$; 100 μM, $P < 0.001$) and in some cases remained facilitated for an additional 20 min (not shown).

Co-application of the D_1 receptor antagonist SCH23390 with either 50 μM (n = 6) or 100 μM (n = 8) dopamine did not significantly affect the peak reduction in synaptic responses (Figs. $2.5B_1$ and $2.6B_1$). Dopamine reduced fEPSPs to 87.3 ±3.4 and 73.4 ±2.9% of control for 50 and 100 μM of dopamine in the presence of SCH23390 (50 μM, $F_{1,5} = 14.68$, $P < 0.05$; 100 μM, $F_{1,7} = 75.70$, $P < 0.001$). However, the delayed facilitation of synaptic responses observed during the end of the washout period following dopamine alone was blocked. Synaptic responses were 88.5 ±3.1 and 95.8 ±7.2% of control levels during the final 5 min of recording following the co-application of SCH23390 with 50 or 100 μM of dopamine.

In contrast, co-application of the D_2 receptor antagonist sulpiride blocked the reduction in synaptic responses induced by 50 μM (n = 7) or 100 μM (n = 6) dopamine (Figs. $2.5B_2$ and $2.6B_2$). Responses were not affected by application of sulpiride alone, and subsequent co-application of either 50 or 100 μM dopamine also had no significant effect (50 μM, 101.8 ±4.0% of control; 100 μM, 90.6 ±7.2% of control). Field responses were facilitated during the final 5 min of these recordings (to 106.4 ±4.6 and 116.2 ±9.4% of control for 50 and 100 μM of dopamine), but these increases were not statistically significant.

DISCUSSION

We demonstrate here that dopamine has powerful modulatory effects on lateral entorhinal cortex responses to inputs from adjacent sensory cortices, and

our findings suggest that the mesocortical dopamine system regulates the sensory and mnemonic functions of the entorhinal cortex. We have utilized both *in vivo* and *in vitro* electrophysiological techniques to determine the effect of dopamine on synaptic function in the entorhinal cortex. Results demonstrate that dopamine has dose-dependent, bidirectional effects on excitatory synaptic transmission in layer II projection neurons of the lateral entorhinal cortex. In awake animals, systemic injections of the dopamine reuptake inhibitor GBR12909 increased extracellular dopamine in the lateral entorhinal cortex, and facilitated synaptic responses evoked by piriform cortex stimulation. Paired-pulse tests can be affected by activation of local inhibition, but results suggested that dopamine facilitates responses via a postsynaptic mechanism. Subsequent *in vitro* tests showed that the effects of dopamine are concentration-dependent; low concentrations of dopamine (10 μM) enhanced fEPSPs mainly via D_1 receptors, and higher concentrations (50 to 100 μM) reduced synaptic responses via D_2 receptors. Reductions in synaptic responses have been observed previously in the medial entorhinal cortex (Pralong & Jones, 1993; Stenkamp et al., 1998) and we observed similar reductions in fEPSPs with high concentrations of dopamine. We demonstrate here, though, that the effect of dopamine at lower concentrations is to facilitate layer II responses to cortical inputs. Moderate activation of the mesocortical dopamine system is therefore most likely to enhance the salience of sensory information processed by the lateral entorhinal cortex, and this may depend on activation of D_1 receptors.

Facilitation of Synaptic Responses in Awake Rats

Systemic injections of the dopamine reuptake inhibitor GBR12909 facilitated evoked fEPSPs in the lateral entorhinal cortex of awake rats (Fig. 2.2). Such facilitation could have resulted, in part, from indirect effects of enhanced dopamine release in other brain areas. Systemic dopamine can enhance firing of raphé neurons (Martin-Ruiz, Ugedo, Honrubia, Mengod, & Artigas, 2001) and the superficial layers of the entorhinal cortex receive serotonergic inputs (Köhler, Chan-Palay, Haglund, & Steinbusch, 1980b), but serotonin inhibits synaptic transmission in superficial layer neurons in both the medial (Schmitz et al., 1998; Schmitz et al., 1999; Sizer, Kilpatrick, & Roberts, 1992) and lateral (Grunschlag, Haas, & Stevens, 1997) divisions *in vitro*. The facilitation of synaptic responses observed here following GBR12909 is therefore unlikely to reflect actions of dopamine on serotonergic inputs to the entorhinal cortex. The findings from microdialysis demonstrated that systemic GBR12909 enhanced extracellular dopamine in the entorhinal cortex (Fig. 2.1) suggesting that there were direct effects on local circuitry. The basal level of dopamine measured here (0.4 pg/10 µl) is comparable to levels in the prefrontal cortex sampled using similar methods (J. Stewart unpublished observations). Although the concentration of dopamine in dialysate (0.21 to 0.51 nM) was substantially lower than the smallest concentration used in *in vitro* experiments (10 µM), dopamine levels fall off extremely rapidly with distance from the release site (Cragg & Rice, 2004), are affected by flow rate, and greatly underestimate actual levels within layer II synapses. Dialysis probes in the entorhinal cortex usually included portions of ventral hippocampus and subiculum which could have contributed to the

dopamine signal. However, dopaminergic projections to ventral hippocampus and subicular complex are much less dense than those to the entorhinal cortex (Gasbarri et al., 1996; Gasbarri, Sulli, & Packard, 1997; Gasbarri, Verney, Innocenzi, Campana, & Pacitti, 1994), and probes clearly included layer II where dopamine afferents surround principal cell islands (Bjorklund & Lindvall, 1984).

The facilitation induced by GBR12909 may have been countered to some degree by activation of the cholinergic system during increased locomotor activity in these animals (Fig. 2.1B2; Nakachi et al., 1995). Forebrain cholinergic neurons are active during movement (Bland & Oddie, 2001) and cholinergic activation can suppress EPSPs in hippocampus and medial entorhinal cortex (Caruana, Hamam, Poirier, & Chapman, 2003; Cheong et al., 2001; Kremin et al., 2006). Cholinergic and dopaminergic systems are likely to be co-activated during appetitive behaviors, but it is not known how these two systems may interact to affect sensory processing within the lateral entorhinal cortex.

In Vitro Slice Experiments

The receptor subtypes involved in the facilitation of glutamate-mediated synaptic responses were evaluated using bath application of receptor blockers in acute slices. Field EPSPs were recorded from layer II in response to stimulation of layer I afferents. Initial experiments with high concentrations of dopamine (50 and 100 μM) resulted in a dose-dependent reduction of synaptic responses. Similar depression effects have been reported at comparable concentrations of dopamine in the medial entorhinal cortex (Pralong & Jones, 1993; Stenkamp et al., 1998), but the reduction observed here was surprising given the facilitation

we observed in the lateral division *in vivo*. However, the lower concentration of 10 μM dopamine caused a D_1 receptor-dependent facilitation that mirrored our findings in awake rats (Fig. 2.4). Responses were also facilitated during washout of higher doses as bath concentrations of dopamine fell (Figs. 2.5 and 2.6). This rebound facilitation could be due to the lower concentration of dopamine present, and activation of dopamine receptors, but could also reflect interactions between D_1 and D_2 receptor activation or a more persistent dopamine-mediated potentiation effect (Y. Y. Huang & Kandel, 1995). The effects of the lower dose of 10 μM dopamine suggest that the normal role of dopamine is to enhance synaptic responses to cortical afferents via a D_1 receptor-mediated mechanism. This is consistent with the higher affinity of D_1 versus D_2 receptors (Seeman & Van Tol, 1993), and with the high density of D_1 receptors in layer II of the entorhinal cortex (Q. Huang et al., 1992; Köhler et al., 1991b; Weiner et al., 1991).

In the prefrontal cortex, although dopamine increases the excitability of pyramidal neurons (e.g., Gorelova & Yang, 2000), dopamine most commonly results in a reduction of AMPA synaptic responses through a D_1 receptor-mediated reduction in transmitter release (Gao, Krimer, & Goldman-Rakic, 2001; Law-Tho, Hirsch, & Crepel, 1994; Seamans, Durstewitz, Christie, Stevens, & Sejnowski, 2001a; Urban, González-Burgos, Henze, Lewis, & Barrionuevo, 2002; Zheng, Zhang, Bunney, & Shi, 1999). However, there are reports of increased AMPA responses in prefrontal cortex layer V (G. Chen, Greengard, & Yan, 2004; Onn, Wang, Lin, & Grace, 2005; Seamans et al., 2001a) and low concentrations (<20 μM) of dopamine can lead to a D_1-mediated increase in AMPA EPSCs by a

postsynaptic mechanism in layers II/III (Bandyopadhyay, Gonzalez-Islas, & Hablitz, 2005; Gonzalez-Islas & Hablitz, 2003). In the hippocampus, activation of D_1 receptors in CA1 pyramidal cells with a selective agonist can also lead to a sustained enhancement of AMPA-mediated EPSCs (S. N. Yang, 1999, 2000). Similarly, increases in NMDA-mediated responses induced by dopamine are also commonly observed in the prefrontal cortex (G. Chen et al., 2004; Gonzalez-Islas & Hablitz, 2003; Seamans et al., 2001a; Zheng et al., 1999) and hippocampus (S. N. Yang, 1999, 2000) and are consistent with the D_1-mediated increase in the mixed fEPSPs observed here.

Bidirectional dose-dependent effects of dopamine have been observed in other areas, and our finding that high concentrations of dopamine suppress fEPSPs via D_2 receptors is consistent with these reports. In the prefrontal cortex, studies have reported both a facilitation of NMDA responses at low doses via D_1 receptors and a suppression of responses at high concentrations via D_2 receptors (Seamans et al., 2001a; Zheng et al., 1999). Activation of D_1 and D_2 receptors has parallel bidirectional effects on evoked IPSCs in layer V (Seamans, Gorelova, Durstewitz, & Yang, 2001b; Trantham-Davidson, Neely, Lavin, & Seamans, 2004). D_2 receptor activation has also been shown to suppress synaptic responses in the CA1 region of the hippocampus (Gribkoff & Ashe, 1984; Y. Y. Huang & Kandel, 1995) while also leading to a lasting D_1 receptor-mediated facilitation. In the medial entorhinal cortex both D_2 and D_1 receptors contribute to the suppression of EPSPs (Gribkoff & Ashe, 1984; Y. Y. Huang & Kandel, 1995; Pralong & Jones, 1993; Stenkamp et al., 1998), and evoked field responses in the CA1 region of the hippocampus are also suppressed by

activation of either D_1 or D_2 receptors (Otmakhova & Lisman, 1999). Here, although D_1 receptor antagonism did not affect the peak suppression of fEPSPs induced by dopamine, it did reduce the time-course of the effect (Figs. $2.5B_1$ and $2.6B_1$). This suggests that strong activation of D_1-like receptors could contribute to the reduction of synaptic responses. A D_1-mediated suppression of responses at high concentrations of dopamine may also account for why a facilitation was not revealed when D_2 receptors were blocked with sulpiride (Figs. $2.5B_2$ and $2.6B_2$).

Changes in intrinsic conductances that affect postsynaptic excitability may also contribute to dopaminergic modulation of evoked responses. Indeed, previous reports have shown that dopamine can reduce input resistance in layer II of the entorhinal cortex, likely by an increased K^+ conductance (Pralong & Jones, 1993), and that dopamine reduces responses to current injection and summation of synaptic responses in layer V cells through an increase in I_h (Rosenkranz & Johnston, 2006). Input resistance was reduced by up to 30% when high concentrations of dopamine (≥500 μM) were applied in layer II (Pralong & Jones, 1993), and it is possible that this may account partially for some of the reduction in field EPSPs observed here with 50 and 100 μM dopamine. Application of dopamine activates I_h and reduces input resistance by about 10% in layer V cells of the lateral entorhinal cortex, and this leads to reduced membrane responses to current injection and a dampening of temporal summation of EPSPs (Rosenkranz & Johnston, 2006). Dopamine at this concentration (10 μM) did not significantly depress responses to single stimulation pulses (Rosenkranz & Johnston, 2006), so while changes in I_h may

contribute to depression in responses observed here at high concentrations, it is unlikely to contribute to the facilitation of responses at the 10 μM concentration.

The effects of dopamine on the mixed EPSPs recorded here may have resulted in part from indirect actions of dopamine on inhibitory inputs to principal neurons. However, Pralong and Jones (1993) found that dopamine did not affect isolated IPSPs in medial entorhinal cortex layer II stellate cells, and although D_1 and D_2 receptor activation has bidirectional effects on IPSCs in prefrontal cortex (Seamans et al., 2001b; Trantham-Davidson et al., 2004), the direction of the effects are opposite to what would be expected here based on effects of dopamine on the EPSP. Nevertheless, dopamine may have substantive activity-dependent modulatory effects on activation of interneurons and/or GABA transmission in lateral entorhinal cortex, and this remains to be investigated more closely.

Conclusions

Dopaminergic inputs to prefrontal cortex are thought to facilitate cognitive processes and promote adaptive responses to physiologically relevant stimuli, and optimal effects are thought to occur during moderate, but not excessive activation of D_1 receptors (Arnsten, 1998; Goldman-Rakic, Muly, & Williams, 2000; Seamans & Yang, 2004). Few behavioral studies bear directly on the function of dopaminergic inputs to the entorhinal cortex (Barros et al., 2001), but our data suggest that optimal activation of D_1 receptors may enhance the impact of sensory inputs to the medial temporal lobe. This may promote the induction of long-term forms of synaptic plasticity that could contribute to memory for reward-

relevant stimuli (Chapman & Racine, 1997a; Fransen et al., 2006; Kourrich & Chapman, 2003). Further, although a role for the superficial layers of the entorhinal cortex in working memory has not been well established, and strong sensory input could indeed disrupt working memory, dopaminergic facilitation of synaptic transmission may promote activation of working memory representations and enhance the impact of sensory feedback on processing of reward-relevant stimuli by the hippocampal formation.

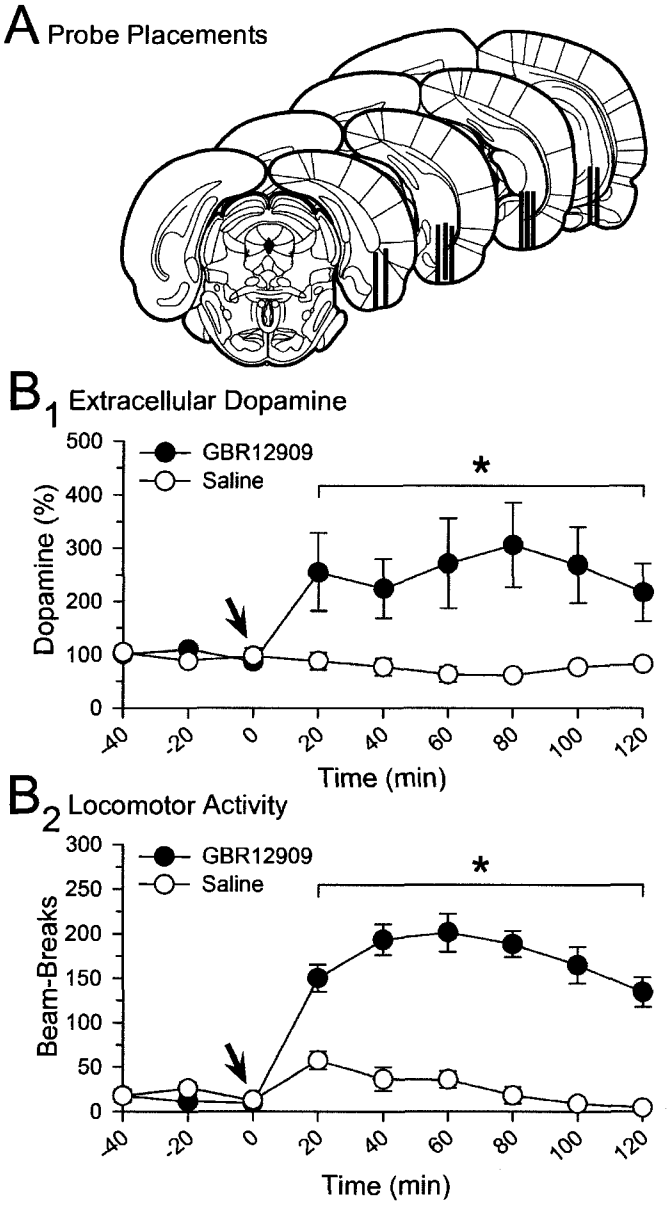

A Probe Placements
B1 Extracellular Dopamine
GBR12909
Saline
*
Dopamine (%)
500
400
300
200
100
0
-40
-20
0
20
40
60
80
100
120
Time (min)
B2 Locomotor Activity
GBR12909
Saline
*
Beam-Breaks
300
250
200
150
100
50
0
-40
-20
0
20
40
60
80
100
120
Time (min)

Figure 2.1. Injections of GBR12909 enhance extracellular levels of dopamine in the lateral entorhinal cortex of awake rats. **A**: Microdialysis probe locations are shown on representative sections taken from the atlas of Paxinos and Watson (1998). **B**: Extracellular dopamine levels following injections of saline (open circles) or GBR12909 (filled circles) are expressed as a percent change (mean ± SEM in this and subsequent figures) from baseline (B_1). Locomotor activity is expressed as the number of photo beam-breaks in 20 minute epochs during dialysis collection (B_2). Note the transient increase in activity following control injections. Asterisks indicate a significant difference from the saline condition ($P < 0.05$) and arrows indicate the time at which systemic injections were administered.

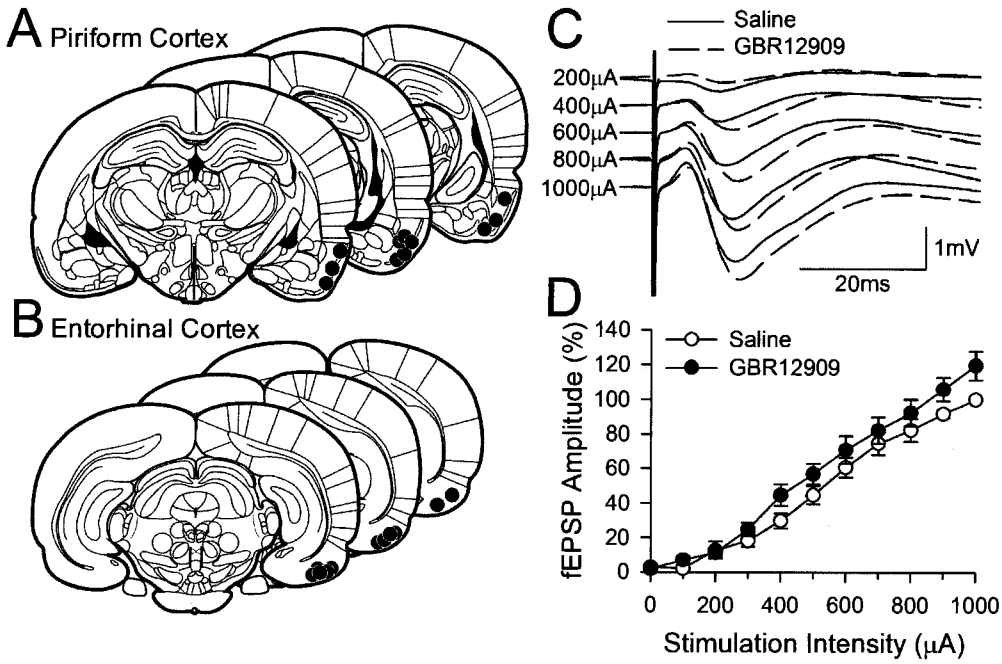

A Piriform Cortex
B Entorhinal Cortex
C
Saline
GBR12909
200μA
400μA
600μA
800μA
1000μA
1mV
20ms
D
fEPSP Amplitude (%)
140
120
100
80
60
40
20
0
Saline
GBR12909
0
200
400
600
800
1000
Stimulation Intensity (μA)

Figure 2.2. Field potential responses in the lateral entorhinal cortex evoked by stimulation of the piriform cortex are enhanced by dopamine. **A** and **B**: Locations of electrode tips in the piriform cortex and lateral entorhinal cortex are shown on representative sections taken from the atlas of Paxinos and Watson (1998) for all rats in chronic recording experiments. **C**: Traces show averaged fEPSPs from a representative animal following an injection of saline (solid lines) or GBR12909 (dashed lines) at the indicated stimulation intensities. **D**: Mean peak amplitudes of fEPSPs are shown as a function of pulse intensity 20 minutes after treatment with saline (open circles) or GBR12909 (filled circles), and are expressed as a percentage of responses to the highest stimulation intensity during the saline condition.

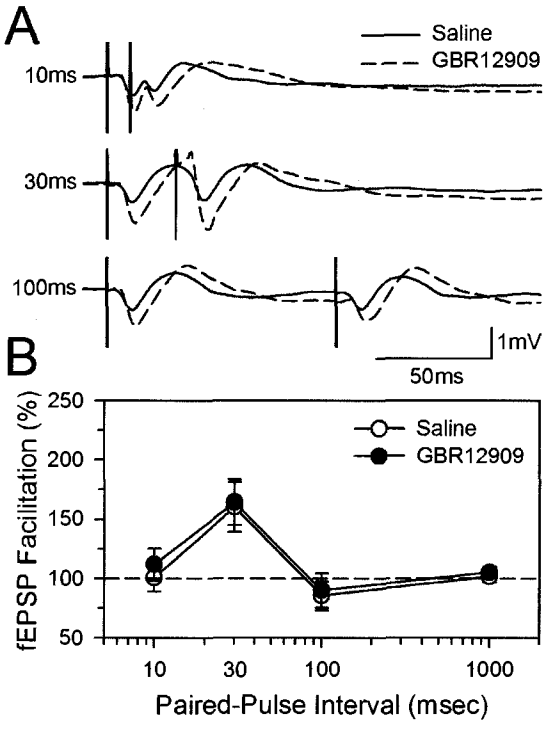

A
Saline
GBR12909
10ms
30ms
100ms
1mV
50ms
B
fEPSP Facilitation (%)
250
200
150
100
50
Saline
GBR12909
10
30
100
1000
Paired-Pulse Interval (msec)

Figure 2.3. Enhancing dopamine transmission with GBR12909 does not affect paired-pulse facilitation in the lateral entorhinal cortex. **A**: Representative traces show responses to stimulation pulses at 10, 30, and 100 ms interpulse intervals following injections of saline (solid lines) or GBR12909 (dashed lines). **B**: Mean amplitudes of fEPSPs evoked by the second of two pulses are expressed as a percentage of responses evoked by the first pulse at the specified interpulse intervals after treatment with saline (open circles) or GBR12909 (filled circles).

