Dopaminergic Enhancement of Excitatory Synaptic Transmission in Layer II of the Lateral Entorhinal Cortex

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ABSTRACT

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Iulia Glovaci

Previous research demonstrated that dopamine produces concentration-dependent changes in synaptic transmission in the entorhinal cortex, wherein high concentrations of dopamine (50 μM) suppress evoked excitatory postsynaptic potentials (EPSPs) and lower concentrations of dopamine (1 to 10 μM) facilitate them. Whole-cell current clamp recordings were used to investigate the dopaminergic facilitation of synaptic responses in layer II neurons of the lateral entorhinal cortex. Surprisingly, the pattern of changes in EPSPs was dependent on cell type. During bath applications of 1 μM dopamine, fan cells showed a facilitation of the amplitude of EPSPs evoked by layer I stimulation. In contrast, pyramidal cells showed mixed modulation of EPSPs in response to dopamine, with different cells showing either facilitation or suppression effects. Voltage clamp recordings of excitatory postsynaptic currents suggest that dopamine facilitates AMPA glutamate receptor-mediated EPSCs. To determine the dopaminegic receptor subtype involved, either the D_1 receptor blocker SCH23390 (50 μM) or the D_2 receptor blocker sulpiride (50 μM) was bath applied to the slices prior to dopamine. Application of SCH23390 blocked the facilitation of EPSCs, whereas application of sulpiride had no significant effect. Therefore, the dopaminergic enhancement of EPSCs is likely to be mediated primarily through D_1-like receptors. D_1 receptors can act through a variety of intracellular signaling pathways to modulate synaptic strength. The role of signaling via protein kinase A was tested by including the PKA inhibitor H-89 in the recording pipette solution. Cells filled with H-89 did not show a

facilitation of EPSCs in response to dopamine application. Thus, the dopamine-induced facilitation of AMPA receptor-mediated synaptic responses in the lateral entorhinal cortex appears to be mediated via a D_1 receptor-dependent increase in PKA activity.

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ACSF	artificial cerebral spinal fluid
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
CA1, CA3	*Cornu Ammonis* fields of the hippocampus
cAMP	3'-5'-cyclic adenosine monophosphate
CNQX	7-nitro-2,3-dioxo-1,4-dihydroquinoxaline-6-carbonitrile
DARPP-32	dopamine- and cyclic AMP