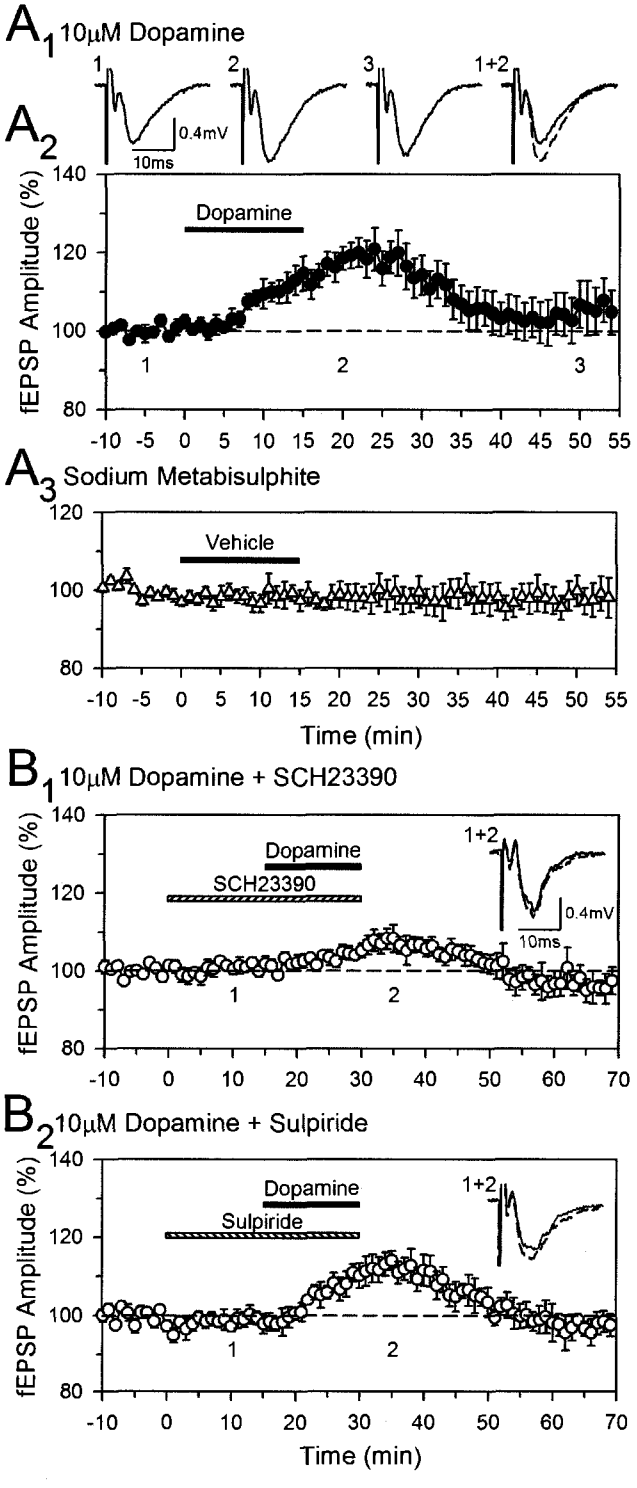

A1 10μM Dopamine
1
2
3
1+2
0.4mV
10ms
A2
fEPSP Amplitude (%)
Dopamine
A3 Sodium Metabisulphite
Vehicle
Time (min)
B1 10μM Dopamine + SCH23390
Dopamine
SCH23390
B2 10μM Dopamine + Sulpiride
Sulpiride

Figure 2.4. Low concentrations of dopamine facilitate synaptic responses in the lateral entorhinal cortex. **A**: Averaged field EPSPs before, during, and after bath-application of 10 μM dopamine. Averaged traces in A_1 show a facilitation of synaptic responses (trace 2) that returns to baseline levels during washout (trace 3). Numbers in A_1 correspond to the time points indicated in A_2. Averaged fEPSP amplitudes are expressed as a percentage of the baseline period and plotted as a function of time for this and subsequent figures. The effects of dopamine application (solid bar; A_2) peaked about 8 min into washout and returned to baseline levels within ≈30 min. The antioxidant sodium metabisulfite was routinely co-applied with dopamine and had no significant effect on synaptic responses when applied alone (A_3). **B**: Application of the D_1 receptor antagonist SCH23390 or the D_2 receptor antagonist sulpiride alone had no effect on synaptic responses. However, co-application of SCH23390 with dopamine attenuated the increase in synaptic responses induced by dopamine alone (B_1). Co-application of sulpiride did not significantly attenuate the increase in fEPSPs induced by dopamine (B_2). Inset traces show averaged overlaid field responses at the indicated times before (solid lines) and after (dashed lines) dopamine administration.

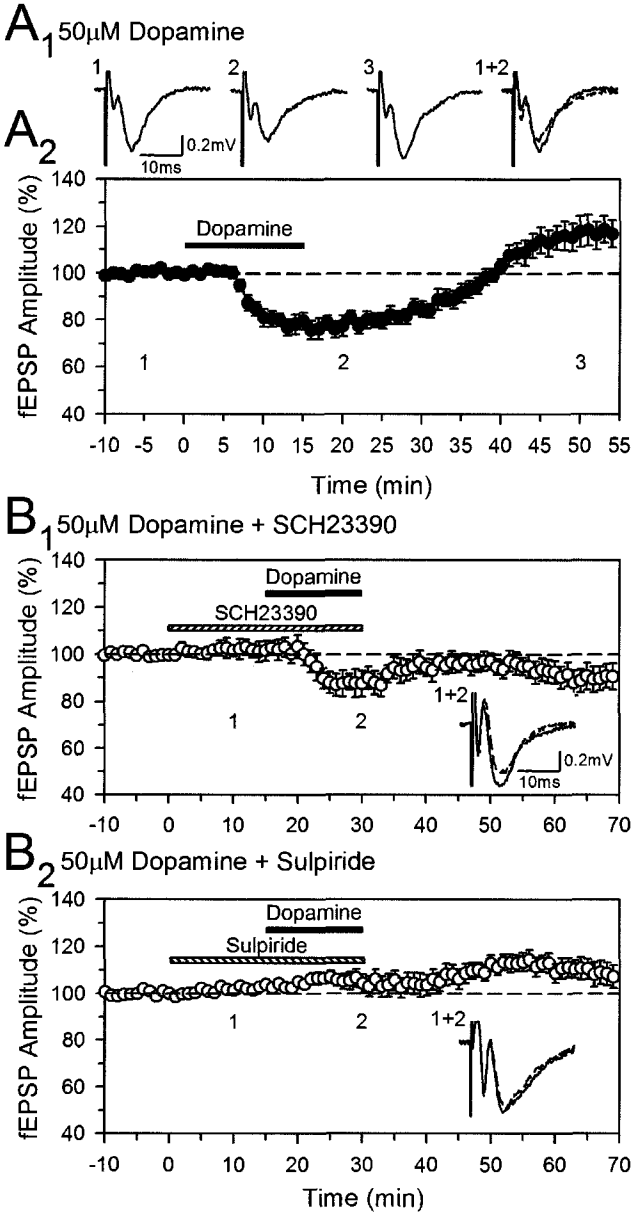

A1 50μM Dopamine
A2
1
2
3
1+2
0.2mV
10ms
fEPSP Amplitude (%)
Dopamine
Time (min)
B1 50μM Dopamine + SCH23390
SCH23390
B2 50μM Dopamine + Sulpiride
Sulpiride

Figure 2.5. A moderate concentration of dopamine suppresses synaptic responses in the lateral entorhinal cortex. **A**: Field potential amplitudes are significantly reduced by dopamine and return to baseline levels within ≈25 min of wash. Synaptic responses rebounded and were significantly facilitated for the remainder of the experiment (A_2, arrow). **B**: Application of sulpiride completely blocked the inhibition of synaptic responses induced by dopamine (B_2), but the effect of SCH23390 on peak amplitudes of responses was not significant (B_1). SCH23390 did, however, significantly attenuate the rebound in fEPSPs typically observed during the last ≈10 min of wash (B_1).

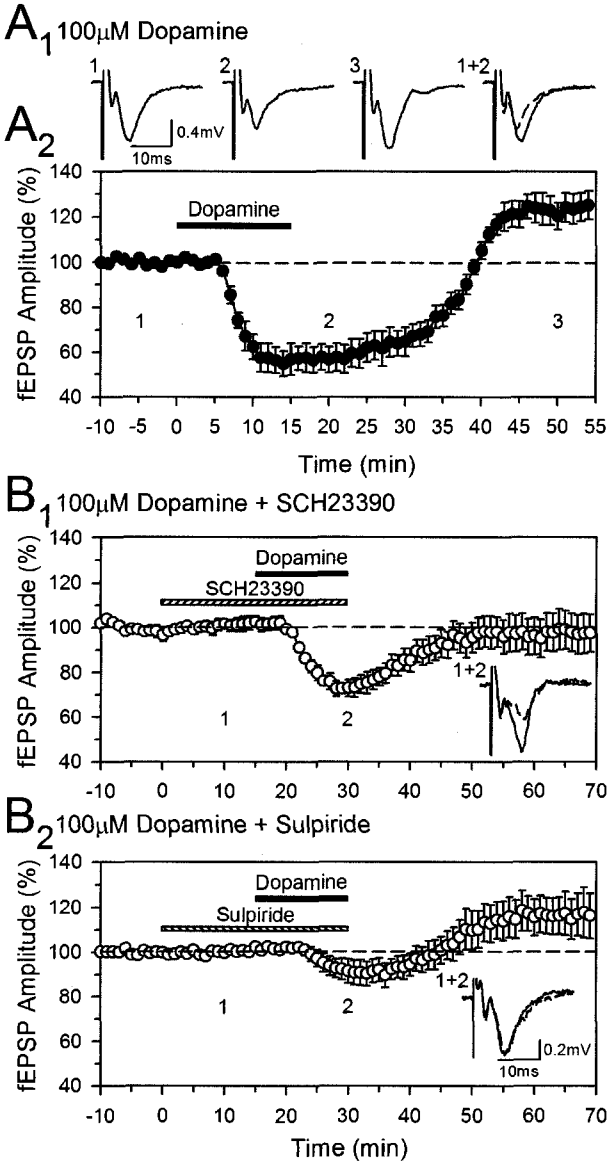

A1 100μM Dopamine
A2
1
2
3
1+2
0.4mV
10ms
fEPSP Amplitude (%)
Dopamine
Time (min)
B1 100μM Dopamine + SCH23390
SCH23390
B2 100μM Dopamine + Sulpiride
Sulpiride
0.2mV

Figure 2.6. A high concentration of dopamine markedly suppresses synaptic responses in the lateral entorhinal cortex. **A**: Field responses are significantly reduced following bath-application of 100 μM dopamine and rebound to amplitudes greater than baseline at the end of washout (A_1, traces 2 and 3). The suppression of synaptic responses peaked 10 to 35 min following application of dopamine (A_2). **B**: Co-application of sulpiride with dopamine almost completely blocked the inhibition of synaptic responses induced by dopamine alone (B_2), but the effect of SCH23390 on responses was not significant (B_1). The D_1 receptor antagonist SCH23990 did however block the rebound in synaptic responses observed during the last ≈10 min of wash (B_1).

CHAPTER 3

DOPAMINERGIC SUPPRESSION OF SYNAPTIC TRANSMISSION IN THE LATERAL ENTORHINAL CORTEX THROUGH REDUCED GLUTAMATE RELEASE

Douglas A. Caruana and C. Andrew Chapman

In the previous chapter, systemic administration of the selective dopamine reuptake inhibitor GBR12909 enhanced extracellular levels of dopamine in the entorhinal cortex and facilitated the amplitude of fEPSPs evoked in the superficial layers following stimulation of the piriform cortex. This effect was mimicked by bath-application of a low 10 μM concentration of dopamine *in vitro*. Interestingly, higher concentrations of 50 and 100 μM dopamine *suppressed* the amplitude of fEPSPs in slices containing the lateral entorhinal cortex. Not only were the effects of dopamine on fEPSPs concentration-dependent and bidirectional, they were also dependent on the activation of *different* dopamine receptor subtypes. In particular, the facilitation was dependent on activation of D_1-like dopamine receptors and the suppression on D_2-like receptors. Although the previous experiments clearly demonstrate that the facilitation and suppression effects require activation of different dopamine receptors, the intracellular mechanisms underlying the facilitatory and inhibitory effects of dopamine on excitatory synaptic transmission in the superficial layers are largely unknown.

Experiments conducted in the next Chapter examine the mechanisms underlying the potent suppression of synaptic transmission induced by high concentrations of dopamine using whole cell current clamp recordings of mixed and isolated EPSPs. Results show that the suppression of synaptic transmission by dopamine is mediated largely by a D_2 receptor-dependent reduction in transmitter release, as well as a D_1 receptor-dependent drop in cellular input resistance.

ABSTRACT

Dopaminergic projections to the superficial layers of the lateral entorhinal cortex can modulate the strength of olfactory inputs that also terminate in this region. We have found that low concentrations of dopamine facilitate field EPSPs, and that higher concentrations of dopamine suppress synaptic responses in the lateral entorhinal cortex. Here, we have used whole-cell current clamp recordings from layer II fan cells to determine the mechanisms of the synaptic suppression. Bath application of dopamine (10 to 50 μM) hyperpolarized fan cells and reversibly suppressed the amplitude of EPSPs evoked by stimulation of layer I afferents. Dopamine suppressed both the isolated AMPA- and NMDA-mediated components of the EPSP, and paired-pulse facilitation was also enhanced, indicating that the suppression of EPSPs is mediated largely by a reduction in glutamate release. Blockade of D_2-like receptors greatly reduced the suppression of EPSPs, and blocked the increase in paired-pulse facilitation. Dopamine also lowered input resistance of fan cells, and reduced the number of action potentials evoked by depolarizing current steps. The drop in input resistance was mediated by activation of D_1-like receptors, and was prevented by blocking K^+ channels with TEA. The dopaminergic suppression of synaptic transmission in the lateral entorhinal cortex is therefore mediated by a D_2 receptor-dependent reduction in transmitter release, and a D_1 receptor-dependent increase in a K^+ conductance. This suppression of EPSPs may dampen the strength of sensory inputs to the lateral entorhinal cortex during periods of elevated mesocortical dopamine activity.

The entorhinal cortex is an important interface that links primary sensory and association cortices to the hippocampal formation, and it is critical for the sensory and mnemonic functions of the medial temporal lobe (Lavenex & Amaral, 2000; Schwarcz & Witter, 2002b; Squire et al., 2004; Squire & Zola-Morgan, 1996). In the rat, the lateral division of the entorhinal cortex receives most of its cortical inputs from the olfactory cortex and perirhinal cortex, and the medial entorhinal cortex receives visual and multimodal inputs mainly via the postrhinal cortex (Burwell, 2000; Burwell & Amaral, 1998; Kerr et al., 2007). This pattern of cortical input to the medial and lateral divisions of the entorhinal cortex contributes to their different roles in sensory and cognitive processing (Hafting et al., 2005; Hargreaves et al., 2005; Sewards & Sewards, 2003). In addition, neuromodulatory transmitters innervate both the medial and lateral entorhinal cortices and can have powerful effects on sensory and mnemonic function in these regions. Acetylcholine and serotonin modulate synaptic transmission and rhythmic EEG activities in the medial entorhinal cortex (Bland & Oddie, 2001; Grunschlag et al., 1997; Hamam et al., 2006; Ma, Shalinsky, Alonso, & Dickson, 2007; Schmitz et al., 1998). Further, midbrain dopamine neurons send one of their largest cortical projections to the superficial layers of the lateral entorhinal cortex where they target principal cells islands (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987; Oades & Halliday, 1987). Relatively little is known, however, regarding the neuromodulatory effects of dopamine in the lateral entorhinal cortex.

The large dopaminergic projection to the prefrontal cortex is known to regulate cellular processes related to working memory (Goldman-Rakic, 1999;

Phillips, Vacca, & Ahn, 2007; Seamans & Yang, 2004), and dopaminergic inputs to the lateral entorhinal cortex are also likely to affect mechanisms of sensory and mnemonic function. In the prefrontal cortex, activation of D_1 receptors can suppress glutamate release in layer V (Gao et al., 2001; Law-Tho et al., 1994; Seamans et al., 2001a), but can enhance glutamatergic transmission in layer III (Bandyopadhyay et al., 2005; Gonzalez-Islas & Hablitz, 2003). Further, the positive effects of D_1 receptor activation on working memory follows an inverted U-shaped function (Arnsten, 1998), and strong or weak stimulation of D_1 receptors can also have opposite effects on NMDA receptor-mediated synaptic currents (Seamans & Yang, 2004; C. R. Yang & Chen, 2005). We have also found that dopamine has dose-dependent bidirectional effects in layer II of the lateral entorhinal cortex. In awake animals, increasing levels of dopamine with a selective reuptake inhibitor facilitates synaptic responses evoked by stimulation of the piriform cortex, and field excitatory postsynaptic potentials (EPSPs) are also facilitated by a low concentration of dopamine *in vitro* (Caruana, Sorge, Stewart, & Chapman, 2006). Higher concentrations of dopamine, however, suppress fEPSPs, and similar suppression effects have been observed by others in medial entorhinal cortex layer II (Pralong & Jones, 1993) and layer III (Stenkamp et al., 1998). Dopamine can also reduce the input resistance of layer II neurons in the medial entorhinal cortex (Pralong & Jones, 1993) and reduce temporal summation in layer V neurons of the lateral division through an increase the I_h current (Rosenkranz & Johnston, 2006). Dopamine may therefore modulate synaptic function in the lateral entorhinal cortex through multiple mechanisms.

We have used whole-cell current clamp recordings to investigate the mechanisms of the suppression of EPSPs by dopamine in electrophysiologically identified fan cells in layer II of the lateral entorhinal cortex. Receptor blockers were used to determine the dopamine receptors that mediate the suppression of EPSPs, and paired-pulse tests were used to assess whether the suppression is expressed pre- or postsynaptically. Changes in the intrinsic excitability of fan cells were also monitored using responses to hyperpolarizing and depolarizing current steps. In addition to a D_2-like receptor-mediated suppression of transmitter release, we show evidence that EPSPs are also reduced by an increased K^+ conductance dependent on activation of D_1 receptors.

MATERIALS AND METHODS

Tissue Slices. Methods for obtaining whole cell current clamp recordings were similar to those described previously (Caruana et al., 2006; Glasgow & Chapman, 2007; Hamam et al., 2006; Mueller, Chapman, & Stewart, 2006). Male Long-Evans rats between 4 and 6 weeks old were anesthetized with halothane, decapitated, and their brains rapidly removed and transferred into cold (4°C) artificial cerebrospinal fluid (ACSF) saturated with 95% O_2 and 5% CO_2 containing (in mM) 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose (pH ≈7.3; 300-310 mOsm). All chemicals were obtained from Sigma. Horizontal slices (300 µm thick) were cut using a vibratome (WPI, Vibroslice), and slices recovered for at least one hour at 22 to 24°C. Slices were transferred individually to a recording chamber and visualized using an upright microscope (Leica, DM-LFS) equipped with differential

interference contrast optics, a 40x water immersion objective, and a near-infrared camera (COHU). Submerged slices were superfused with oxygenated ACSF at a rate of 1.5 to 2.0 ml/min. Slices containing the lateral entorhinal cortex were taken from ventral sections about 1.9 to 3.4 mm above the interaural line (Paxinos & Watson, 1998). Layer II was identified based on the presence of cell "islands" about 150 µm from the cortical surface (Blackstad, 1956; Carboni & Lavelle, 2000; Steward, 1976; Wyss, 1981).

Stimulation and Recording. Patch recording pipettes for whole cell recordings were prepared from borosilicate glass (1.0 mm OD, 4 to 8 MΩ) using a horizontal puller (Sutter Instr., P-97), and were filled with a solution containing (in mM) 140 K-gluconate, 5 NaCl, 2 $MgCl_2$, 10 HEPES, 0.5 EGTA, 2 ATP-Tris, and 0.4 GTP-Tris (pH adjusted to 7.24-7.32 with KOH; 270-280 mOsm). Pipettes were placed in contact with somata of layer II neurons and gentle suction was applied under voltage clamp to form a tight seal (1-3 GΩ). Whole cell configuration was achieved by increased suction, and experiments began after 3 to 5 min. Current clamp recordings were obtained using an Axopatch 200B amplifier (Axon Instr.) and displayed on a digital oscilloscope (Gould 1604). Recordings were filtered at 10 kHz and digitized at 20 kHz (Axon Instr., Digidata 1322A) for storage on computer hard disk. Recordings were accepted if the series resistance was ≤25 MΩ (mean = 16.9 ±0.9 MΩ) and if input resistance and resting potential were stable. A bipolar stimulating electrode made from two tungsten electrodes (FHC, 1.0 MΩ) was positioned to span layer I near the border with layer II approximately 0.2 to 0.6 mm rostral to the recording electrode. Synaptic responses were evoked with 0.1 ms constant current pulses

delivered using a stimulus timer and isolation unit (WPI, Models A300 and A360). Stimulation intensity was adjusted to evoke responses approximately 75% of maximal.

All neurons (n = 118) included for analyses were identified as "fan" cells based on electrophysiological characteristics described previously (Tahvildari & Alonso, 2005; Wang & Lambert, 2003). In comparison to stellate cells of the medial entorhinal cortex, fan cells show modest inward rectification during hyperpolarizing current steps, a small depolarizing afterpotential following single spikes, and do not show prominent theta-frequency membrane potential oscillations at subthreshold voltages (Alonso & Klink, 1993; Tahvildari & Alonso, 2005; Wang & Lambert, 2003).

Dopaminergic Modulation of Synaptic Responses. The effects of dopamine on glutamate-mediated synaptic transmission were assessed by recording excitatory postsynaptic potentials (EPSPs) evoked by stimulation of layer I before and after 5-min bath-application of 1, 10, or 50 μM dopamine. Responses were evoked once every 20 sec and the mean of 10 responses was obtained for analysis. Baseline responses were obtained at resting potential and, because dopamine usually hyperpolarizes fan cells, constant current was often required to return cells to the original membrane potential for recordings in the presence of dopamine. Sodium metabisulfite (50 μM) was co-applied to slow the oxidation of dopamine (Caruana et al., 2006; Stenkamp et al., 1998; C. R. Yang & Seamans, 1996) and ambient lighting was also reduced. Possible effects of sodium metabisulfite were assessed with a vehicle control group. Drugs were

routinely stored at –20°C as concentrated stock solutions until needed, but dopamine HCl was dissolved just prior to bath application.

Paired-pulse tests were used to determine whether dopamine modulates EPSPs through a pre- or postsynaptic mechanism (Hamam et al., 2006). Pairs of stimulation pulses separated by an interval of 30 ms were delivered before and after 5-min bath-application of 1, 10, or 50 μM dopamine. Stimulation intensity was adjusted to evoke EPSPs approximately 60% of maximal and ten responses were averaged for analyses. Paired-pulse facilitation was quantified by expressing the amplitude of the second response as a percentage of the first response.

Mechanisms mediating the suppression of EPSPs by high concentrations of dopamine were investigated by assessing the effects of 50 μM dopamine on isolated components of synaptic responses. After baseline recordings in normal ACSF, AMPA receptor-mediated responses were isolated with bath application of 50 μM 2-amino-5-phosphonovaleric acid (APV) and 25 μM bicuculline methiodide, or NMDA receptor-mediated responses were isolated with 20 μM 7-nitro-2,3-dioxo-1,4-dihydroquinoxaline-6-carbonitrile (CNQX) and 25 μM bicuculline. GABA-mediated IPSPs were isolated with either 1 mM kynurenic acid or 20 μM CNQX with 50 μM APV. Isolated synaptic responses were recorded before and after 5-min application of 50 μM dopamine. Isolated AMPA receptor-mediated responses were also used to determine if dopamine suppresses EPSPs primarily through D_1- or D_2-like receptors. Baseline responses were recorded in the presence of either the D_1 receptor antagonist SCH23390 (50 μM) or the D_2 receptor antagonist sulpiride (50 μM; Caruana et

al., 2006; Pralong & Jones, 1993; Stenkamp et al., 1998), and 50 µM dopamine was then applied for 5 min. Sulpiride was prepared daily in a stock solution of 6% DMSO in ACSF titrated with 0.1 N HCl, and there was final concentration of 0.1% DMSO with sulpiride.

The effects of dopamine on the intrinsic excitability of fan cells was assessed by monitoring responses to hyperpolarizing and depolarizing current steps. Changes in action potentials, afterhyperpolarizations, input resistance and inward rectification were examined before and after 5-min bath application of 1, 10, or 50 µM dopamine. The number of action potentials elicited in response to suprathreshold current injection can be used to characterize neuronal excitability (Rosenkranz & Johnston, 2006), and we therefore determined the number of spikes fired in response to a 500 ms-duration depolarizing current pulse from a constant holding potential (typically rest) using a pulse amplitude that elicited 3 to 5 action potentials (Rosenkranz & Johnston, 2006). Receptors that mediate the dopamine-induced change in input resistance were investigated by co-application of either SCH23390 or sulpiride, and the ionic conductances involved were assessed using 0.5 µM tetrodotoxin (TTX) or 30 mM tetraethylammonium (TEA).

Data Analysis. Electrophysiological characteristics of fan cells and changes in synaptic responses were analyzed using the software program Clampfit 8.2 (Axon Instr.). The amplitudes of averaged EPSPs were measured relative to the prestimulus baseline, and paired-pulse facilitation was determined by expressing the amplitude of the second response as a proportion of the amplitude of the first response. Action potential height was measured from resting potential, and action potential width and fast and medium

afterhyperpolarizations were measured from threshold. Input resistance was calculated by measuring peak and steady-state voltage responses to –200 pA current steps (500 ms) and inward rectification was quantified by expressing the peak input resistance as a proportion of the steady-state resistance (rectification ratio). All data were expressed as the mean ±SEM for plotting, and changes in response properties were assessed using paired samples t-tests or mixed design ANOVAs.

RESULTS

Electroresponsiveness of Layer II Fan Cells. A total of 118 fan cells in layer II of the lateral entorhinal cortex were identified electrophysiologically and included for analysis, and the characteristics of these cells were similar to those reported previously (Tahvildari & Alonso, 2005; Wang & Lambert, 2003). Fan cells had a mean resting membrane potential of –58.8 ±0.6 mV, and a peak input resistance of 99.1 ±2.1 MΩ. Most cells (108 of 118) demonstrated a small delayed inward rectification in response to hyperpolarizing current steps (rectification ratio: 1.10 ±0.01). Action potentials (amplitude: 128.8 ±0.7 mV, width: 4.1 ±0.1 ms, threshold: –44.1 ±0.8 mV) were typically followed by fast and medium afterhyperpolarizations (–3.3 ±0.3 mV and –5.8 ±0.3 mV) with a small depolarizing afterpotential. Averaged EPSPs evoked by stimulation of layer I had a mean amplitude of 4.4 ±0.2 mV. Continuous recordings of membrane potential were obtained in a subset of 28 cells to assess subthreshold membrane potential oscillations and, similar to findings of Tahvildari and Alonso (2005), fan cells did not display prominent oscillations (data not shown).

Dopaminergic Modulation of EPSPs. We previously found concentration-dependent effects of dopamine on field EPSPs in layer II *in vitro*, in which 10 μM dopamine facilitated fEPSPs and 50 to 100 μM dopamine suppressed fEPSPs (Caruana et al., 2006). We obtained similar concentration-dependent effects in whole cell EPSPs recorded here before and after 5-min bath application of dopamine. Application of 50 μM dopamine resulted in a strong suppression of synaptic response to 38.5 ±5.8% of baseline levels (Fig. 3.1A; $t_8 = 7.75$, $P < 0.001$; $n = 9$) that could be reversed by 15 min washout in normal ACSF (3 cells). We initially expected 10 μM dopamine to facilitate EPSPs (Caruana et al., 2006), but found that 10 μM dopamine instead caused a small synaptic suppression (to 87.0 ±5.8% of baseline; Fig. 3.1B; $t_{15} = 2.31$, $P < 0.05$; $n = 18$). However, a lower concentration of 1 μM dopamine significantly enhanced responses to 132.7 ±4.4% of baseline levels (Fig. 3.1C; $t_6 = 5.04$, $P < 0.01$; $n = 7$). In our previous study using a gas-fluid interface chamber, a larger bath volume and slower flow-rate may have increased dopamine oxidation and reduced the effective concentration of dopamine at the slice, and this may account for why a higher applied concentration facilitated responses in that study (Caruana et al., 2006). Bath application of the antioxidant sodium metabisulfite alone had no significant effect on the amplitude of whole cell EPSPs (Fig. 3.1D; $n = 8$).

Paired-pulse tests were used to determine if synaptic suppression and facilitation effects were likely expressed pre- or postsynaptically. Pairs of pulses were delivered before and after 5-min dopamine application, and a 30 ms interpulse interval was used that results in optimal paired-pulse facilitation (Bouras & Chapman, 2003; Caruana & Chapman, 2004; Hamam et al., 2006;

Kourrich & Chapman, 2003). If EPSPs are reduced through a reduction in transmitter release, then a greater amount of transmitter should be available for release in response to the second stimulation pulse and paired-pulse facilitation should be enhanced (Manabe, Wyllie, Perkel, & Nicoll, 1993; Zucker, 1989; Zucker & Regehr, 2002). Changes in EPSPs mediated by alterations in postsynaptic receptors, however, should not be associated with changes in paired-pulse ratio. High concentrations of dopamine that reduced EPSP amplitude were also found to enhance paired-pulse facilitation (Fig. 3.2A,B; t_{13} = 2.78, $P < 0.05$ for 10 µM; $t_8 = 2.97$, $P < 0.05$ for 50 µM), suggesting that dopamine reduced EPSPs by suppressing glutamate release. In contrast, the low concentration of 1 µM dopamine that facilitated EPSPs had no significant effect on paired pulse facilitation (Fig. 3.2C), suggesting that the facilitation of EPSPs was mediated primarily by an increased postsynaptic response to glutamate. A similar dopaminergic facilitation of fEPSPs with no effect on paired-pulse ratio has been observed in the entorhinal cortex *in vivo* (Caruana et al., 2006).

Isolated Synaptic Responses. The suppression of EPSPs by high concentrations of dopamine was examined more closely using pharmacologically isolated synaptic responses. Consistent with a suppression of glutamate release from presynaptic terminals, bath application of 50 µM dopamine significantly attenuated both the isolated AMPA- and NMDA-mediated responses. The NMDA component was reduced to 26.0 ±7.5% of baseline (Fig. 3.3B; $t_7 = 3.32$, $P < 0.05$; $n = 8$) and the AMPA component was reduced to 41.7 ±5.6% of baseline (Fig. 3.3A; $t_5 = 3.50$, $P < 0.05$; $n = 6$).

Dopamine receptor subtypes underlying the suppression of AMPA-mediated synaptic responses were investigated by applying 50 μM dopamine in the presence of either the D_1 receptor antagonist SCH23390 (50 μM) or the D_2 receptor antagonist sulpiride (50 μM). Similar to previous reports that have used selective agonists in the medial (Pralong & Jones, 1993; Stenkamp et al., 1998) and lateral (Caruana et al., 2006) entorhinal cortex, application of either the D_1 agonist SKF38393 (25 to 50 μM; n = 9) or the D_2 agonist quinpirole (20 to 40 μM; n = 10) had no effect on EPSPs (data not shown) and we therefore used receptor blockers known to affect synaptic responses in the lateral entorhinal cortex (Caruana et al., 2006). Application of antagonists alone had no effect on EPSPs, and the D_1 antagonist SCH23390 did not block the suppression of AMPA-mediated EPSPs (Fig. 3.4A; $t_4 = 3.0$, $P < 0.05$; n = 5), suggesting that D_1 receptors do not mediate the suppression. However, application of dopamine in the presence of the D_2 antagonist sulpiride resulted in a non-significant suppression of synaptic responses, and the size of the suppression was significantly smaller than that observed with dopamine alone (79.8 ±7.2% versus 41.7 ±5.6% of baseline; $F_{1,9} = 18.10$, $P < 0.001$; Fig. 3.4B_1). Sulpiride also prevented the enhancement of paired-pulse facilitation induced by 50 μM dopamine (Fig. 3.4B_2). Although this indicates that the dopaminergic suppression of EPSPs is largely dependent upon activation of D_2-like receptors, the suppression of responses in the presence of sulpiride was close to statistical significance ($t_4 = 2.65$, $P = 0.06$), suggesting that a non-D_2 receptor-mediated mechanism mediates the residual suppression.

Dopaminergic Suppression of IPSPs. Biphasic IPSPs were recorded from fan cells held near action potential threshold (–51 to –48 mV) and exposed to either 1 mM kynurenic acid or a combination of 50 µM APV and 20 µM CNQX to block ionotropic glutamate transmission. Dopamine suppressed both the early $GABA_A$- and late $GABA_B$-mediated components of the IPSP. The early IPSP was reduced to 84.5 ±8.7% of baseline levels, and the late IPSP was reduced to 62.3 ±11.1% of baseline levels (Fig. 3.5B; early, $t_8 = 2.41$, $P < 0.05$, $n = 9$; late, $t_7 = 2.46$, $P < 0.05$, $n = 8$). The dopaminergic suppression of GABA synapses indicates that the reduction of EPSPs by dopamine is unlikely to be due to increased GABAergic inhibition of fan cells.

Modulation of Intrinsic Excitability. Bath application of dopamine also hyperpolarized resting membrane potential and reduced the input resistance of fan cells. Membrane potential was reduced from –56.1 ±2.0 to –59.7 ±1.4 mV (Fig. 3.6A; $t_8 = 4.73$, $P < 0.001$; $n = 9$), and peak input resistance was reduced from 90.3 ±7.6 to 68.9 ±3.1 MΩ by 50 µM dopamine (Fig. 3.6B; $t_7 = 4.27$, $P < 0.01$; $n = 8$). Similar changes in membrane potential and input resistance were observed for 10 µM dopamine (not shown) and have also been reported following application of high concentrations of dopamine in whole-cell recordings from medial entorhinal cortex stellate cells (Pralong & Jones, 1993). Changes were not due to the vehicle, because control cells and cells exposed to 1 µM dopamine did not show a drop in input resistance or hyperpolarization of membrane potential.

In layer V entorhinal cortex cells dopamine causes a reduction in excitability and a drop in input resistance through an increase in the

hyperpolarization-activated current I_h (Rosenkranz & Johnston, 2006), and changes in I_h were therefore assessed in layer II fan cells. However, dopamine did not significantly affect the amount of inward rectification, and the rectification ratio remained stable (Fig. 3.6D; 1.09 ±0.02 in ACSF and in 50 µM dopamine, t_7 =0.00, P = 1.00).

Dopamine suppressed the excitability of fan cells, and application of 10 and 50 µM dopamine reduced the number of action potentials evoked by brief 500 ms depolarizing current pulses (Fig. 3.7). The number of spikes was reduced from 4.1 ±0.1 to 2.8 ±0.5 spikes by 10 µM dopamine (Fig. 3.7B; t_{17} = 2.54, P < 0.05; n = 18). Fifty µM dopamine caused a similar reduction in the number of spikes (from 3.9 ±0.2 to 2.8 ±0.6) that was not statistically significant (t_8 = 1.82, P = 0.11; n = 9). The reduction in spiking could result in part from reduced input resistance, but it was not due to membrane hyperpolarization because cells were tested at the same membrane potential both before and after dopamine application.

The drop in input resistance induced by 50 µM dopamine was blocked by co-application of the D_1 receptor antagonist SCH23390 (and there was actually a very small but reliable *increase* in R_{in} in 4 of 5 cells; t_4 = 2.60, P = 0.06; Fig. 3.8A). The drop in input resistance was not affected by co-application of the D_2 receptor antagonist sulpiride (t_4 = 9.71, P < 0.001; n = 5; Fig. 3.8B). The reduction in input resistance induced by dopamine is therefore dependent on activation of D_1, but not D_2, receptors.

The conductances that mediate the reduced input resistance were investigated using blockers of Na^+ and K^+ channels. The Na^+ channel blocker

TTX was used to verify that reductions in input resistance were not due to an increase in action potential-dependent synaptic inputs to fan cells, or due to an altered Na^+ conductance. Blockade of Na^+ channels with TTX did not prevent the drop in input resistance induced by dopamine (Fig. 3.9A; peak, $t_4 = 6.02$, $P < 0.01$; steady-state, $t_4 = 8.21$, $P < 0.01$; $n = 5$). It has been suggested that the reduced input resistance induced by dopamine in medial entorhinal cortex stellate cells might be mediated by an increased K^+ conductance (Pralong & Jones, 1993), and we therefore assessed the effects of dopamine on input resistance in the presence of the K^+ channel blocker TEA (30 mM; $n = 5$). Co-application of TEA blocked the reduction in input resistance induced by dopamine (Fig. 3.9B), indicating that the D_1 receptor-dependent reduction in input resistance is due to an increased K^+ conductance. The increased K^+ conductance is likely to underlie the hyperpolarization of membrane potential induced by dopamine, and may also account for the reduced excitability of fan cells (Fig. 3.7). The reduced input resistance may also contribute to the dopamine-induced suppression of EPSPs; the D_2 receptor blocker sulpiride did not fully prevent the suppression of AMPA-mediated EPSPs (Fig. 3.4B_1), and the D_1 receptor-mediated reduction in input resistance could contribute to part of the EPSP suppression.

DISCUSSION

We show here that dopamine has powerful suppressive effects on glutamate-mediated synaptic transmission in layer II fan cells of the lateral entorhinal cortex. The suppression of EPSPs is mediated by a combined D_2

receptor-mediated reduction in neurotransmitter release and a D_1 receptor-mediated increase in a K^+ conductance that reduces cellular input resistance. Previously, we found that field EPSPs were enhanced by low concentrations of dopamine *in vitro*, and by blocking dopamine reuptake in awake animals (Caruana et al., 2006). This suggested that moderate increases in dopamine release might facilitate synaptic responses in the entorhinal cortex, and enhance transmission of sensory information to the rest of the hippocampal formation. Here, we have replicated the synaptic facilitation with a low 1 μM concentration of dopamine, and have also shown that high concentrations of dopamine induce a strong and reversible suppression of intracellular EPSPs. Similar suppression effects have been observed in the medial entorhinal cortex (Pralong & Jones, 1993; Stenkamp et al., 1998) and prefrontal cortex (Gao et al., 2001; Law-Tho et al., 1994; Urban et al., 2002; Zheng et al., 1999) using comparable doses of dopamine.

Suppression of Glutamate Release

The suppression of EPSPs by high concentrations of dopamine was found to be largely dependent on D_2 receptors since co-application of the D_2 receptor antagonist sulpiride blocked most of the reduction. Dopamine also enhanced paired-pulse facilitation which suggests that the suppression of EPSPs resulted from a reduction in presynaptic glutamate release (Manabe et al., 1993; Zucker & Regehr, 2002). The suppression of both AMPA- and NMDA-mediated components of the synaptic response is also consistent with reduced transmitter release. Although similar reductions in EPSPs have been shown in stellate cells

of the medial entorhinal cortex, the suppression was dependent on D_1, and not D_2, receptor activation (Pralong & Jones, 1993). However, Stenkamp et al. (1998) showed a reduction in synaptic responses in layer III of the medial entorhinal cortex through activation of both D_1 *and* D_2 receptors, and results of paired-pulse tests in their study suggested that the suppression was also mediated by reduced glutamate release.

Dopamine has been shown to suppress AMPA-mediated synaptic responses in the prefrontal cortex through a D_1 receptor-mediated suppression of transmitter release (Gao et al., 2001; Law-Tho et al., 1994; Seamans et al., 2001a). Strong activation of D_1 receptors can also suppress synaptic responses through a retrograde signaling cascade. Weak D_1 receptor activation can enhance NMDA responses, but stronger D_1 receptor activation can lead to more intense NMDA receptor activation and the release of adenosine that suppresses transmitter release by acting on presynaptic A_1 receptors that suppress voltage-gated Ca^{2+} channels (Craig, Temple, & White, 1994; Scholz & Miller, 1996; C. R. Yang & Chen, 2005). In the striatum, activation of presynaptic D_2 receptors suppresses N-type Ca^{2+} currents and inhibits acetylcholine release from striatal cholinergic interneurons (Yan, Song, & Surmeier, 1997). D_2 receptors have also been linked to a suppression of responses in the parabrachial nucleus (X. Chen, Kombian, Zidichouski, & Pittman, 1999), ventral tegmental area (Koga & Momiyama, 2000), and striatum (Hsu, Huang, Yang, & Gean, 1995; Levine, Li, Cepeda, Cromwell, & Altemus, 1996) via a D_2-mediated reduction in glutamate release. A similar D_2-mediated mechanism underlies the suppression of GABA release from striatal inhibitory cells onto cholinergic interneurons (Pisani, Bonsi,

Centonze, Calabresi, & Bernardi, 2000). Similar mechanisms may mediate the dopaminergic suppression of glutamate release in the entorhinal cortex.

The dopaminergic suppression of EPSPs observed here cannot be explained by increased transmission at GABA synapses because we found that dopamine *reduced* monosynaptic $GABA_A$ and $GABA_B$ IPSPs. The suppression is also unlikely to be due to increased activation of feedback inhibition (Finch, Tan, & Isokawa-Akesson, 1988) because dopamine reduced both glutamatergic transmission and the number of spikes in fan cells (Fig. 3.7). The suppression of monosynaptic IPSPs that we observed may have resulted from a D_2-mediated reduction in GABA release (Pisani et al., 2000; Seamans et al., 2001b) and reduced input resistance in fan cells could also have contributed. These possibilities are consistent with the parallel reductions observed in $GABA_A$ and $GABA_B$ IPSPs. Recordings of spontaneous and/or miniature IPSCs would be useful to determine the mechanisms of the reduced IPSPs.

Modulation of Intrinsic Excitability

In addition to the D_2-mediated suppression of transmitter release, high concentrations of dopamine also appear to suppress synaptic transmission through a D_1-receptor dependent mechanism. Sulpiride did not completely block the suppression of EPSPs (Fig. 3.4B_1) and a D_1 receptor-dependent activation of a TEA-sensitive K^+ conductance appears to mediate the residual suppression via a reduction in input resistance. Blockade of synaptic transmission and voltage-gated Na^+ channels with TTX did not prevent the drop in input resistance induced by dopamine indicating that it is not due to increased spontaneous synaptic drive

or to an increased Na^+ conductance. However, the broadly acting K^+ channel blocker TEA prevented the drop in input resistance, indicating that dopamine activates a K^+ conductance. The drop in input resistance was also prevented by blockade of D_1, but not D_2, receptors, indicating that dopamine activates K^+ channels via D_1 receptors. High concentrations of dopamine also hyperpolarize membrane potential and reduce input resistance in stellate cells of the medial entorhinal cortex, and it was also suggested that these changes might be mediated by an increased K^+ conductance (Pralong & Jones, 1993).

A large number of K^+ conductances are affected by TEA, and it is therefore not clear which type(s) may be responsible for the drop in input resistance observed here. Background leak channels are insensitive to TEA (Lesage, 2003), and are therefore not likely to contribute. Voltage-gated K^+ currents are blocked by TEA, but dopamine in the prefrontal cortex tends to enhance neuronal excitability by *suppressing* these currents (Dong & White, 2003; see also C. R. Yang & Seamans, 1996). Several reports in CA1 pyramidal cells have found that dopamine hyperpolarizes membrane potential, reduces input resistance, and increases afterhyperpolarizations through a D_1-receptor mediated increase in Ca^{2+}-activated K^+ currents (Benardo & Prince, 1982; Berretta et al., 1990; see also Hernandez-Lopez, Bargas, Reyes, & Galarraga, 1996), but others have found an increase in the excitability of CA1 neurons due to a *suppression* of Ca^{2+}-activated K^+ currents (Malenka & Nicoll, 1986; Pedarzani & Storm, 1995; see also Rosenkranz & Johnston, 2006). Here, there was no clear increase in afterhyperpolarizations, suggesting that Ca^{2+}-dependent K^+ currents do not mediate the change in input resistance. Activation of D_1

receptors can also have dose-dependent effects on activation of inward rectifying K^+ currents (IRKC). In the prefrontal cortex, D_1 receptor activation typically *inhibits* IRKC by direct effects of cAMP on IRK channels, but strong activation can enhance IRKC via phosphorylation of the channels by elevated levels of PKA (Dong, Cooper, Nasif, Hu, & White, 2004). This could explain why a significant reduction in input resistance was observed here only at the higher concentrations of dopamine. Clearly, however, further experiments will be required to determine the nature of the D_1 receptor-dependent K^+ conductance in fan cells.

We observed a decrease in fan cell firing in response to depolarizing current steps after dopamine, and the reduced spiking is likely due primarily to reduced input resistance. A surprising finding was that while the D_1 receptor antagonist SCH23390 prevented the dopamine-induced reduction in input resistance it did not completely eliminate the reduction in the number of spikes, suggesting that reduced input resistance cannot entirely account for the reduction in spiking, and that other mechanisms may also contribute. D_1 receptor activation can *increase* spiking in prefrontal neurons by enhancing the persistent Na^+ current (I_{NaP}) and suppressing a slowly-inactivating K^+ conductance (Gorelova & Yang, 2000; C. R. Yang & Seamans, 1996), but a suppression of spiking via a reduction in I_{NaP} has also been observed (Geijo-Barrientos & Pastore, 1995). In layer V entorhinal cortex neurons, dopamine reduces input resistance and leads to a reduction of spiking though an increase in I_h (Rosenkranz & Johnston, 2006). Here, there was no significant change in I_h in fan cells, and action potential threshold and afterhyperpolarizations were not affected, suggesting that the underlying currents were not modified.

Dopaminergic effects on I_{NaP} were not directly assessed in the present study, and the drop in input resistance could mask possible reductions in depolarizing responses to current injection related to I_{NaP}. However, in tests in tests in which SCH23390 prevented a change in input resistance we found no reduction in the response to +20 pA pulses. This rules out a D_1-mediated reduction in I_{NaP}, but it is still possible that dopamine may contribute to reduced spiking via D_2 receptor-mediated reduction in I_{NaP} (Geijo-Barrientos & Pastore, 1995).

Conclusions

We have shown here that dopamine has concentration-dependent, bidirectional effects on glutamate-mediated synaptic transmission in principal cells of layer II of the lateral entorhinal cortex. The lateral entorhinal cortex receives a major input from the piriform cortex (Burwell, 2000; Burwell & Amaral, 1998; Kerr et al., 2007), and dopaminergic innervation of the superficial layers is likely to have a strong modulatory effect on olfactory processing. In the prefrontal cortex, moderate activation of dopaminergic inputs promotes working memory function, but excessive dopamine activation leads to a decrement in performance (Arnsten, 1998; Seamans & Yang, 2004). In the entorhinal cortex, moderate increases in dopamine concentration may enhance the salience of olfactory representations carried to the lateral entorhinal cortex (Fig. 3.1C; see also Caruana et al., 2006), but large increases in dopamine associated with drug effects or acute stress (Arnsten, 1998) may dampen synaptic inputs to the superficial layers and suppress working memory function (McGaughy et al., 2005; Tahvildari et al., 2007; Young et al., 1997) or induction of lasting synaptic

plasticity (Caruana, Reed, Sliz, & Chapman, 2007). The dopaminergic suppression of synaptic transmission in layer II is also likely to inhibit the propagation of sensory information to the rest of the hippocampal formation such that only strong and synchronous inputs to the entorhinal region may be sufficient to activate entorhinal projection neurons.

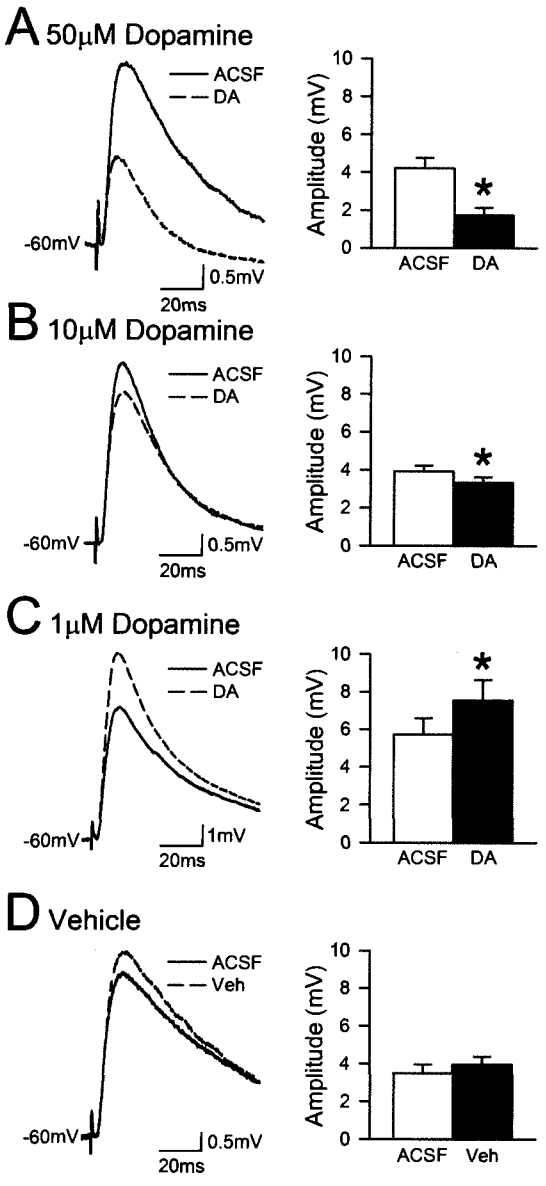

A 50μM Dopamine
ACSF
DA
-60mV
0.5mV
20ms
Amplitude (mV)
10
8
6
4
2
0
*
ACSF
DA
B 10μM Dopamine
ACSF
DA
-60mV
0.5mV
20ms
Amplitude (mV)
10
8
6
4
2
0
*
ACSF
DA
C 1μM Dopamine
ACSF
DA
-60mV
1mV
20ms
Amplitude (mV)
10
8
6
4
2
0
*
ACSF
DA
D Vehicle
ACSF
Veh
-60mV
0.5mV
20ms
Amplitude (mV)
10
8
6
4
2
0
ACSF
Veh

Figure 3.1. Dopamine has dose-dependent and bidirectional effects on the amplitude of mixed EPSPs in layer II fan cells. **A.** Fifty μM dopamine significantly reduces the amplitude of synaptic responses. Traces show averaged EPSPs before (ACSF) and after 5-min bath application of dopamine (DA) in a representative cell. Group data indicate the mean amplitude of EPSPs before and after dopamine (*, $P < 0.001$). Bars indicate ±1 SEM in this and subsequent figures, and * indicates $P < 0.05$ unless otherwise indicated. **B.** A lower concentration of 10 μM dopamine causes a smaller suppression of synaptic responses. **C.** The low 1 μM concentration of dopamine enhances the amplitude of synaptic responses (*, $P < 0.01$). **D.** Bath application of vehicle (50 μM sodium metabisulfite; Veh) does not significantly affect synaptic transmission.

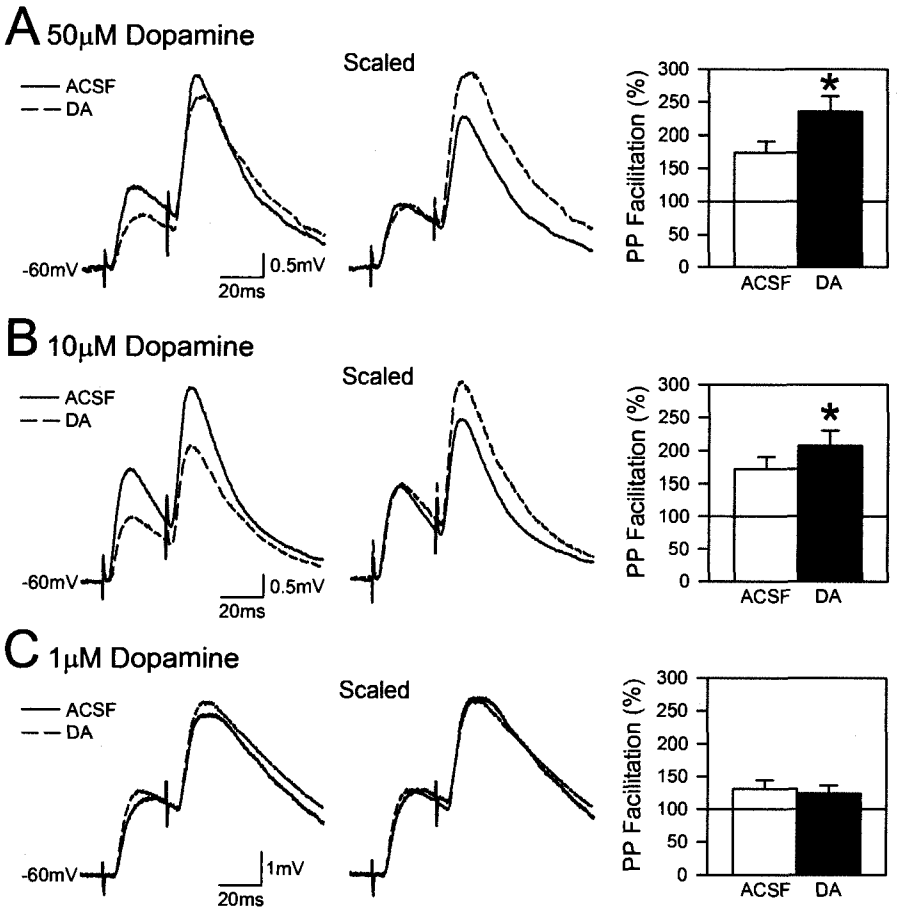

A 50μM Dopamine
ACSF
DA
-60mV
0.5mV
20ms
Scaled
PP Facilitation (%)
300
250
200
150
100
50
0
ACSF
DA
*
B 10μM Dopamine
ACSF
DA
-60mV
0.5mV
20ms
Scaled
PP Facilitation (%)
300
250
200
150
100
50
0
ACSF
DA
*
C 1μM Dopamine
ACSF
DA
-60mV
1mV
20ms
Scaled
PP Facilitation (%)
300
250
200
150
100
50
0
ACSF
DA

Figure 3.2. High concentrations of dopamine increase paired-pulse facilitation. **A.** Pairs of stimulation pulses with a 30 ms interpulse interval were delivered before and after 5-min bath application of 50 μM dopamine. Averaged traces at left show responses recorded before (ACSF) and after (DA) dopamine from a representative cell. Note the suppression of the response to the first pulse and the large facilitation of the second response following dopamine (dotted line). Traces at right have been scaled to the amplitude of the first response in normal ACSF to aid comparison. Group data are shown on the right. **B.** Paired-pulse facilitation was also enhanced by 10 μM dopamine. **C.** In contrast, the low concentration of 1 μM dopamine that enhances the amplitude of synaptic responses does not affect paired-pulse ratio.

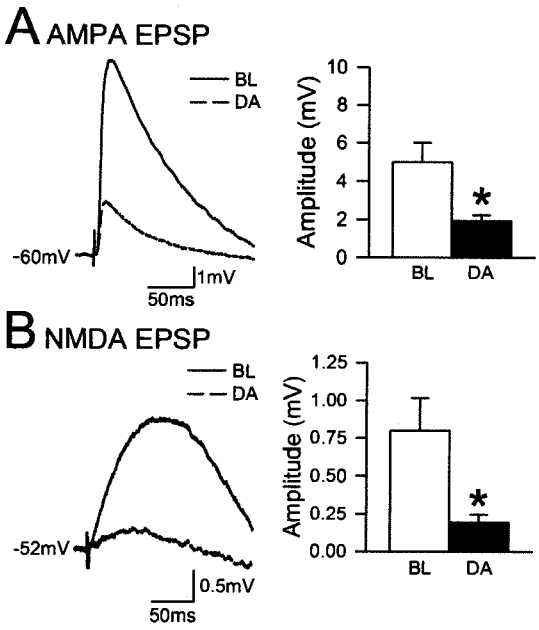

A AMPA EPSP
BL
DA
-60mV
1mV
50ms
Amplitude (mV)
10
8
6
4
2
0
*
BL
DA
B NMDA EPSP
BL
DA
-52mV
0.5mV
50ms
Amplitude (mV)
1.25
1.00
0.75
0.50
0.25
0.00
*
BL
DA

Figure 3.3. Dopamine suppresses the amplitude of both AMPA- and NMDA receptor-mediated components of EPSPs. **A.** AMPA-mediated EPSPs recorded in the presence of APV and bicuculline were suppressed by 50 μM dopamine. Averaged traces show EPSPs recorded before (BL) and after (DA) dopamine application, and group data are shown at right. **B.** Isolated NMDA receptor-mediated EPSPs recorded in the presence of CNQX and bicuculline are also suppressed by a high concentration of dopamine. Group data show a consistent suppression of the small isolated NMDA response.

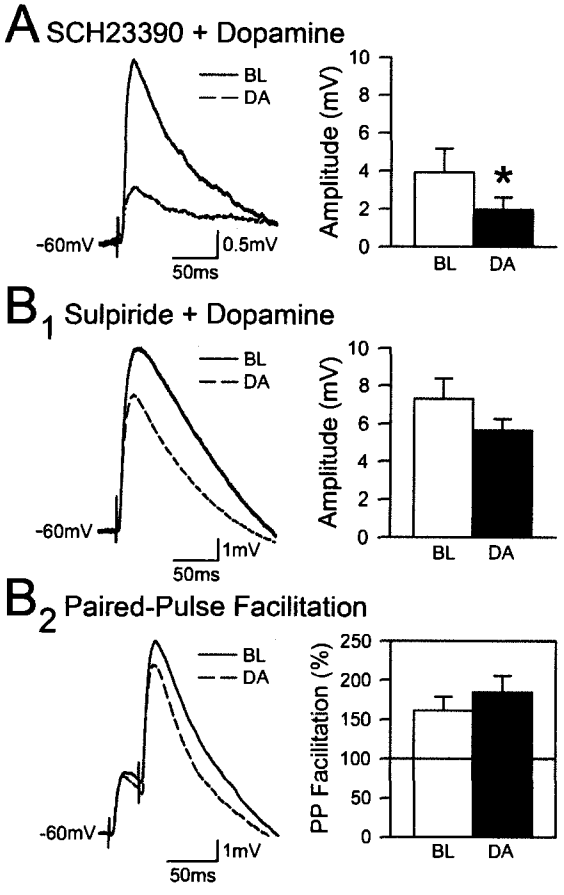

A SCH23390 + Dopamine
BL
DA
-60mV
0.5mV
50ms
Amplitude (mV)
10
8
6
4
2
0
*
BL
DA
B1 Sulpiride + Dopamine
BL
DA
-60mV
1mV
50ms
Amplitude (mV)
10
8
6
4
2
0
BL
DA
B2 Paired-Pulse Facilitation
BL
DA
-60mV
1mV
50ms
PP Facilitation (%)
250
200
150
100
50
0
BL
DA

Figure 3.4. Dopamine suppresses isolated AMPA-mediated EPSPs via a D_2 receptor-dependent mechanism. **A.** Co-application of the D_1 receptor antagonist SCH23390 (50 μM) did not prevent the dopamine-induced reduction in EPSP amplitude. **B.** However, co-application of the D_2 receptor antagonist sulpiride (50 μM) significantly attenuated the dopaminergic suppression of EPSPs. Sulpiride also prevented the enhancement of paired-pulse facilitation induced by dopamine (B_2).

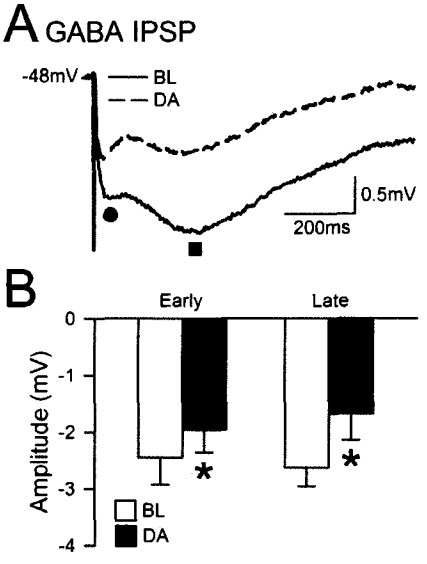

A GABA IPSP
-48mV
BL
DA
0.5mV
200ms
B
Early
Late
0
-1
-2
-3
-4
Amplitude (mV)
BL
DA
*
*

Figure 3.5. Dopamine suppresses both the fast and slow components of the mixed monosynaptic IPSP in fan cells. **A.** GABA-mediated IPSPs were isolated pharmacologically with ionotropic glutamate receptor blockers and recorded at membrane potentials just below action potential threshold. Both the early (circle) and late (square) components of the biphasic IPSP were suppressed by 50 μM dopamine (DA). **B.** Group data reflect a significant suppression of both the early and late IPSPs.

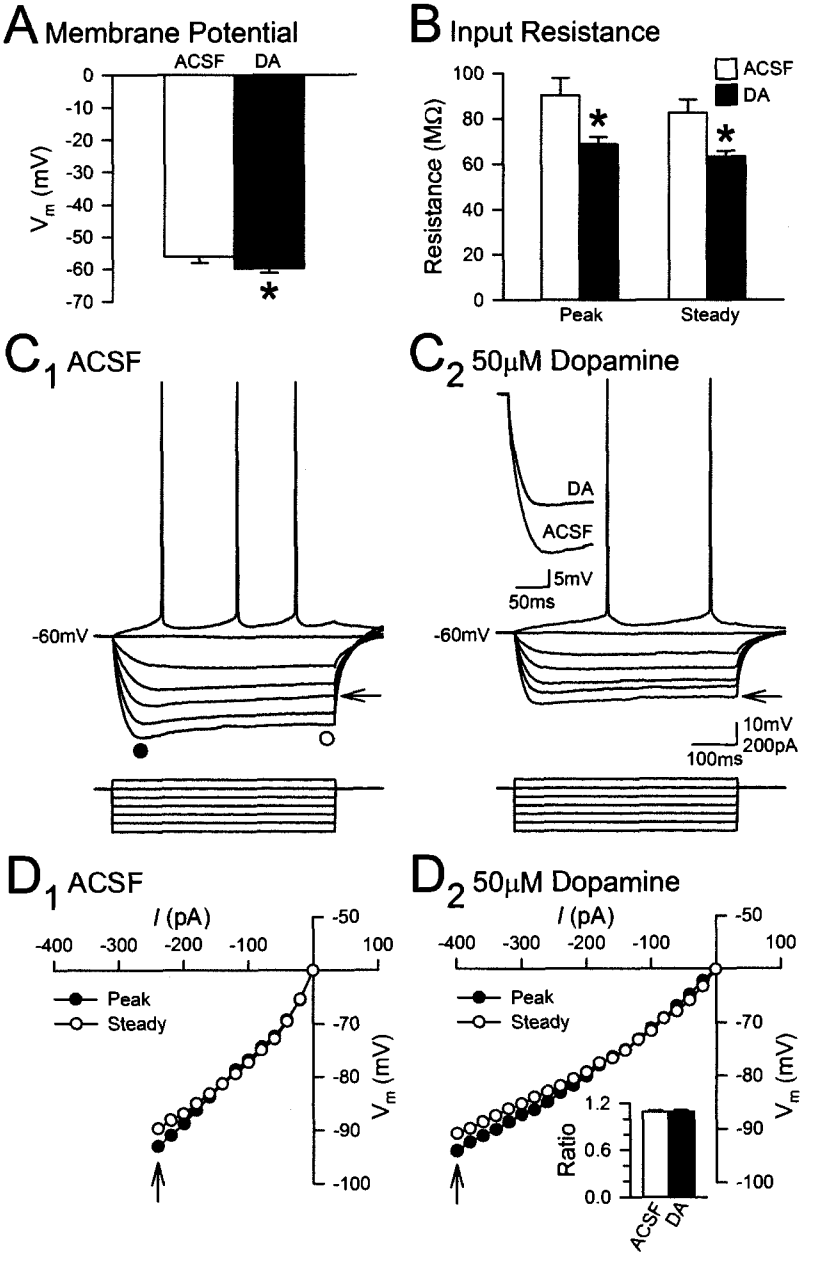

A Membrane Potential
ACSF
DA
0
-10
-20
-30
-40
-50
-60
-70
V_m (mV)
*
B Input Resistance
ACSF
DA
100
80
60
40
20
0
Resistance (MΩ)
*
*
Peak
Steady
C_1 ACSF
-60mV
C_2 50μM Dopamine
DA
ACSF
5mV
50ms
-60mV
10mV
200pA
100ms
D_1 ACSF
I (pA)
-400
-300
-200
-100
-50
100
Peak
Steady
-70
-80
-90
-100
V_m (mV)
D_2 50μM Dopamine
I (pA)
-400
-300
-200
-100
-50
100
Peak
Steady
-70
-80
-90
-100
V_m (mV)
1.2
0.6
0.0
Ratio
ACSF
DA

Figure 3.6. Dopamine hyperpolarizes membrane potential and reduces the input resistance of layer II fan cells. **A**. Membrane potential was shifted to more hyperpolarized potentials by dopamine (*, $P < 0.001$). **B**. Dopamine also reduced both peak and steady-state input resistance (*, $P < 0.01$). **C**. Voltage responses to applied current steps before (C_1) and after (C_2) bath application of 50 μM dopamine in a representative cell. Action potentials are truncated. Circles in C_1 indicate the latencies at which peak and steady-state input resistance were measured. Inset traces in C_2 compare the initial voltage deflection to a –200 pA current step before and after application of dopamine. Arrows indicate voltage responses before and after dopamine that were similar in amplitude and which allow comparison of the magnitude of the inward rectification. **D**. Current-voltage plots show peak and steady-state responses to current steps of increasing size. Arrows indicate points at which a comparable degree of inward rectification was observed during hyperpolarization to similar voltages before and after dopamine application.

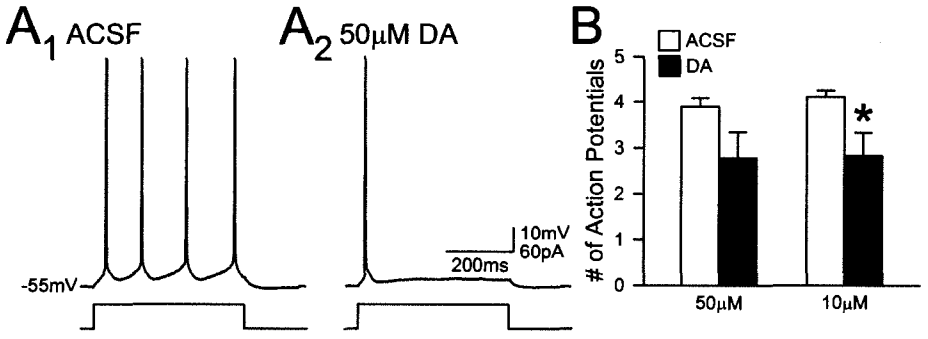

A1 ACSF
A2 50μM DA
-55mV
10mV
60pA
200ms
B
ACSF
DA
# of Action Potentials
0
1
2
3
4
5
*
50μM
10μM

Figure 3.7. The number of action potentials elicited by positive current steps is reduced by dopamine. **A**. Traces show action potentials generated in response to 500 ms duration, 60 pA current steps before and after application of 50 μM dopamine. Action potentials are truncated. **B**. Group data show a reduction in firing for both the 10 and 50 μM conditions but only the reduction in the 10 μM condition was significant.

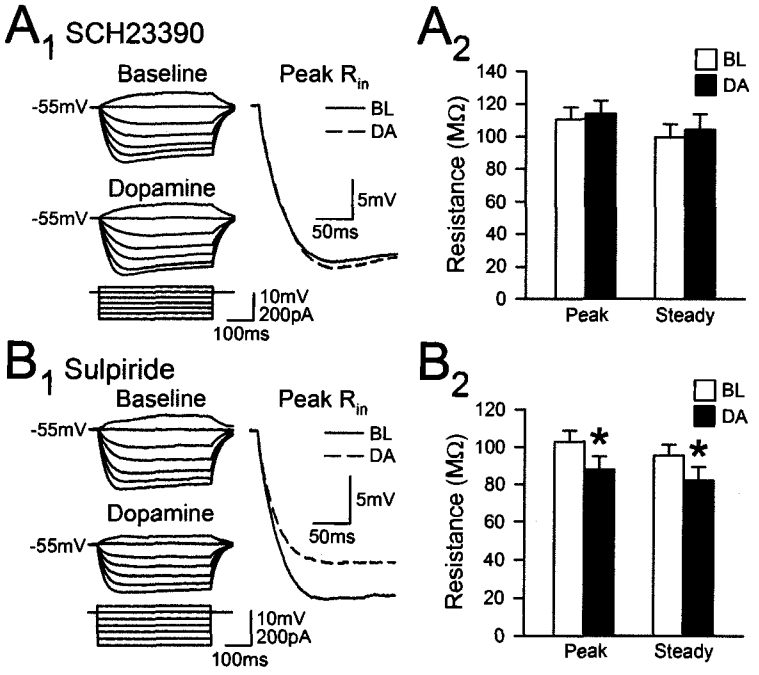

A1 SCH23390
Baseline
Peak Rin
-55mV
BL
DA
Dopamine
-55mV
5mV
50ms
10mV
200pA
100ms
A2
BL
DA
Resistance (MΩ)
140
120
100
80
60
40
20
0
Peak
Steady
B1 Sulpiride
Baseline
Peak Rin
-55mV
BL
DA
Dopamine
-55mV
5mV
50ms
10mV
200pA
100ms
B2
BL
DA
Resistance (MΩ)
120
100
80
60
40
20
0
*
*
Peak
Steady

Figure 3.8. Blockade of D_1, but not D_2, receptors prevents the dopamine-induced reduction in input resistance. **A.** Bath-application of the D_1 receptor antagonist SCH23390 (50 µM) prevented the reduction in input resistance induced by 50 µM dopamine. Traces at left show voltage responses to a series of current steps during baseline recordings in SCH23390 and during subsequent dopamine application. Traces at right compare the initial voltage responses to –200 pA steps before and after dopamine application. Note that input resistance is unchanged when D_1 receptors are blocked. **B.** The D_2 receptor blocker sulpiride (50 µM) does not prevent changes in input resistance induced by dopamine (*, $P < 0.001$).

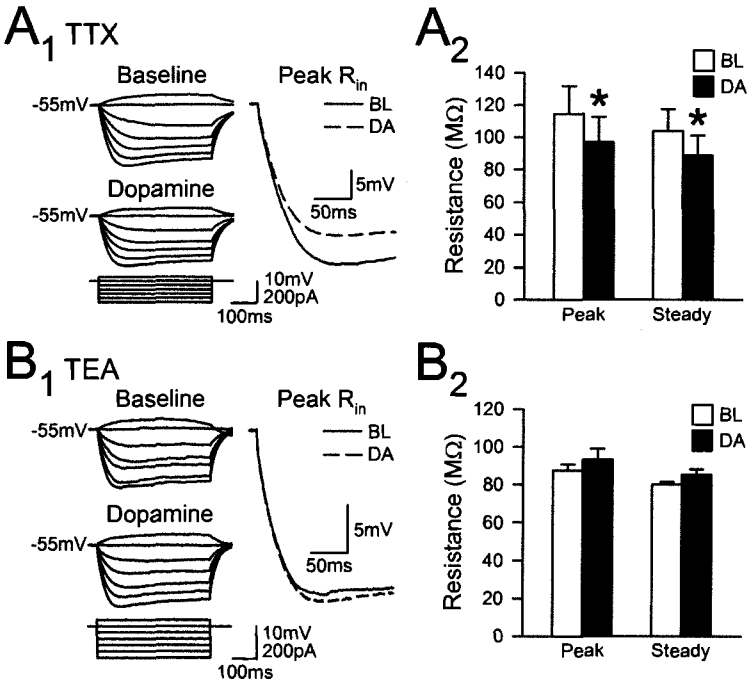

A1 TTX
Baseline
Peak Rin
-55mV
BL
DA
Dopamine
-55mV
5mV
50ms
10mV
200pA
100ms
A2
BL
DA
Resistance (MΩ)
140
120
100
80
60
40
20
0
Peak
Steady
B1 TEA
Baseline
Peak Rin
-55mV
BL
DA
Dopamine
-55mV
5mV
50ms
10mV
200pA
100ms
B2
BL
DA
Resistance (MΩ)
120
100
80
60
40
20
0
Peak
Steady

Figure 3.9. Blocking potassium channels prevents the dopamine-induced reduction in input resistance. **A.** Blockade of Na^+ channels with 0.5 μM TTX does not prevent the reduction of peak or steady state input resistance induced by 50 μM dopamine (*, $P < 0.01$). Conventions are as in Figure 3.8. **B.** In contrast, co-application of the K^+ channel blocker TEA (30 mM) prevented the dopamine-induced reduction in input resistance.

CHAPTER 4

INHIBITING DOPAMINE REUPTAKE BLOCKS THE INDUCTION OF LONG-TERM POTENTIATION AND DEPRESSION IN THE LATERAL ENTORHINAL CORTEX OF AWAKE RATS

Douglas A. Caruana, Sean J. Reed, Diane J. Sliz, and C. Andrew Chapman

Although Chapter 3 focused primarily on the mechanisms underlying the dopamine-induced suppression of excitatory synaptic responses in the lateral entorhinal cortex by high concentrations of dopamine, the results of experiments conducted in Chapter 2 demonstrate that systemic administration of the selective dopamine reuptake inhibitor GBR12909 *enhances* the transmission of olfactory inputs to the superficial layers of the lateral entorhinal cortex in awake rats. This effect was also shown in field potential recordings of synaptic responses (Chapter 2) and in intracellular recordings of EPSPs (Chapter 3) following bath-application of low concentrations of dopamine. The facilitation of synaptic responses induced by dopamine may serve to enhance the propagation of sensory information to the hippocampal formation or facilitate the induction of persistent forms of synaptic plasticity in sensory inputs to the entorhinal cortex.

Experiments conducted in Chapter 4 examine the effects of dopamine reuptake inhibition on the induction of long-term potentiation (LTP) and long-term depression (LTD) in piriform cortex inputs to layer II of the lateral entorhinal cortex in awake rats. Both LTP and LTD are widely-studied cellular models of memory storage in the brain, but the effects of dopamine on the induction of LTP and LTD in the entorhinal cortex are not known. Different groups of rats received systemic administration of saline or GBR12909 prior to the delivery of low or high frequency stimulation to induce LTP or LTD in piriform cortex inputs to the lateral entorhinal cortex. Results show that pretreatment with GBR12909 blocks the induction of both LTP and LTD in olfactory inputs to the lateral entorhinal cortex.

ABSTRACT

Synaptic plasticity in olfactory inputs to the lateral entorhinal cortex may result in lasting changes in the processing of olfactory stimuli. Changes in dopaminergic tone can have strong effects on basal evoked synaptic responses in the superficial layers of the entorhinal cortex, and the current study investigated whether dopamine may modulate the induction of long-term potentiation (LTP) and depression (LTD) in piriform cortex inputs to layer II of the lateral entorhinal cortex in awake rats. Groups of animals were pretreated with either saline or the selective dopamine reuptake inhibitor GBR12909 prior to low or high frequency stimulation to induce LTD or LTP. In saline-treated groups, synaptic responses were potentiated to 122.4 ±6.4% of baseline levels following LTP induction, and were reduced to 84.5 ±4.9% following induction of LTD. Changes in synaptic responses were maintained for up to 60 minutes and returned to baseline levels within 24 hours. In contrast, induction of both LTP and LTD was blocked in rats pretreated with GBR12909. Dopaminergic suppression of synaptic plasticity in the entorhinal cortex may serve to restrain activity-dependent plasticity during reward-relevant behavioral states or during processing of novel stimuli.

The entorhinal cortex provides an interface between cortical association areas and the hippocampus and is involved in the formation of olfactory memory. Monosynaptic projections from the olfactory bulb and piriform cortex carry olfactory information directly to the entorhinal cortex (Burwell, 2000), and lesions of the parahippocampal region that include the entorhinal cortex can produce deficits on olfactory tasks involving odor discrimination (Petrulis et al., 2000), delayed non-matching-to-sample performance (Otto & Eichenbaum, 1992; Stäubli et al., 1984), and social recognition (Bannerman et al., 2002). The entorhinal region, therefore, may make important contributions to olfactory memory, and persistent changes in synaptic strength in the entorhinal cortex may provide a mechanism for the modification of processing of olfactory information (Bouras & Chapman, 2003; Chapman & Racine, 1997b; de Curtis & Llinas, 1993; Kourrich & Chapman, 2003).

Midbrain dopamine neurons of the ventral tegmental area and substantia nigra project to the superficial layers of the entorhinal cortex (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987; Oades & Halliday, 1987) and are well-poised to modulate responses to olfactory inputs that also terminate in these layers. Indeed, our recent work has demonstrated that increasing dopaminergic tone in the entorhinal cortex can facilitate synaptic responses to olfactory inputs in the superficial layers *in vitro* at low doses via D_1 receptors, and can suppress responses at higher concentrations mainly via D_2 receptors (Caruana et al., 2006). However, the effect of dopamine on lasting forms of synaptic plasticity in olfactory afferents to the entorhinal cortex is not known. Dopamine can have strong modulatory effects on both long-term potentiation (LTP) and depression

(LTD) in cortical regions. In the hippocampus, dopamine facilitates the induction of LTD (Z. Chen et al., 1995) and is also required for the long-term maintenance of LTP (Swanson-Park et al., 1999). Similarly, dopamine facilitates both LTD (Otani et al., 1998) and LTP (Jay, Burette, & Laroche, 1996) in the prefrontal cortex. In most reports the enhanced plasticity in these areas has been linked to activation of D_1-like receptors (Jay, 2003). Although dopamine typically facilitates both LTP and LTD, it can also inhibit plasticity; D_1 receptor activation can prevent LTP of the population spike in the dentate gyrus (Yanagihashi & Ishikawa, 1992) and block maintenance of LTD in the CA1 region (Mockett, Guevremont, Williams, & Abraham, 2007).

To determine the effects of dopamine on the induction and maintenance of synaptic plasticity in the lateral entorhinal cortex, rats were pretreated with either saline or the selective dopamine reuptake inhibitor GBR12909 and field excitatory postsynaptic potentials (fEPSPs) evoked by stimulation of the piriform cortex were recorded before and after low or high frequency stimulation to induce LTD or LTP. We have shown previously that systemic administration of GBR12909 enhances extracellular levels of dopamine in the lateral entorhinal cortex *in vivo* (Caruana et al., 2006) and the chronic field potential recording techniques used here provide a way to assess the effects of dopamine on synaptic plasticity in the awake rat.

MATERIALS AND METHODS

Experiments adhered to the guidelines of the Canadian Council on Animal Care, and surgical procedures were conducted according to methods described

previously (Caruana et al., 2006). Briefly, male Long-Evans rats (300 to 350 g; n =34) were anesthetized with sodium pentobarbital (65 mg/kg, i.p.), and placed in a stereotaxic frame. A bipolar stimulating electrode was lowered into the right piriform cortex (P, 3.6 mm; L, 6.5 mm; V, 9.0 mm relative to bregma), and a bipolar recording electrode was lowered into the superficial layers of the lateral entorhinal cortex (P, 6.5 mm; L, 6.5 mm; V, 7.5 to 8.5 mm). Vertical placements were adjusted to optimize evoked responses. A stainless-steel screw in the contralateral frontal bone served as a reference electrode, and a screw in the occipital bone served as ground. Electrode leads were mounted in a connector and the assembly was embedded in dental cement.

Biphasic constant current square-wave pulses (0.1 ms) were delivered via a stimulus isolator (A-M Systems, Model 2200) using a computer DAC channel or pulse generator (AMPI, Master 8 or A-M Systems, Model 2100). Evoked responses were filtered (0.1 Hz to 5 kHz passband), amplified (A-M Systems, Model 1700), and digitized at 20 kHz for storage on computer hard disk (Datawave Tech.).

Animals were placed in a 40 x 40 x 60 cm Plexiglas chamber inside a Faraday cage, and recordings were obtained after animals had habituated. Stability of responses was assessed using input/output tests every 2 days over a 5-day baseline period. During each test, 10 responses to stimulation of the piriform cortex were recorded and averaged at each of 6 intensities (0 to 1000 μA) using a 10 sec inter-trial interval.

Following the final baseline input/output test, animals were injected with either the selective dopamine reuptake inhibitor GBR12909 (10 mg/kg, i.p.;

Sigma) or physiological saline (0.9%, 1 ml/kg, i.p.) followed by another input/output test 20 min later. GBR12909 was prepared fresh by dilution in distilled water. Stability of responses was monitored over a 20 min baseline period prior to high frequency stimulation to induce LTP. During the baseline period, single stimulation pulses were delivered every 30 sec at an intensity set to evoke responses ≈50% of maximal. To induce LTP, ten high-frequency stimulation trains (16 pulses at 400 Hz) were delivered every 2 min (Chapman & Racine, 1997b). Post-tetanic effects were assessed during the 2 min inter-train intervals by delivering single pulses every 10 sec. Responses following LTP induction were monitored every 30 sec for a 60-min follow-up period, and input/output tests were administered 1 hour, and 1, 3, and 5 days post-tetanization.

Procedures to induce LTD were similar. Animals were pretreated with saline or GBR12909, and synaptic responses were monitored during the baseline period by delivering single stimulation pulses at an intensity that evoked responses ≈75% of maximal. Low frequency stimulation to induce LTD consisted of 900 pairs of stimulation pulses (30 ms inter-pulse interval) delivered at 1 Hz over a 15 min period (Bouras & Chapman, 2003).

Electrode placements were verified by light microscopy (Caruana et al., 2006) and showed stimulating electrodes in the piriform cortex, and recording electrodes in superficial layers (I to III) of the lateral entorhinal cortex (not shown). Peak amplitudes of evoked fEPSPs were measured relative to the prestimulus baseline (Bouras & Chapman, 2003; Chapman & Racine, 1997b) and responses evoked during each input/output test were normalized to

responses obtained at the highest stimulation intensity during the last baseline test. Field EPSPs recorded during LTP and LTD induction were normalized to the mean of responses obtained during the baseline period. Repeated measures ANOVAs compared averaged responses during the baseline period to responses evoked during the first and last 10 min of the 60-min follow-up period. Repeated measures ANOVAs also compared pre- and post-induction input/output tests.

RESULTS

Pretreatment with GBR12909 blocked the induction of LTP in piriform cortex inputs to the lateral entorhinal cortex (Fig. 4.1). Baseline fEPSPs had onset and peak latencies of 5.0 ±0.4 and 12.6 ±0.4 ms and a mean peak amplitude of 1.03 ±0.14 mV (e.g. Fig. 4.1A_1). High frequency stimulation potentiated synaptic responses in animals pretreated with saline ($F_{2,16} = 7.75$, $P < 0.01$; $n = 9$) but did not significantly affect responses in animals pretreated with GBR12909 ($n = 8$). The amplitudes of synaptic responses in saline-treated rats increased to 122.4 ±6.4% of baseline levels during the first 10 min following tetanization (Tukey, $P < 0.05$) and were maintained at 125.1 ±7.0% of baseline during the last 10 min of the 60-min follow-up period ($P < 0.01$). In contrast, responses in GBR12909-treated rats were stable and remained at 105.0 ±4.1% and 97.8 ±3.0% of baseline levels during the first and last 10 min of the follow-up period. Synaptic responses remained potentiated in saline-treated rats during the first follow-up input/output test ($F_{5,40} = 4.08$, $P < 0.01$; Fig. 4.1C_1) but responses decayed to baseline levels within 24 hours (not shown).

To determine whether pretreatment with GBR12909 influenced post-tetanic potentiation in the 2-min periods after each train, amplitudes of the first responses evoked after each of the 10 trains were compared between saline- and GBR12909-treated rats (Fig. 4.1B; black bar). Significant post-tetanic potentiation was evidenced in both groups by a decay in the amplitude of responses during the 2-min inter-train intervals ($F_{11,165} = 5.91$, $P < 0.001$; not shown) but there was no significant difference between groups in the responses evoked immediately following each train. The development of LTP in saline-treated rats, however, resulted in larger overall averaged responses during the 2-min inter-train intervals ($F_{1,15} = 5.45$, $P < 0.05$; not shown).

Pretreatment with GBR12909 also blocked induction of LTD (Fig. 4.2). Low frequency paired-pulse stimulation depressed synaptic responses in saline-treated rats ($F_{2,18} = 5.87$, $P < 0.05$; n =10) but had no significant effect on fEPSPs in animals pretreated with GBR12909 (n =7). In control animals, responses were significantly reduced to 84.5 ±4.9% of baseline levels during the first 10 min (Tukey, $P < 0.05$) and remained depressed at 82.8 ±6.8% of baseline after 60 min ($P < 0.05$). In contrast, responses in GBR12909-treated animals remained stable at 94.7 ±5.9% and 101.2 ±5.0% of baseline after 10 and 60 min, respectively. Synaptic responses remained depressed during the first follow-up input/output test in saline-treated rats ($F_{5,45} = 2.30$, $P < 0.05$; Fig. 4.2C_1) but returned to baseline levels within 24 hours (not shown). Responses to conditioning pulses tended to be larger in control animals during LTD induction, but this difference was not significant (Fig. 4.2B, black bar), and GBR12909 also

did not significantly affect the amount of paired-pulse facilitation during LTD induction.

DISCUSSION

We have found here that dopamine has a suppressive effect on the induction of both long-term potentiation and depression in olfactory inputs to the lateral entorhinal cortex of awake rats. Animals pretreated with saline showed levels of LTP and LTD that were comparable to previous reports that used similar stimulation protocols (Bouras & Chapman, 2003; Chapman & Racine, 1997b; Kourrich & Chapman, 2003). However, increasing dopamine levels in the entorhinal cortex with the selective dopamine reuptake inhibitor GBR12909 suppressed the induction of both LTP and LTD. In contrast to reports that have shown a facilitatory effect of dopamine on LTP and LTD in the hippocampus (Z. Chen et al., 1995; Swanson-Park et al., 1999) and prefrontal cortex (Jay et al., 1996; Otani et al., 1998), the suppression observed here suggests that synaptic plasticity is normally dampened in the entorhinal cortex during behaviors associated with increased activity in dopaminergic inputs. We have shown previously that dopamine has concentration-dependent biphasic effects on basal synaptic transmission in the entorhinal cortex; although high concentrations suppressed synaptic transmission, responses were facilitated by a lower concentration of dopamine (Caruana et al., 2006). This, together with our current findings, suggests that moderate elevations in extracellular dopamine may promote transmission of olfactory patterns into the hippocampus, while simultaneously limiting activity-dependent synaptic modifications in the entorhinal

cortex. Although increased dopamine might be expected to enhance learning-related plasticity, a dopaminergic suppression of plasticity might be useful during periods of increased network excitability to prevent excessive changes in synaptic strength, and to maintain stable processing of physiologically relevant olfactory signals. Of course, the systemic injections used here may have elevated dopamine concentrations beyond physiologically relevant levels, and further work with moderate, temporally controlled elevations in cortical dopamine both *in vivo* (e.g., Jay et al., 1996) and *in vitro* (Caruana & Chapman, 2006) is necessary.

Systemic administration of GBR12909 could have enhanced dopamine availability in terminal regions throughout the brain, but the suppression of plasticity observed here was likely due to effects of GBR12909 within the entorhinal cortex. Olfactory inputs from the piriform cortex terminate in the superficial layers of the lateral entorhinal cortex (Burwell, 2000) where fibers from midbrain dopamine neurons also terminate (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987; Oades & Halliday, 1987). Further, we previously monitored the effects of GBR12909 at the dose used here with *in vivo* microdialysis, and found that extracellular levels of dopamine in the entorhinal cortex were increased to 306% of basal levels (Caruana et al., 2006). However, GBR12909 injections could also have resulted in increased acetylcholine and/or serotonin in the entorhinal cortex. GBR12909 increases locomotor activity in rats (Caruana et al., 2006; Nakachi et al., 1995) that is associated with cholinergic-dependent theta activity in the entorhinal cortex (Mitchell & Ranck, 1980) and, although cholinergic activation can promote synaptic plasticity in some areas, we have

recently found that muscarinic receptor activation suppresses glutamatergic transmission in the entorhinal cortex (Hamam et al., 2006). This suppression could have contributed to the block of LTP and LTD shown here. Similarly, systemic administration of GBR12909 can increase firing of raphé neurons (Martin-Ruiz et al., 2001), and serotonin inhibits synaptic transmission in the lateral entorhinal cortex *in vitro* (Grunschlag et al., 1997). Thus, although the block of synaptic plasticity observed here was likely due primarily to the effects of increased dopamine levels on local entorhinal circuitry (Caruana et al., 2006), effects of systemic injections are always difficult to interpret, and the current results will have to be extended using *in vitro* recordings.

The induction of both LTP and LTD in the entorhinal cortex is dependent on NMDA receptors (Alonso et al., 1990; Deng & Lei, 2006; Kourrich & Chapman, 2003), and it is likely that GBR12909 may have interfered with plasticity by reducing postsynaptic depolarization required for NMDA receptor activation. We showed previously that low concentrations of dopamine facilitate synaptic responses in the lateral entorhinal cortex (Caruana et al., 2006), and this suggested to us that dopamine might enhance the induction of LTP. However, although 10μM dopamine facilitates responses via a D_1 receptor-mediated mechanism, higher concentrations of 50 and 100 μM suppress AMPA and NMDA responses via a D_2-mediated reduction in glutamate release (Caruana & Chapman, 2006; Caruana et al., 2006). A suppression of transmitter release could help block plasticity by reducing postsynaptic depolarization during stimulation trains. In the present study, pretreatment with GBR12909 did not significantly enhance basal responses, and this could be due to a D_2-mediated

suppression of transmitter release (Caruana & Chapman, 2006; Caruana et al., 2006; Pralong & Jones, 1993; Stenkamp et al., 1998). Strong activation of D_1 receptors could also have reduced levels of postsynaptic depolarization during trains (Caruana et al., 2006); while D_1 receptors mediate the facilitation of responses at low-concentrations of dopamine, they also contribute to the suppression of EPSPs observed at higher concentrations. This is similar to the inverted U-shaped relationship described for prefrontal cortex responses (Arnsten, 1998; Goldman-Rakic et al., 2000). We cannot know what the effective concentration of dopamine was during LTP and LTD induction in the present groups of animals, but both D_1 and D_2 receptor activation may have contributed to the block of plasticity observed here. In the prefrontal cortex and hippocampus, dopamine typically enhances plasticity through an intracellular signal cascade involving a D_1 receptor-mediated increase in cAMP via activation of adenylate cyclase and resultant activation of PKA (Jay, 2003). However, D_1 receptor activation can also inhibit NMDA-mediated synaptic currents in cultured hippocampal neurons through a direct coupling of D_1 receptors to NMDA receptors (Castro, de Mello, de Mello, & Aracava, 1999), and selective D4 receptor activation in prefrontal cortex slices and cultures can also suppress NMDA receptor currents (Wang, Zhong, Gu, & Yan, 2003). D_4 receptors are also present in the entorhinal cortex (Defagot et al., 1997; Primus et al., 1997; Tarazi, Kula, & Baldessarini, 1997) and a D_4-mediated suppression of NMDA receptor currents may have blocked the induction of LTP and LTD in the current study.

Relative to control animals, there was a non-significant reduction in responses evoked following each high-frequency train in GBR12909-treated rats

(Fig. 4.1B), as well as a non-significant reduction in responses to conditioning pulses during low frequency paired-pulse stimulation to induce LTD (Fig. 4.2B). In layer V neurons of the lateral entorhinal cortex dopamine increases the I_h current, and this reduces postsynaptic excitability during repetitive synaptic stimulation by reducing temporal summation of EPSPs (Rosenkranz & Johnston, 2006). Layer II neurons also show inward rectification that reflects I_h (Caruana & Chapman, 2006; Sewards & Sewards, 2003; Tahvildari & Alonso, 2005) but we have found that dopamine significantly reduces I_h in layer II neurons rather than enhancing it (Caruana & Chapman, 2006). It is not yet clear, then, if these effects on temporal summation may be expressed in the superficial layers.

We have used systemic injections here, but the precise timing of dopamine application relative to LTP or LTD induction has been a critical factor in other studies. Plasticity is generally enhanced when transient D_1 receptor activation occurs before or during stimulation (Z. Chen et al., 1995; Jay et al., 1996; Otani et al., 1998; Swanson-Park et al., 1999) but the maintenance of LTD is blocked when D_1 receptors are activated shortly after the trains (Mockett et al., 2007). Multiple intracellular mechanisms are likely to have been activated by the injections used here, and it is unknown if similar time-dependent effects control the modulatory actions of dopamine in the entorhinal cortex.

Activation of midbrain dopamine neurons during appetitive behaviors is likely to have complex effects on the processing and encoding of olfactory representations by the entorhinal cortex. We found previously that dopamine has bidirectional effects on synaptic transmission via D_1 and D_2 receptors (Caruana et al., 2006), and we have shown here that enhancing dopaminergic tone with

GBR12909 blocks the induction of LTP and LTD in the lateral entorhinal cortex of awake animals. In the hippocampus, dopamine efflux is triggered in response to novelty, and it has been suggested recently that this can enhance the encoding of new information within CA3 projections to the CA1 region (Lisman & Grace, 2005). Thus, enhanced basal transmission in the entorhinal cortex could promote transfer of sensory information into the hippocampus and enhance the integration of this information into elaborated representations carried by the CA3 and CA1 regions (Caruana et al., 2006; Lisman & Grace, 2005; Swanson-Park et al., 1999). At the same time, the inhibitory effect of dopamine on LTP and LTD that we have observed here suggests that dopamine may protect the entorhinal cortex from plasticity that could follow from increased neuronal activity during intense sensory processing, and may also shift the site of plasticity in novel or reward-relevant situations to the hippocampal region (Lisman & Grace, 2005).

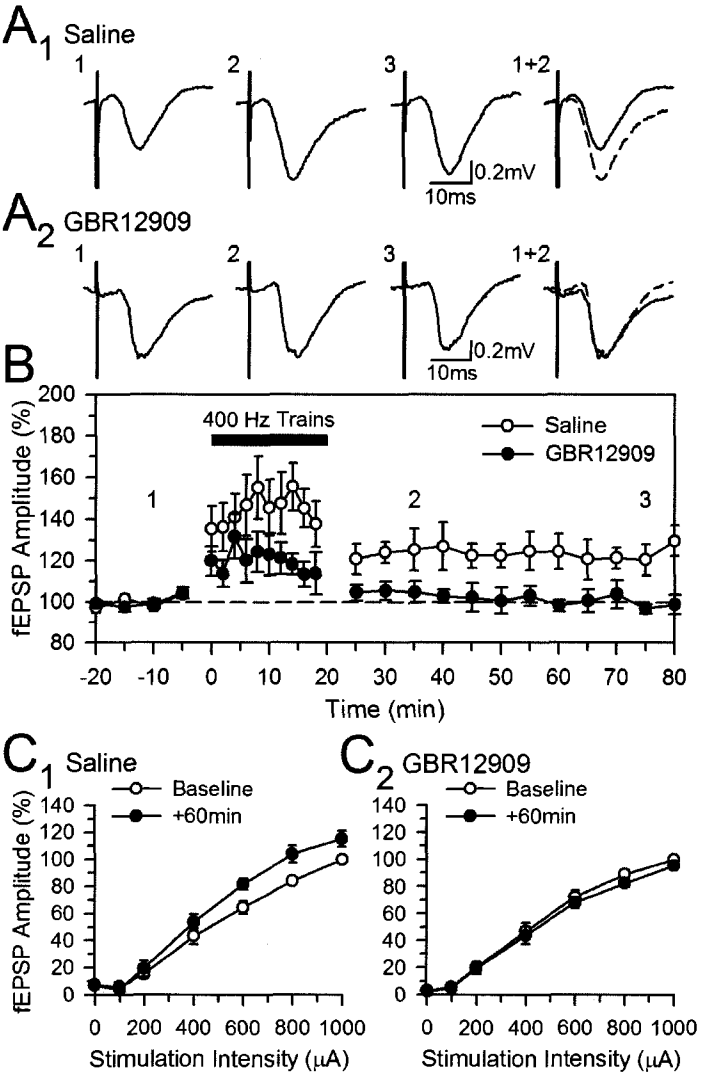
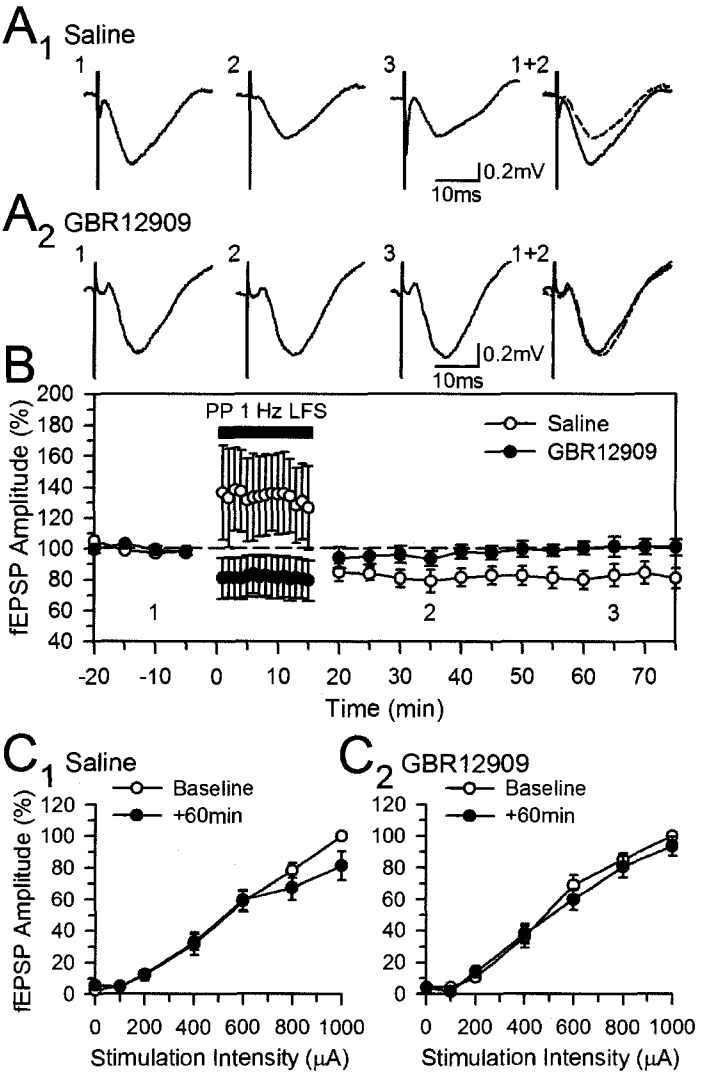

A1 Saline
1
2
3
1+2
0.2mV
10ms
A2 GBR12909
1
2
3
1+2
0.2mV
10ms
B
400 Hz Trains
Saline
GBR12909
1
2
3
fEPSP Amplitude (%)
200
180
160
140
120
100
80
-20
-10
0
10
20
30
40
50
60
70
80
Time (min)
C1 Saline
Baseline
+60min
fEPSP Amplitude (%)
140
120
100
80
60
40
20
0
0
200
400
600
800
1000
Stimulation Intensity (μA)
C2 GBR12909
Baseline
+60min
140
120
100
80
60
40
20
0
0
200
400
600
800
1000
Stimulation Intensity (μA)

Figure 4.1. Enhancing extracellular dopamine with GBR12909 blocks the induction of long-term potentiation in olfactory inputs to the lateral entorhinal cortex. **A**: Representative traces from rats pretreated with saline (A_1) or GBR12909 (A_2) before or after high frequency stimulation to induce LTP. Numbered traces in A correspond to time points indicated in B. Note the potentiation observed in the saline-treated animal (A_1; 1+2) but not the GBR12909-treated rat (A_2; 1+2). **B**: Mean response amplitudes (±SEM) recorded before, during, and after high-frequency stimulation trains in rats pretreated with saline (open circles) or GBR12909 (filled circles). Amplitudes of fEPSPs were expressed as a percentage of the entire baseline period and averaged every 5-min for plotting. Averaged responses recorded immediately following each stimulation train showed no significant difference between groups in post-tetanic potentiation (black bar). **C**: Synaptic responses remained potentiated for 60 min in saline-treated rats (C_1) and were stable in rats pre-treated with GBR12909 (C_2). Responses in C are expressed as a percentage of responses to the highest stimulation intensity during the last baseline test.


A1 Saline
1
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3
1+2
0.2mV
10ms
A2 GBR12909
1
2
3
1+2
0.2mV
10ms
B
PP 1 Hz LFS
Saline
GBR12909
fEPSP Amplitude (%)
200
180
160
140
120
100
80
60
40
1
2
3
-20
-10
0
10
20
30
40
50
60
70
Time (min)
C1 Saline
Baseline
+60min
fEPSP Amplitude (%)
120
100
80
60
40
20
0
0
200
400
600
800
1000
Stimulation Intensity (μA)
C2 GBR12909
Baseline
+60min
120
100
80
60
40
20
0
0
200
400
600
800
1000
Stimulation Intensity (μA)

Figure 4.2. Pretreatment with GBR12909 blocks induction of long-term depression in the lateral entorhinal cortex. **A**: Representative fEPSPs from saline- (A_1) and GBR12909-treated (A_2) rats were recorded before and after repetitive low frequency paired-pulse stimulation to induce LTD. Depression of the fEPSP was observed in the control animal (A_1), but not in the animal pretreated with GBR12909 (A_2). **B**: Mean fEPSP amplitudes before, during, and after low frequency stimulation in saline- (open circles) and GBR12909-treated (filled circles) animals. Amplitudes of responses to conditioning pulses during repetitive paired-pulse low frequency stimulation (PP LFS, black bar) were not significantly different between groups. **C**: LTD was maintained for 60 min in saline-treated animals in response to the highest stimulation intensities (C_1). Amplitudes of fEPSPs remained stable in animals pretreated with GBR12909 (C_2).

CHAPTER 5

DOPAMINE DEPLETION IN THE ENTORHINAL CORTEX BY 6-OHDA LESION IMPAIRS REACQUISITION OF A PREVIOUSLY-LEARNED OLFACTORY NON-MATCH-TO-SAMPLE TASK

Douglas A. Caruana, Sean J. Reed, Lila S.J. Karpowicz, Uri Shalev, and C. Andrew Chapman

The previous 3 chapters demonstrate that dopamine has powerful modulatory effects on glutamate-mediated synaptic transmission in layer II of the lateral entorhinal cortex. The results show that dopamine can act through a variety of mechanisms to enhance *or* suppress basal synaptic transmission, as well as regulate the induction of persistent forms of synaptic plasticity. Such changes at the synaptic level may play an important role in the transmission of sensory information to the hippocampal formation or in the encoding/maintenance of sensory representations by the entorhinal cortex. However, from the methods used and the findings obtained in the previous experimental chapters, it is not possible to infer how dopamine-induced changes at the synaptic level might contribute to behavioral performance on tasks requiring olfactory memory.

Experiments conducted in Chapter 5 assess the effects of dopamine depletion in the entorhinal cortex on the behavior of rats performing an olfactory working memory task. Results show that rats with 6-OHDA lesions to the entorhinal cortex make more errors and take nearly twice as long to reacquire criterion performance relative to control animals during postsurgical retraining. However, once criterion performance is re-attained, the behavior of lesioned animals is indistinguishable from controls on a version of the task involving longer delay periods.

ABSTRACT

Midbrain dopaminergic inputs to the superficial layers of the entorhinal cortex may contribute to memory processing by modulating the strength of olfactory inputs that also terminate in this region. The role of dopaminergic inputs to the entorhinal cortex in olfactory working memory was assessed here using a non-match-to-sample (NMTS) task in which food-restricted rats were trained to discriminate between different odors to obtain a food reward buried in cups filled with scented sand. Upon reaching criterion performance on a version of the task with a minimal delay, animals were pretreated with desipramine and received bilateral infusions of either 6-hydroxydopamine (6-OHDA) or saline into the entorhinal cortex. When retrained on the task 2 weeks later, lesioned rats made significantly more errors and took longer to respond during the first 2 days of retraining relative to sham-operated controls. Further, while control animals required 2.7 ±0.4 days to re-attain criterion levels of performance, lesioned rats required nearly twice as long and took 4.8 ±0.8 days. However, once lesioned rats reached criterion, their behavior was indistinguishable from controls on a version of the task that used a set of 4 sample odors with variable delay periods. Accuracy for both groups was above 80% at the 15 min delay, but was not significantly above chance levels at longer delays of 30, 60, or 180 min. Response latencies were also similar between groups at all delays. Thus, 6-OHDA lesions of the entorhinal cortex cause a transient disruption of olfactory NMTS performance in the period following surgery, but do not lead to permanent impairments in performance.

The entorhinal cortex is a major component of the medial temporal lobe that plays an important role in sensory processing and declarative memory (Lavenex & Amaral, 2000; Schwarcz & Witter, 2002b; Squire et al., 2004; Squire & Zola, 1996). Olfactory inputs originating from the primary olfactory (piriform) cortex terminate in the superficial layers of the entorhinal cortex (Burwell, 2000; Kerr et al., 2007), and this suggests that these layers may contribute to olfactory memory processing. Modulation of synaptic efficacy in the entorhinal cortex is also likely to affect processes central to olfactory memory, and neuromodulatory transmitters such as acetylcholine and serotonin have been shown to have strong effects on basal synaptic transmission in the entorhinal cortex (Grunschlag et al., 1997; Hamam et al., 2006; Schmitz et al., 1998). In addition, although there is a large dopaminergic projection to the entorhinal cortex (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987; Oades & Halliday, 1987), and dopaminergic inputs can have both facilitatory (Caruana et al., 2006) *and* inhibitory effects on basal synaptic transmission in the superficial layers (Caruana et al., 2006; Pralong & Jones, 1993; Stenkamp et al., 1998), the functional role of dopaminergic inputs to the entorhinal cortex on olfactory memory has not been assessed (but see Gauthier & Soumireu-Mourat, 1981).

In the prefrontal cortex, dopamine modulates working memory function for visual stimuli by enhancing the sustained activity of deep layer neurons during delayed-response tasks (Goldman-Rakic, 1999; Seamans & Yang, 2004). Similarly, neurons in the entorhinal cortex increase their firing rates during the delay period of an olfactory non-match-to-sample task (Young et al., 1997), and the entorhinal cortex is also known to contribute to the short-term maintenance of

novel odors (McGaughy et al., 2005; Ranganath & D'Esposito, 2001; Schon, Hasselmo, Lopresti, Tricarico, & Stern, 2004; Stern, Sherman, Kirchhoff, & Hasselmo, 2001). Further, neurons in both the deep and superficial layers of the entorhinal cortex show persistent firing activity that could support working memory (Egorov et al., 2002; Fransen et al., 2006; Tahvildari et al., 2007). These findings indicate that the entorhinal cortex is likely to play an important role in olfactory working memory, and it is also likely that dopaminergic inputs to the superficial layers may modulate synaptic processes that contribute to mechanisms required for the short-term maintenance of olfactory representations.

We have shown previously that dopamine has bidirectional, concentration-dependent effects on basal synaptic transmission in the lateral entorhinal cortex; moderate increases in dopamine facilitate glutamate-mediated EPSPs, while larger increases suppress responses (Caruana et al., 2006). Increases in dopaminergic tone within the entorhinal cortex are likely to occur in response to appetitive or aversive stimuli, and may enhance working memory by facilitating the transmission of olfactory inputs to the superficial layers. Although the persistent firing of superficial layer neurons in the entorhinal cortex is dependent on cholinergic inputs (Tahvildari et al., 2007), dopamine might contribute to working memory by increasing the likelihood that salient odors will initiate sustained firing in principal cells. Further, McGaughy et al. (2005) have proposed that changes in synaptic strength in the superficial layers of the entorhinal cortex may be required for the temporary maintenance of olfactory representations during long delay intervals (see also Hasselmo & Stern, 2006).

Thus dopaminergic innervation of the superficial layers may also affect mechanisms of synaptic plasticity that may be involved in olfactory working memory (Caruana et al., 2007; Hasselmo & Stern, 2006).

Although dopamine is likely to contribute to the modulation of synaptic function in the entorhinal cortex during appetitively motivated olfactory tasks, there are no published reports on the effects of altering entorhinal dopamine levels on olfactory working memory. In the current study, animals were trained to perform an olfactory discrimination task (Dudchenko et al., 2000; McGaughy et al., 2005) before receiving either sham or 6-hydroxydopamine (6-OHDA) lesions of the entorhinal cortex. Animals were trained to dig in cups filled with scented sand to obtain buried food rewards and were required to remember trial-specific odors across variable delay intervals in order to choose the correct scented cups during the test phase. This task was selected because it likely promotes activation of mesocortical dopaminergic inputs to the entorhinal cortex, it depends on working memory for olfactory stimuli, and the digging response required is a natural behavior for the rat. Results indicate that rats with 6-OHDA lesion are impaired during postsurgical retraining on the task, and that they are able to regain performance on this task with extended training.

METHODS

Behavioral Testing

Materials and Apparatus. Subjects were 17 male Long-Evans rats weighing 320 to 350 g. One week prior to shaping animals were placed on a restricted feeding schedule (18g of chow per day) that allowed animals to

maintain 80% of their free-feeding body weight. Animals were housed individually and tested during the lights-on phase of a 12-hour light-dark cycle.

An open field constructed from black Plexiglas (92 x 92 cm with 3.2 cm-high walls) was elevated 92 cm from the floor. A series of 24 Velcro strips (4 x 2.5 cm) were affixed to the floor of the open field at 13-cm intervals around the perimeter, 9 cm from the outer wall. An additional Velcro strip was affixed to the center of the field. Commercially available spices used as olfactory cues during training and testing were allspice, basil, celery, cinnamon, cloves, cocoa, coffee, cumin, dill, garlic, ginger, lemon gelatin, marjoram, mint, nutmeg, orange gelatin, oregano, paprika, parsley, peach gelatin, poultry, sage, tea, and thyme. One g of spice was mixed with 100 g of dampened and unscented playground sand in semi-transparent cups (6 cm tall; 8 cm diameter; Fisher Scientific; 0.5 g cloves and 2 g assorted gelatin were also used). Velcro strips on the bottom of the cups allowed them to be attached to positions in the open field, and prevented rats from toppling the cups. One-quarter pieces of Froot Loops cereal were used as food reward. The experiment was conducted in a small room equipped with a fume hood so that stimuli could ventilated constantly.

Shaping. Animals were shaped to dig in scented sand to obtain a buried reward during 6 daily 20-min sessions. Rats were placed in the center of the open field with a single baited cup in a random location. The reward was placed on the top of unscented sand for the first 3 days, and rats were allowed to obtain as many rewards as possible during the 20-min session. The reward was placed progressively deeper within the sand over these days, but always remained visible. Rats were then shaped for 3 days using rewards buried in sand scented

with a different odor on each day (onion, lemon gelatin, and strawberry gelatin). All animals reliably obtained rewards by the end of the sixth day. The open field and cups were wiped clean with 20% alcohol between sessions and the entire apparatus was also rotated 90°.

Non-Match-to-Sample Training. Training for the non-match-to-sample (NMTS) rule began the day after shaping. Each NMTS trial consisted of a sample phase and a test phase. During the sample phase, a single scented and baited cup was placed in the center of the field, and rats were allowed up to 2 min to obtain the reward, and were allowed to finish consuming the reward before being removed from the maze. Rats that did not obtain the reward were removed from the maze until the next trial and a latency of 120 sec was recorded. Rats were placed in a holding cage for about 20 sec while the test phase was set up. The open field was rotated 90°, wiped clean with alcohol, and two new cups were placed randomly along the perimeter. An un-baited cup contained sand with the same scent used in the sample trial, and a baited cup contained a different scent. Rats were required to remember the sample odor in order to obtain the buried reward from the cup containing the non-matching odor. Rats were placed in the open field perpendicular to the two cups and had 2 min to obtain the reward. If a rat began to displace sand with his forepaws in the cup containing the sample odor he was removed from the field and the trial ended. Rats that obtained the reward from the cup with the non-matching odor were allowed to consume it before being removed. The latency to make either a correct or incorrect choice was recorded (a 120 sec latency was scored for animals that did not approach a cup). Twelve trials were conducted each day

until a criterion of at least 83% correct (10 of 12 trials) was maintained over 2 days. Twelve spices served as sample odors on the first day of training and the remaining 12 were used as test odors. The odors were reversed on the next day so that all 24 spices were used as both sample and test odors over each 2-day period. Presenting each spice only once per day ensured that all 24 odors were used, and prevented any particular spice from appearing more often than another. At the end of a trial, the open field and the outside of each cup was wiped down with a 20% alcohol solution and the open field was rotated 90°. Following surgery (see below) and a 2-week recovery period, animals were retrained on the olfactory NMTS task using the same 24 olfactory cues. Procedures were identical to those described above.

Varying Set-Size. Rats can remember a single familiar sample odor for up to 3 hours in a version of the NMTS task used here (McGaughy et al., 2005), but increasing the number of sample items to be retained for variable delays can increase the efficiency of testing, and can increase demands on working memory and make the task more sensitive to potential lesion-induced impairments (Dudchenko et al., 2000). We therefore conducted preliminary tests using a series of 2, 4, 8, or 12 scented sample cups to determine the largest number of sample items that both sham- and 6-OHDA-lesioned animals could retain well at a short (≤20 sec) delay intervals. This set-size could then be used in subsequent tests using a variable delay interval.

The sample phase began by allowing the rat to retrieve the reward from a single baited cup placed in the center of the field. After consuming the reward the rat remained in the open field as the first sample cup was replaced by a

second baited cup with a different odor, and this was repeated until either 2, 4, 8, or 12 cups had been presented. The rat was then placed in a holding cage. In the test phase, the rat was placed perpendicular to two randomly located cups. One cup contained one of the sample odors and the second cup contained a different odor and was baited. After choosing one of the cups, the cups were replaced with 2 new randomly located cups. The rat remained in the open field during the sequential presentation of either 2, 4, 8, or 12 pairs of cups from which the rat was required to discriminate between a previous sample odor and a non-matching odor in order to obtain a reward. Animals were tested twice with the same set-size on a given day and the order of set-sizes tested was 4, 8, 2, 12, 4, 8, 2, and 12 over eight days.

Varying Delay Interval. The results of varying set-size showed that sham- and 6-OHDA-lesioned rats could remember a sequence of 4 sample items over short delay intervals. Thus, the effects of dopamine lesions on working memory performance at longer delay intervals were therefore tested using a sample-set of 4 different odors on each trial. The procedures for the delayed NMTS task were identical to those described above except that rats had to remember the 4 sample odors during delay periods of 15, 30, 60, and 180 min. Rats were tested twice on each day using the same delay interval. The order of the delays tested was 15, 60, 30, 180, 15, 60, 30, and 180 min over eight days. Animals were placed in a holding cage in a quiet and darkened room during the delay period.

Surgery

The day after attaining criterion performance on the one-sample task, rats received either bilateral 6-OHDA lesions of the medial and lateral entorhinal cortex or sham lesions. Group assignment was quasi-random to ensure roughly equal NMTS performance in both groups. Rats were pretreated with desipramine (2.5 mg/kg, i.p.) 60 min prior to anesthesia and atropine methylnitrite (0.1 mg/kg, i.p.) 15 min before surgery. Rats were anesthetized with a 5% isoflurane and 95% oxygen mixture and placed in a stereotaxic frame with bregma and lambda leveled. Two stainless steel cannulae (26 gauge) were used to inject either sterile saline (0.9%) or 6-OHDA (4 µg/ml) bilaterally into each of 5 sites along the rostral-caudal axis of the entorhinal cortex (from bregma; site 1: P -6.3, L ±4.4, V -8.0 mm; site 2: P -6.8, L ±4.4, V -8.0 mm; site 3: P -7.3, L ±4.4, V -7.4 mm; site 4: P -7.8, L ±4.4, V -7.2 mm; site 5: P -8.3, L ±4.4, V -5.5 mm). Infusions were made using two Hamilton syringes (10 µl; 1800 Series) connected to a Harvard Apparatus microinfusion pump (Model 22), and syringes were attached to infusion cannulae by short lengths of PE-20 tubing. A volume of 1 µl was delivered to sites 1, 3, and 5 over a 5 min period and 0.5 µl was delivered to sites 2 and 4 over 2.5 min. Cannulae were left in place for 4 min after each infusion. The catecholaminergic toxin 6-OHDA (Sigma) was prepared fresh daily by dilution in sterile saline and ascorbic acid (5 mg/ml). Buprenorphine (0.02 mg/kg, s.c.) was administered as a postsurgical analgesic. Recovery from surgery lasted for 2 weeks; animals had free access to food and water during the first week, but the food restriction schedule was reinstated during the second week.

Locomotor Activity.

To determine if 6-OHDA lesions induced any lasting changes in locomotor behavior, and to assses possible recovery of the dopamine system involving receptor supersensitivity, a series of measures of locomotor activity were taken in response to novelty and to an amphetamine challenge. Animals were placed in 43 x 43 x 40 cm clear Plexiglas boxes with 2 photosensor grids located around the perimeter of the box 5 and 15 cm from the floor (Coulbourn, Models E63-20 and -22). Each box was enclosed in a sound attenuating foam chamber with an exhaust fan and a house light at the top. Photosensor grids were connected to a personal computer and measures of locomotor activity were acquired using the software package TruScan v2.01 (number of movements, distance traveled, and time spent moving; Coulbourn). Testing occurred during 3 sessions conducted on the same day. The first 30 min session reflected the animals response to being placed in the novel locomotor boxes for the first time. Animals then received a systemic injection of saline (0.9%; 1 ml/kg, i.p.), and activity was recorded for 30 min after a 20 min delay. Rats then received a systemic injection of amphetamine (1.5 mg/kg, i.p.) and activity was monitored again after a 20 min delay for a period of 60 min.

High Performance Liquid Chromatography

The assay of tissue-levels of dopamine was performed according to methods described previously (Moroz, Pecina, Schallert, & Stewart, 2004; Moroz, Rajabi, Rodaros, & Stewart, 2003). Both sham and lesioned animals were killed by decapitation and their brains were rapidly removed and placed in isopentane

cooled on dry ice, and were then frozen overnight at –80°C. The brains were then sliced on a cryostat into 300 µm sections and punches (1 or 2 mm in diameter) were taken from the left and right entorhinal cortices and bilaterally from the caudate/putamen, prefrontal cortex, nucleus accumbens, ventral tegmental area, and substantia nigra. Tissue was stored at –80°C. The tissue punches were then suspended in artificial cerebrospinal fluid containing (in mM) 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose and frozen overnight. The following day the samples were thawed and centrifuged at 4000 rpm for 15 min. The supernatant was removed and assayed for dopamine content using high performance liquid chromatography (HPLC) with electrochemical detection as described previously (Caruana et al., 2006). Tissue pellets were suspended in sodium hydroxide and analyzed for protein content using spectrophotometry.

For HPLC analysis, a 5-µl volume (caudate/putamen and nucleus accumbens) or 10-µl volume (entorhinal and prefrontal cortices, substantia nigra, and ventral tegmental area) was extracted from each sample and loaded onto a C-18 reverse-phase column (5 µm, 15 cm) through a manual injection port (Rheodyne, Model 7125, 20 µl loop), and the redox current for dopamine was measured with a dual-channel coulometric detector (ESA Biosciences, Coulochem III with a Model 5011 analytical cell). The mobile phase (20% acetonitrile, 0.076 M SDS, 0.1 M EDTA, 0.058 M $NaPO_4$, and 0.27 M citric acid; pH = 3.35) was circulated through the system at a rate of 1.1 ml/min by a Waters 515 HPLC pump and the peak for dopamine was quantified by EZChrom Chromatography Data System (Scientific Software Inc.). Measures of dopamine

content were adjusted for protein quantity using custom software and expressed in µg/mg of protein for analysis and plotting.

RESULTS

Non-Match-to-Sample Training. Animals typically required 6 to 9 days to meet the criterion level of 83% correct during initial training on the NMTS task. Discrimination between the sample and test odors was at chance levels during the first few days of training (47.7 ±2.8% on day 1, n = 18; Fig. 5.1A), and accuracy improved gradually over a period of 6 to 9 days (78.1 ±2.9% on day 6). Response latencies were longest during the sample phase on the first day as animals learned to approach the cup in the center of the maze to obtain the reward (30.0 ±5.6 sec), and latencies for both sample and test phases stabilized after 3 days and remained less than ≈20 sec for the remainder of training (sample latency, 17.6 ±3.0 sec on day 4; test latency, 17.3 ±3.4; Fig 5.1B). All but two animals reached criterion performance by the tenth day of training (Fig. 5.1C).

Postsurgical Retraining. Rats with 6-OHDA lesions of the entorhinal cortex were impaired in the re-acquisition of the NMTS task when they were retrained two weeks following surgery. Lesioned animals (n = 9) made significantly more errors than control rats (n = 8) during the first 2 days of retraining (64.8 ±6.9% versus 82.3 ±5.5% accuracy on day 1; $F_{1,15} = 6.81$, $P < 0.05$; Fig. 5.2A) and it also took lesioned rats significantly longer than controls to re-attain criterion performance (4.8 ±0.8 versus 2.7 ±0.4 days; $t_{15} = 2.31$, $P < 0.05$; Fig 5.2C_2). Response latencies during the first 2 days of retraining show

that lesioned rats also took significantly longer than controls to either retrieve the reward during the sample phase or choose a cup during the test phase (sample, $F_{1,15} = 5.55$, $P < 0.05$; test, $F_{1,15} = 3.61$, $P < 0.05$; Fig. 5.2B$_3$). Lesioned animals took 36.7 ±8.7 sec on average to retrieve the reward from the sample cup on the first day of retraining, whereas control animals required only 12.8 ±3.7 sec. Similarly, lesioned animals required 26.3 ±5.9 sec to respond on the test phase as compared to 14.8 ±2.4 sec in control animals. These findings indicate that dopamine-depletion in the entorhinal cortex impairs re-acquisition of a previously-learned olfactory NMTS task.

Delayed Non-Match-to-Sample Performance. In contrast to the poor performance of lesioned rats during NMTS retraining with one sample odor per trial, the performance of lesioned and sham rats was indistinguishable during subsequent testing with a delayed version of the task that used multiple sample odors. Sham and lesioned rats performed similarly in preliminary tests used to determine the appropriate number of sample items for retention during delay testing. Both groups performed at ≈80% accuracy with a 4-item set and a delay ≤20 sec (80.4 ± 4.4 and 83.3 ± 4.4% for sham and lesioned animals, respectively; data not shown). In addition, there was no significant difference between sham and lesioned rats in subsequent tests in which animals were required to retain the 4 sample items for delay intervals of 15, 30, 60, or 180 min (Fig. 5.3). Both groups performed well at the 15 min delay (sham, 82.1 ±4.6%; 6-OHDA, 84.7 ±5.4%), but were at chance levels at longer intervals. This was reflected in a significant main effect of delay interval that was due to above-chance performance only at the 15 min interval ($F_{3,42} = 5.37$, $P < 0.01$; sham, $t_6 =$

6.97, $P < 0.001$; 6-OHDA, $t_8 = 6.40$, $P < 0.001$). Moreover, there was no significant difference between sham and lesioned rats in response latencies at any interval. Lesions therefore had no lasting effect on performance in the 4-item NMTS task at any delay interval.

Locomotor Activity. Lasting changes in general motor behavior induced by 6-OHDA lesions were assessed in a subset of the rats tested. Animals were placed in novel chambers equipped with sensors to monitor spontaneous locomotor activity to determine if there was any lasting change in motor output or response to novelty that might be related to the impaired performance of lesioned animals. Both sham (n = 4) and lesioned (n = 5) animals, however, showed similar activity patterns during initial exposure to the arena and for the 30 min following a saline injection (Fig. 5.4). Lesioned animals therefore had no lasting impairment in general motor behavior. We observed an interesting effect, however, when animals were administered 1.5 mg/kg amphetamine, and found significantly higher measures of activity in the lesioned group during the 60-min test session as compared to the control group. Lesioned animals showed an increase in the total number of movements (4855 ±100 versus 4272 ±182 movements; $t_7 = 2.98$, $P < 0.05$), the total time spent moving (1390 ±42 versus 1004 ±123 sec; $t_7 = 3.24$, $P < 0.05$), and the total distance traveled (27.7 ±1.6 versus 18.0 ±2.8 m; $t_7 = 3.18$, $P < 0.05$; Fig. 5.4). This suggests that a sensitized response to amphetamine developed in lesioned animals.

Histology and HPLC. Tissue-punches from multiple brain regions containing dopamine terminal fields were available from roughly half of the animals, and were used to assess the effect of lesions on dopamine levels.

Although statistics indicate no significant difference between sham and lesioned animals in the amount of dopamine contained in tissue punches in any region (Fig. 5.5), the mean levels were lower in lesioned rats in the entorhinal cortex (left, 0.6 ±0.3 versus 1.4 ±0.3 µg/µl; right, 0.6 ±0.1 versus 0.9 ±0.6 µg/µl), prefrontal cortex (0.9 ±0.3 versus 1.4 ±0.1 µg/µl), ventral tegmental area (11.1 ±3.5 versus 18.9 ±6.6 µg/µl), nucleus accumbens (4.5 ±1.3 versus 7.1 ±2.3 µg/µl), and caudate/putamen (8.8 ±1.4 versus 26.0 ±11.2 µg/µl). Interestingly, dopamine levels appeared to increase in the substantia nigra in lesioned rats (4.3 ±1.4 versus 3.7 ±0.7 µg/µl), though this difference was not significantly significant.

DISCUSSION

There is a growing body of evidence indicating that the entorhinal cortex is likely to play a substantial role in working memory (Egorov et al., 2002; Fransen et al., 2006; Hasselmo & Stern, 2006; McGaughy et al., 2005; Staubli et al., 1986; Staubli et al., 1995; Tahvildari et al., 2007) and although the entorhinal cortex plays a major role in olfactory processing (Burwell, 2000; Kerr et al., 2007) and is a major target of the mesocortical dopamine system (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987; Oades & Halliday, 1987), this is the first study aimed at determining if dopaminergic inputs to the entorhinal cortex are required for successful completion of an olfactory working memory task. Here, we have used an olfactory task that is appetitively motivated in order to increase the likelihood that the dopaminergic input to the entorhinal cortex would contribute (Dudchenko et al., 2000; McGaughy et al., 2005).

We show here that infusion of 6-OHDA into the entorhinal cortex results in impaired performance on an olfactory NMTS task that was learned prior to surgery. Lesioned animals made more errors than sham animals during the first 2 days of retesting after surgery, showed longer latencies to approach the cups, and took nearly twice as long to reach criterion performance. It is unclear, however, whether these deficits resulted from amnesia for the NMTS rule, disrupted motivation to perform the task, disrupted sensory processing, or from other cognitive deficits related to successful NMTS performance. However, our data reflect a significant impairment in performance of an appetitively motivated olfactory task in the first three weeks following lesion of dopaminergic inputs to the entorhinal cortex.

Once the lesioned animals had been retrained on the one-sample version of the task with a minimal delay period, they performed just as well as control animals on a 4-sample version of the task with a variable delay interval. This suggests that dopaminergic inputs to the entorhinal cortex do not play a substantial role in olfactory working memory. Dopamine levels in tissue samples obtained after testing were shown to be non-significantly reduced in lesioned animals relative to controls, but it is common for dopamine levels to recover in the weeks following 6-OHDA lesions (Altar, Marien, & Marshall, 1987; Finkelstein et al., 2000; Kostrzewa, 1995; Neve, Kozlowski, & Marshall, 1982; Robinson, Castaneda, & Whishaw, 1990; Schwarting & Huston, 1996; Zigmond, 1997). Thus, although the early performance deficits are likely due to loss of dopamine cells that project to the entorhinal cortex, it is possible that recovery of

dopaminergic function may account for the normal performance of the task with multiple sample stimuli and a variable delay interval.

Initial Impairments in Non-Match-to-Sample Performance

There were deficits on the one-sample NMTS task in 6-OHDA-lesioned animals during retraining following surgery. Deficits were not simply due to recovery from the surgical procedure because animals were tested a full two weeks after surgery when response latencies and performance of control animals were similar to presurgical levels. We have shown previously that dopamine has bidirectional effects on synaptic transmission in layer II of the entorhinal cortex, such that moderate increases in dopamine facilitate glutamate-mediated synaptic responses in inputs from the olfactory cortex, and high doses suppress transmission (Caruana et al., 2006). The relative roles of synaptic facilitation and suppression effects in olfactory processing and memory function are not known. However, as in the prefrontal cortex, it is the *lower* concentrations of dopamine which may contribute most strongly to memory function in the entorhinal cortex (Arnsten, 1998; Seamans & Yang, 2004), perhaps by enhancing the salience of olfactory stimuli carried by piriform cortex inputs or by promoting mechanisms within the entorhinal cortex that maintain working memory representations (Egorov et al., 2002; Fransen et al., 2006; Tahvildari et al., 2007). Here, we have used 6-OHDA lesions to disrupt dopaminergic inputs to the entorhinal cortex, and the impairments in performance observed may have resulted in part from the loss of dopaminergic modulation of olfactory inputs to the entorhinal cortex and hippocampal formation.

If the synaptic facilitation induced by dopamine normally serves to increase the salience of reward-relevant stimuli by increasing the associated motivational valence, then the loss of dopamine in lesioned animals may have affected performance by interfering with the detection and discrimination of stimuli during the task (Bannerman et al., 2002). This could prevent the adequate processing of olfactory cues within the entorhinal cortex, the integration of these representations with other task-related stimuli, or the propagation of these representations to the hippocampus. Similar to the prefrontal cortex, dopamine may normally enhance working memory function in the entorhinal cortex by promoting the maintenance of olfactory representations in working memory (Goldman-Rakic, 1999). The performance deficits observed here may therefore be due in part to loss of working memory function, and that this may be related to an inability of olfactory cues to initiate or maintain persistent firing in entorhinal cortex networks (Egorov et al., 2002; Fransen et al., 2006; Tahvildari et al., 2007).

Although the present results can be interpreted in terms of reward-relevant dopaminergic modulation of the salience of sensory and memory function in the entorhinal cortex, it is clear that other factors may contribute. It is notable that lesioned animals had a much longer latency to approach the sample cup for a reward during initial retraining, and this is not easily attributed solely to an impairment in working memory. Sham animals, in contrast, showed response latencies very similar to those observed prior to surgery. Performance deficits resulting from 6-OHDA lesions could be due to impaired olfactory sensation (Bannerman et al., 2002) impaired spatial navigation (Fyhn et al., 2004; Hafting

et al., 2005; Witter & Moser, 2006), loss of memory for the requirements of the task, or loss of the ability to coordinate behaviors to perform the task. Further, lesions that result in a substantial loss of dopamine neurons in the ventral tegmental area can produce a generalized reduction in appetitive motivation (Martinez-Hernandez, Lanuza, & Martinez-Garcia, 2006; Shimura, Kamada, & Yamamoto, 2002; Winter et al., 2007), and this may have reduced the motivational value of the food reward (Wise, 2006). Thus, although lesions may have acted to reduce the motivational salience of olfactory stimuli through effects in the entorhinal cortex, and this is consistent with the longer response latencies observed, the long response latencies are also consistent with a more general motivational or attentional deficit that interfered with task performance.

The performance deficits observed in lesioned animals likely resulted primarily from disrupted dopaminergic transmission within the entorhinal cortex, but effects in other brain areas may have contributed. Dopamine lesions to the entorhinal cortex destroy the cells of origin as well as the local dopamine terminals (Liang et al., 2004), and 6-OHDA lesions in the entorhinal cortex are also known to reduce dopamine levels in the nucleus accumbens (Louilot & Choulli, 1997). In the present study, measures of dopamine in tissue samples were available only for about half of the animals and there were no statistically significant differences detected between sham and lesioned groups with this small number of animals in any region tested. However, the mean level of dopamine measured was almost always less in lesioned animals, not only in the entorhinal cortex but in other terminal regions as well. Thus, although effects observed here on retraining are most likely due to direct effects of dopamine

within the entorhinal cortex, it is also possible that depletion of dopamine in other regions including the prefrontal cortex or nucleus accumbens may have contributed to the deficits, perhaps by disruption of working memory function or motivation (Brozoski et al., 1979; Winter et al., 2007).

Recovery of Function

The deficits displayed by 6-OHDA lesioned animals were transient and, once lesioned rats re-attained criterion levels of performance on the one-sample version of the task, there were no further deficits during testing with multiple sample odors and longer delay intervals. The improvement in performance could be due in part to a recovery of function within the mesocortical dopamine system including dopaminergic projections to the entorhinal cortex. In the striatum, dopamine lesions that preserve at least 20% of dopaminergic fibers do not significantly reduce extracellular dopamine levels (Robinson et al., 1990) and the small percentage of intact dopamine fibers can maintain dopamine-dependent behaviors. Incomplete lesions could account for the performance of several animals during retraining that was not substantially impaired (Fig. $2B_2$). More complete lesions of striatal dopamine that destroy 80 to 95% of inputs are known to activate compensatory mechanisms that upregulate dopamine production and release, as well as facilitate the insertion of postsynaptic dopamine receptors (Altar et al., 1987; Finkelstein et al., 2000; Kostrzewa, 1995; Neve et al., 1982; Robinson et al., 1990; Schwarting & Huston, 1996; Zigmond, 1997). This recovery can take between 3 and 18 days, so that the behavioral recovery of lesioned animals to a level that was indistinguishable from controls may be

explained in part by similar mechanisms in the entorhinal cortex (Altar et al., 1987). This is also consistent with the lack of a reduction in spontaneous locomotor activity in lesioned rats. Further, the elevated locomotor activity of lesioned animals in response to the amphetamine injection is consistent with a lesion-induced dopamine receptor supersensitivity in motor regions (Kostrzewa, 1995; Neve et al., 1982).

It is also possible that the recovery of NMTS performance was due to the development of alternative behavioral strategies that do not depend on the entorhinal cortex. The prefrontal cortex plays a central role in a variety of working memory tasks (Funahashi & Kubota, 1994; Goldman-Rakic, 1999) and orbitofrontal cortex cells show increased firing during the delay period of a similar olfactory NMTS task (Ramus & Eichenbaum, 2000). The prefrontal cortex can also contribute to the recall of familiar stimuli (Stern et al., 2001) and might have played a role in the performance of the current task which repeatedly used the same set of olfactory stimuli during the testing period (Hasselmo & Stern, 2006; McGaughy et al., 2005). Thus, animals may have shifted to a reliance on other brain regions such as the prefrontal cortex for successful completion of the delayed versions of the task.

Conclusions

The initial impairments observed in the one-sample version of the NMTS task seen 2 weeks following 6-OHDA infusions into the entorhinal cortex are likely due to a disruption in neuronal processes in the entorhinal cortex required for performance of the NMTS task. Although the lesions most certainly induced

changes in other brain regions, the deficits observed here are likely attributable in part to a reduction in the salience of reward-related cues or reduced memory function within the entorhinal cortex (McGaughy et al., 2005; Young et al., 1997). Because compensatory increases in dopamine turnover and dopamine receptor supersensitivity may have prevented any working memory deficits during later tests on the four-sample version of the task with longer delay intervals (Altar et al., 1987; Finkelstein et al., 2000; Kostrzewa, 1995; Neve et al., 1982; Robinson et al., 1990; Schwarting & Huston, 1996; Zigmond, 1997), it is not clear how a disruption of dopaminergic inputs to the entorhinal cortex might have affected performance on this task. Further work on the role of dopamine in the sensory and mnemonic functions of the entorhinal cortex could employ additional behavioral tests to assess the early effects of lesions on sensory and motor functions.

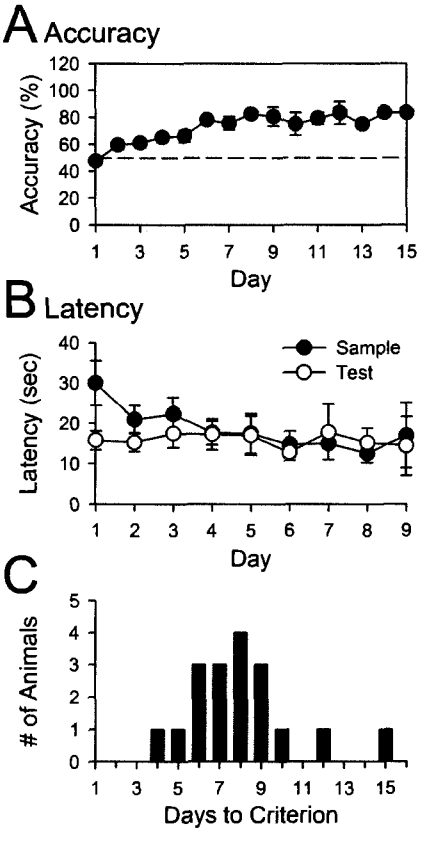
A Accuracy
Accuracy (%)
120
100
80
60
40
20
0
1 3 5 7 9 11 13 15
Day
B Latency
Latency (sec)
40
30
20
10
0
Sample
Test
1 2 3 4 5 6 7 8 9
Day
C
# of Animals
5
4
3
2
1
0
1 3 5 7 9 11 13 15
Days to Criterion

Figure 5.1. Rats learn to perform the one-sample olfactory non-match-to-sample (NMTS) task within about 9 days of training. **A**. The mean accuracy of responses during the 12 trials conducted on each day are shown as a function of training day (mean ±SEM in this and subsequent figures). **B**. The average latency to obtain the reward during the sample phase and to choose a cup during the test phase are shown for the first 9 days of training. **C**. A frequency distribution shows the number of days required for animals to reach criterion performance of 83% correct on two consecutive days.

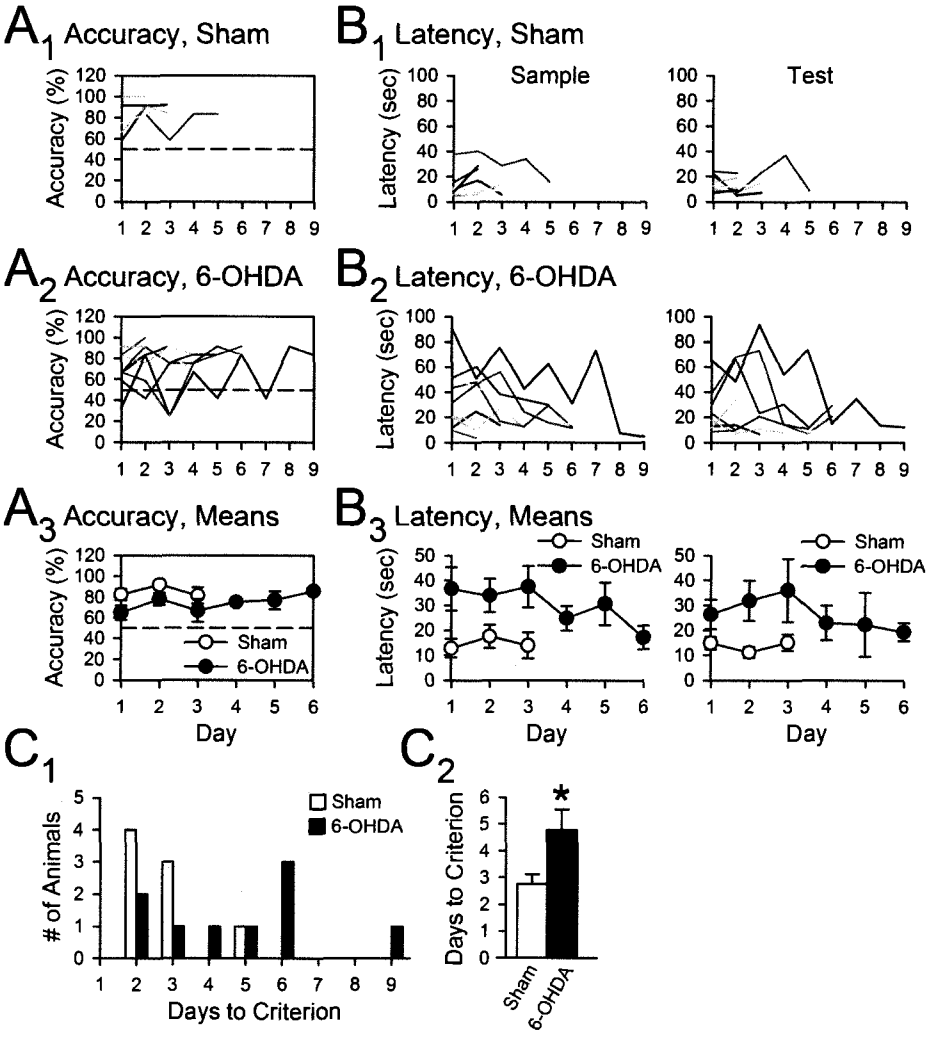
A1 Accuracy, Sham
Accuracy (%)
B1 Latency, Sham
Sample
Test
Latency (sec)
A2 Accuracy, 6-OHDA
B2 Latency, 6-OHDA
A3 Accuracy, Means
B3 Latency, Means
Sham
6-OHDA
Day
C1
# of Animals
Days to Criterion
C2
Days to Criterion
*

Figure 5.2. Rats with 6-OHDA lesions of the entorhinal cortex make more errors and take longer to reach the criterion level of performance during retraining following surgery. **A.** Mean accuracy during the 12 trials on each day is shown for each sham (A_1) and 6-OHDA-lesioned (A_2) rat. Group data for the first six days are shown in A_3. **B.** The latency to obtain the reward from the sample cup (left) and to make a choice between cups in the test phase (right) are shown for each sham (B_1) and lesioned rat (B_2). Group data are shown in B_3. **C.** Lesioned animals took longer to re-attain criterion performance during retraining (*, $P < 0.05$).

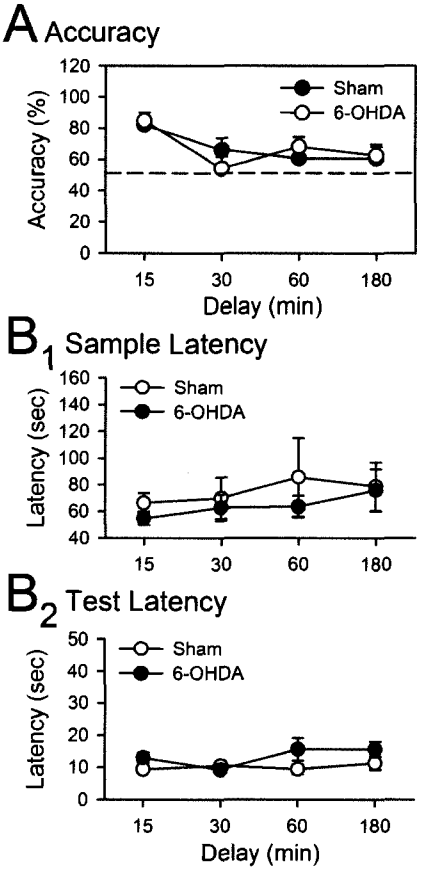
A Accuracy
Accuracy (%)
120
100
80
60
40
20
0
Sham
6-OHDA
15
30
60
180
Delay (min)
B1 Sample Latency
Latency (sec)
160
140
120
100
80
60
40
Sham
6-OHDA
15
30
60
180
B2 Test Latency
Latency (sec)
50
40
30
20
10
0
Sham
6-OHDA
15
30
60
180
Delay (min)

Figure 5.3. Rats with 6-OHDA lesions to the entorhinal cortex retained four sample odors in working memory for 15 minutes. **A**. Both sham and lesioned rats performed at above 80% accuracy when tested using a 15 min delay. Performance dropped to chance levels as the delay interval was increased to 30 min, and there was no significant difference between groups at any interval. **B**. Response latencies were similar in sham and lesioned animals during both the sample (B_1) and test (B_2) phases of the task regardless of delay interval.

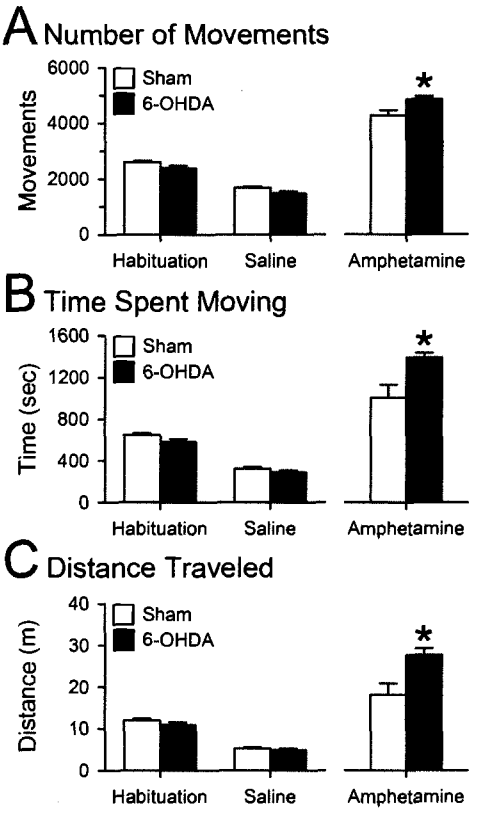
A Number of Movements
Movements
6000
4000
2000
0
Sham
6-OHDA
*
Habituation
Saline
Amphetamine
B Time Spent Moving
Time (sec)
1600
1200
800
400
0
Sham
6-OHDA
*
Habituation
Saline
Amphetamine
C Distance Traveled
Distance (m)
40
30
20
10
0
Sham
6-OHDA
*
Habituation
Saline
Amphetamine

Figure 5.4. Spontaneous locomotor activity recorded 9 weeks following surgery did not differ between animals that received either 6-OHDA or sham lesions. Both sham- and dopamine-lesioned rats were placed in a novel recording chamber and the total number of movements (**A**), time spent moving (**B**), and total distance traveled (**C**) were measured. Spontaneous activity during the initial 30 min (Habituation) and following an injection of saline (Saline) did not differ between groups. However, lesioned rats showed an enhanced locomotor response in the 60 min following injection of amphetamine for each of the behaviors examined.

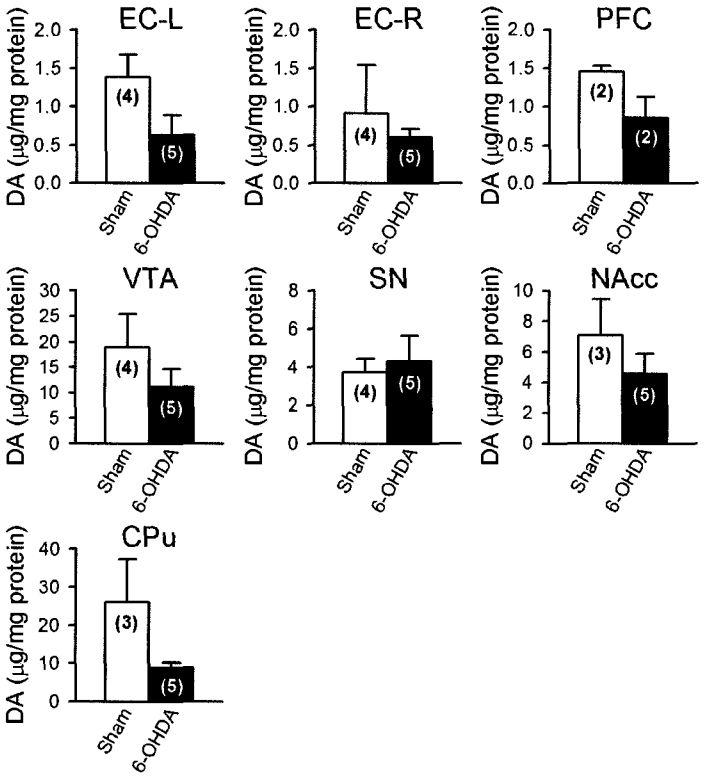
EC-L
EC-R
PFC
VTA
SN
NAcc
CPu
DA (µg/mg protein)
Sham
6-OHDA
(4)
(5)
(2)
(3)

Figure 5.5. Results of assays conducted on tissue punches obtained from roughly half of the animals tested 12 weeks after either sham or 6-OHDA lesions reflect comparable levels of dopamine in both sham and lesioned animals. There was a reduced level of dopamine (DA) in all terminal regions examined, but the difference was not statistically significant. Note that the mean tissue dopamine level was non-significantly lower in the ventral tegmental area (VTA), but not in the substantia nigra (SN). Abbreviations indicate the left and right entorhinal cortex (L-EC, R-EC) the caudate/putamen (CPu), nucleus accumbens (NAcc), and prefrontal cortex (PFC). Numbers in parentheses represent the number of animals from which samples were obtained for each region assessed.

CHAPTER 6

GENERAL DISCUSSION

A number of diverse experimental techniques have been used here to investigate the role of dopamine in modulating sensory and mnemonic functions of the entorhinal cortex. Experiments have demonstrated that changes in dopaminergic tone in the superficial layers of the lateral entorhinal cortex can have powerful effects on glutamate-mediated synaptic transmission (Chapters 2 and 3), induction of persistent forms of synaptic plasticity (Chapter 4), and performance on an olfactory memory task (Chapter 5). These findings are consistent with the known anatomy of midbrain dopaminergic projections to the entorhinal cortex (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987; Oades & Halliday, 1987), the laminar distribution of dopamine receptors in the superficial layers (Q. Huang et al., 1992; Köhler et al., 1991b; Weiner et al., 1991), and the role of the entorhinal cortex in olfactory memory (McGaughy et al., 2005; Otto & Eichenbaum, 1992; Staubli et al., 1995; Young et al., 1997). Taken together, these findings suggest that dopaminergic inputs to the entorhinal cortex may play an important role in shaping the content of sensory representations processed and maintained by networks within the entorhinal cortex, and in modulating the flow of sensory information to the hippocampal formation. Thus, behavior-related alterations in dopaminergic inputs to the entorhinal cortex are likely to have strong effects on sensory and mnemonic functions mediated by the entire medial temporal lobe.

Summary of Main Findings

Initial experiments examined the effects of inhibiting the reuptake of dopamine on excitatory synaptic responses in olfactory inputs to the superficial

layers of the lateral entorhinal cortex in awake rats (Chapter 2). Systemic administration of the selective dopamine reuptake inhibitor GBR12909 enhanced extracellular levels of dopamine in the entorhinal cortex and facilitated synaptic responses in the superficial layers of the lateral entorhinal cortex evoked by stimulation of the piriform cortex. These are the first experiments to use *in vivo* microdialysis techniques to sample dopamine levels in the entorhinal cortex before and after the inhibition of reuptake of dopamine, as well as the first experiments to show a facilitation of basal synaptic transmission in the entorhinal cortex induced by dopamine. Subsequent experiments, in slices of lateral entorhinal cortex maintained in a gas-fluid interface recording chamber *in vitro*, showed that the effects of dopamine on glutamate-mediated synaptic transmission are dose-dependent and bidirectional; bath application of low doses of dopamine enhanced transmission, similar to experiments in awake rats, and higher concentrations of dopamine *suppressed* responses. Experiments using selective dopamine receptor antagonists demonstrated that the facilitation was dependent on activation of D_1-like receptors and that the suppression is dependent on D_2-like receptors. Although others have shown that dopamine can suppress synaptic responses in the medial entorhinal cortex (Pralong & Jones, 1993; Stenkamp et al., 1998), this is the first study to demonstrate the *bidirectional* modification of synaptic transmission in the lateral entorhinal cortex dependent on different receptor subtypes.

The mechanisms underlying the suppression of synaptic responses induced by high concentrations of dopamine were examined more closely using whole cell current clamp recordings of intracellular EPSPs from principal cells in

layer II of the lateral entorhinal cortex *in vitro* (Chapter 3). These experiments demonstrated that dopamine suppresses synaptic transmission through combined actions on mechanisms that mediate presynaptic glutamate release and intrinsic neuronal excitability. Activation of D_2 receptors by high concentrations of dopamine enhanced paired-pulse facilitation and reduced both the AMPA- and NMDA-mediated components of the excitatory synaptic response. At the same time, stimulation of D_1 receptors by dopamine increased a K^+ conductance which in turn reduced input resistance and contributed to the suppression of EPSPs. These are the first experiments to describe the mechanisms underlying the suppression of synaptic transmission by high concentrations of dopamine. These findings highlight a complex interaction between synaptic and membrane conductances that are modulated by dopamine to dampen synaptic transmission in layer II of the lateral entorhinal cortex.

Based on the finding that low concentrations of dopamine can facilitate basal synaptic transmission, it was hypothesized initially that enhancing extracellular levels of dopamine with GBR12909 might promote the induction of lasting forms of synaptic plasticity in the entorhinal cortex of awake rats (Chapter 4). However, systemic administration of GBR12909 at a dose that typically enhances basal synaptic transmission blocked the induction of both LTP and LTD in olfactory inputs to layer II. There are no other studies of the effects of dopamine on synaptic plasticity in the entorhinal cortex, and it is unclear why LTP and LTD were blocked in these experiments. Although administration of the same dose of GBR12909 has been shown to enhance extracellular levels of dopamine by 305% in the entorhinal cortex, we cannot be certain of the effective

concentration of dopamine at entorhinal synapses, and it is possible that the injection resulted in a much higher concentration that may have suppressed plasticity by suppression of basal synaptic transmission (Pralong & Jones, 1993; Stenkamp et al., 1998). However, the results may also suggest that dopaminergic innervation of the lateral entorhinal cortex prevents activity-dependent synaptic modifications from occurring and shifts the site of plasticity from the entorhinal cortex to other regions in the hippocampal formation (Lisman & Grace, 2005).

The experiments described in the previous chapters used electrophysiological techniques to assess the role of dopamine in modulating synaptic efficacy in the lateral entorhinal cortex. These experiments focused on the monosynaptic projection from the piriform cortex to the superficial layers of the entorhinal cortex which is often used as a model sensory system to examine the synaptic mechanisms underlying olfactory processing in the entorhinal cortex (Alonso et al., 1990; Caruana & Chapman, 2004; Caruana et al., 2007; Chapman & Racine, 1997b; de Curtis & Llinas, 1993). From the methods used and the findings obtained it was not possible to infer how dopamine-induced changes at the synaptic level within this pathway might contribute to behavioral performance on tasks requiring olfactory memory. Experiments described in the final chapter of this dissertation were designed to test how changes in dopaminergic tone within the entorhinal cortex influenced memory processing on an olfactory memory task that is known to involve the entorhinal cortex (Chapter 5; Dudchenko et al., 2000; McGaughy et al., 2005). Before receiving either sham or 6-OHDA lesions to the entorhinal cortex, food restricted rats were trained to

criterion performance on an olfactory non-match-to-sample task. Lesioned rats were significantly impaired relative to sham-operated controls during retraining two weeks following surgery. However, although it took nearly twice as long for the dopamine-depleted group to re-attain criterion levels of performance as the control group, the performance of the dopamine-depleted group was indistinguishable from the control group during subsequent testing when a version of the task with variable delays between the sample and test phases was used. These findings may imply that dopamine-depletion in the entorhinal cortex does not permanently impair performance on a memory task that requires maintenance of olfactory representations during a variable delay. Alternatively, there may have been sufficient recovery of basal levels of dopamine in the entorhinal cortex to permit normal functioning in the absence of major challenge to the dopamine system (Finkelstein et al., 2000; Robinson et al., 1990). Alternatively, the deficits during postsurgical retraining could also have resulted from a disruption of a variety of cognitive processes including olfactory sensory processing, attention, motor production, appetitive motivation, and loss of memory for the nature of the task.

Control of Sensory Input to the Hippocampal Formation

The entorhinal cortex is commonly thought of as a "gatekeeper" that regulates the flow of sensory information to the hippocampal formation (Fernández & Tendolkar, 2006; Kerr et al., 2007; Pinto et al., 2006; Witter et al., 1989; Witter et al., 1986; Wyss, 1981; Young et al., 1997). Dopaminergic inputs to the entorhinal cortex may, therefore, play an important role in opening or

closing this gate, and thereby filtering the sensory inputs that are ultimately conveyed to the hippocampus. Results obtained here indicate that changes in dopaminergic tone within the superficial layers of the lateral entorhinal cortex can affect the responsiveness of principal cells to afferent sensory drive, and this may affect subsequent spiking and the propagation of information to the hippocampal formation. Thus, dopaminergic inputs to the entorhinal cortex may function to open or close the gate in a manner determined by the level of extracellular dopamine driven by midbrain dopamine neuron output during various behavioral states.

The idea that dopamine merely serves to enhance or constrain entorhinal cortex output to the hippocampus may be too simplistic, and it is likely that dopamine interacts with other neuromodulatory transmitters to affect processes involved in the active maintenance of sensory representations in short-term memory. In particular, dopamine may work synergistically with acetylcholine to contribute to working memory processing in the entorhinal cortex. As noted in the *General Introduction*, there is considerable evidence to suggest that entorhinal cortex networks can support working memory (Egorov et al., 2002; Fransen, Alonso, & Hasselmo, 2002; Fransen et al., 2006; Klink & Alonso, 1997; Tahvildari et al., 2007; Young et al., 1997). In particular, stimulation of muscarinic receptors by acetylcholine activates a Ca^{2+}-sensitive nonspecific cation current in layer V neurons of the medial entorhinal cortex, and this promotes graded and persistent firing in these cells (Egorov et al., 2002; Fransen et al., 2006). Further, muscarinic receptor activation also permits layer III neurons in the lateral entorhinal cortex to be toggled into a persistent firing mode

following brief excitatory drive (Tahvildari et al., 2007). Interestingly, similar excitatory input can turn off the persistent activity and switch the cells back into a silent state. These data are also consistent with observations that unit activity in the lateral entorhinal cortex increases during the delay period of an olfactory non-match-to-sample task (Young et al., 1997). More recently, it has been shown that cholinergic lesions to the entorhinal cortex impair recall of novel sample odors on an olfactory non-match to sample task with a 15 min delay (McGaughy et al., 2005). These studies suggest that activation of cholinergic inputs to the entorhinal cortex promote the entrance of sensory representations into short-term working memory by triggering persistent firing activity dependent on muscarinic receptor activation. Although dopamine is not known to interact with currents that mediate persistent firing, it is possible that the facilitation of synaptic drive associated with moderate increases in dopamine might promote the activation of networks underlying olfactory working memory. Of course, the present data also suggest that excessive dopamine may suppress synaptic input to the entorhinal cortex, and it is likely that this might interfere with normal working memory function.

Interestingly, it has been proposed that the sustained spiking observed in the entorhinal cortex during a short 30-sec delay period of a delayed non-match-to-sample task (Young et al., 1997) is not likely to be maintained during longer delays lasting 15 to 180 min (McGaughy et al., 2005). It has therefore been suggested that increased delay period firing promotes the synaptic modifications required for longer-term maintenance of trial-specific information that needs to be retained for performance of the task (McGaughy et al., 2005). It is possible that

facilitation of glutamatergic transmission induced by dopamine may contribute to working memory by facilitating activity-dependent synaptic strengthening. However, this possibility rests on the assumption that the block of synaptic plasticity observed in Chapter 4 was due to an excess of dopamine induced by GBR12909, and that dopamine at more modest concentrations would enhance plasticity. Further, as noted in the Discussion of Chapter 2, a facilitation of extrinsic synaptic inputs to the entorhinal cortex might actually be expected to disrupt working memory representations maintained by intrinsic circuitry of the entorhinal cortex. It would interesting, therefore, to determine if dopamine has a facilitatory effect on induction of LTP and LTD under more controlled conditions *in vitro* in which concentrations can be well-regulated, and it would also be useful to determine if dopamine has differential effects on basal transmission in intrinsic versus extrinsic inputs to entorhinal neurons.

In addition to a general role in the gating of the flow of sensory information to the hippocampal formation, it is also interesting to speculate how dopaminergic inputs could shape the content of sensory information processed by the entorhinal cortex. If moderate levels of dopamine facilitate synaptic transmission of particular sensory inputs to the entorhinal cortex, then those inputs would be more likely to depolarize entorhinal cortex neurons and toggle them into a persistent mode of firing. Similarly, dopamine at higher concentrations might dampen levels of synaptic activation, and make it less likely for persistent firing states to be initiated in the entorhinal cortex. In order for dopaminergic inputs to have a content-specific effect on the facilitation or suppression of particular sensory inputs, however, it would be required that small

subsets of dopamine neurons terminate on groups of entorhinal cells that were processing semantically related sensory input, and that the subsets of dopamine neurons be selectively activated by inputs to the midbrain. This might help determine which patterns of activity would be maintained "in working memory" in the entorhinal cortex so that information would remain available for transmission to the hippocampus for further processing.

Concentration-Dependent Effects of Dopamine

One of the main findings to come out of the studies conducted for this thesis is that dopamine has concentration-dependent, bidirectional effects on basal synaptic transmission in the entorhinal cortex. This relationship between dopamine concentration and synaptic efficacy resembles the inverted U-shaped curve that has been proposed to describe the relationship between dopamine levels and D_1 receptor activation to working memory function in the prefrontal cortex (Arnsten, 1998; Seamans & Yang, 2004). A number of the results obtained in this thesis suggest that a similar inverted U-shaped function could underlie the complex effects of dopamine in the entorhinal cortex. If this were the case, moderate increases in dopamine would facilitate basal glutamatergic transmission, whereas synaptic transmission would be attenuated by excessive increases in dopaminergic tone or by loss of dopaminergic inputs (Fig. 6.1).

Facilitation of Transmission by Moderate Dopamine Levels. The inverted U-function model assumes that dopamine has an optimal facilitatory effect on synaptic transmission when dopaminergic tone increases moderately such as might be the case in behavioral states associated with appetitive motivation or

aversion (Seamans & Yang, 2004). This is consistent with the finding in the present experiments that administration of the dopamine reuptake inhibitor GBR12909 caused a facilitation of synaptic transmission, and with the finding that low concentrations of dopamine, *in vitro*, facilitated fEPSPs. It was initially expected that administration of GBR12909, *in vivo*, would promote the induction of LTP by enhancing basal synaptic transmission. However, there was no clear increase in basal responses in GBR12909-treated animals, and induction of both LTP and LTD were blocked, not enhanced. One interpretation is that GBR12909 in these animals induced a sufficiently high concentration of dopamine to cause competing D_1 and D_2 receptor-mediated effects and, thus, did not result in a net synaptic facilitation that could enhance induction of LTP or LTD. To sort out these possibilities, *in vitro* experiments using multiple, known concentrations of dopamine will be required.

Suppression of Transmission by Excessive Dopamine. *In vitro* results using both field potential recordings and whole-cell EPSPs showed that high concentrations of 50 and 100 µm dopamine suppress basal glutamatergic transmission. A suppression of the strength of piriform cortex inputs to the lateral entorhinal cortex is likely to disrupt olfactory sensory input to the entire entorhinal-hippocampal circuit. In addition, because the entorhinal cortex is thought to contribute to olfactory working memory (Egorov et al., 2002; Fransen et al., 2006; Tahvildari et al., 2007; Young et al., 1997), the suppression of glutamatergic transmission by high concentrations of dopamine would likely disrupt working memory function within the entorhinal cortex. Similarly, working memory function of the prefrontal cortex is also thought to be disrupted by

excessive dopamine concentration (Arnsten, 1998; Seamans & Yang, 2004) and has been linked to a suppression of synaptic transmission (Gao et al., 2001; Law-Tho et al., 1994; Seamans et al., 2001a; Urban et al., 2002; Zheng et al., 1999).

It is not clear how the suppression of synaptic transmission by dopamine may contribute to information processing within the entorhinal cortex. In models of prefrontal cortex function, the large increases in dopaminergic tone that result in a suppression of cortical function are thought to be brought about by periods of stress or even pathology in the mesocortical dopamine system (Arnsten, 1998), but it is not clear how a suppression of glutamatergic transmission may be considered "adaptive" at such times, either in the prefrontal cortex or in the entorhinal cortex. One possibility, however, dependent on the idea that stressful events may be associated with elevated temporal summation of sensory inputs to the entorhinal cortex, is that the concurrent dopaminergic suppression of transmission prevents induction of spurious synaptic plasticity. This may serve to protect the entorhinal cortex and to maintain stable online processing of sensory information within entorhinal cortex networks.

Suppression of Transmission by Reduced Dopamine Tone. One assumption of the inverted-U model is that extremely low concentrations of dopamine in the lateral entorhinal cortex can disrupt sensory processing by suppressing synaptic responses in layer II. Although there is no direct electrophysiological evidence to support this, and, in fact, bath-application of dopamine receptor antagonists alone during *in vitro* experiments had no effect on basal synaptic transmission, dopamine-depletion in the entorhinal cortex did

impair performance on an olfactory non-match-to-sample task during retraining two weeks following surgery. This suggests that a loss of dopaminergic tone in the entorhinal cortex disrupted synaptic processes related to the non-match-to-sample task. Specifically, the salience of reward-relevant stimuli may have been blunted in 6-OHDA-lesioned animals. This idea is also consistent with models of working memory function in the prefrontal cortex in which dopamine depletion or direct infusion of dopamine receptor antagonists can impair working memory (Arnsten, 1998; Seamans & Yang, 2004).

Concluding Remarks

In contrast to brain regions clearly linked to sensory or motor functions, the entorhinal cortex has an extensive and complex interconnectivity with a number of other areas that contribute to multiple sensory and cognitive functions. This makes determining the function(s) of the entorhinal cortex difficult to evaluate experimentally, and also makes observed changes in synaptic efficacy within the entorhinal cortex difficult to assign to a specific function. Similarly, it is difficult to determine the significance of the bidirectional modifications of synaptic strength induced by dopamine. As noted above, electrophysiological analyses of sensory pathways to the superficial layers of the lateral entorhinal cortex allows one to examine the initial steps of sensory processing within the entorhinal cortex. Thus, although the results of this dissertation can be reasonably interpreted in terms of dopaminergic modulation of olfactory processing, they provide only a glimpse into the role played by the entorhinal cortex in memory processing. However, new information regarding the function of the entorhinal

cortex is rapidly emerging. Recent data from behavioral and pharmacological studies of memory processing, electrophysiological analyses examining the mechanisms of working memory function, and studies examining the differential contributions of the medial versus the lateral entorhinal areas are significantly advancing our understanding of the functioning of the entorhinal cortex (Hafting et al., 2005; Hargreaves et al., 2005; McGaughy et al., 2005; Tahvildari et al., 2007; Young et al., 1997). Future experiments similar to those reported in Chapter 5 should also be useful in determining how the entorhinal cortex contributes to olfactory memory processing. Together, these studies focusing on the effects of dopaminergic inputs on synaptic processing within layer II, a relatively little studied question, may help to disentangle the role of neuromodulators in the overall functioning of the entorhinal cortex.

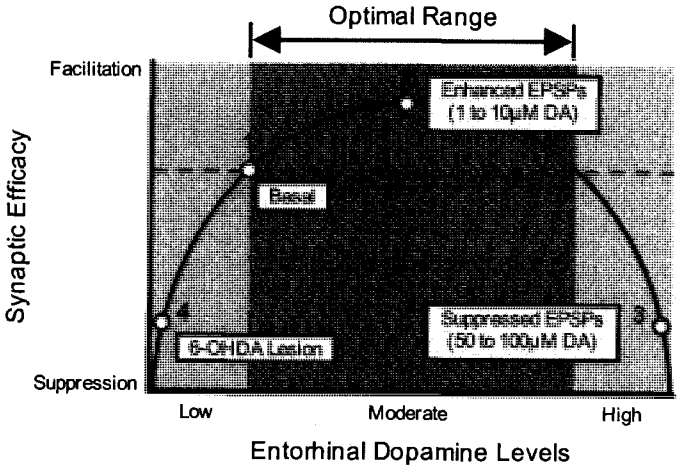
Optimal Range
Facilitation
Synaptic Efficacy
Suppression
Enhanced EPSPs
(1 to 10μM DA)
Basal
6-OHDA Lesion
Suppressed EPSPs
(50 to 100μM DA)
Low
Moderate
High
Entorhinal Dopamine Levels

Figure 6.1. Model indicating the proposed relationship between dopamine concentration in the entorhinal cortex and efficacy of glutamatergic synaptic transmission. The model indicates an optimal range for the concentration of extracellular dopamine that enhances synaptic transmission, and which may contribute to cellular processes involved in working memory function.

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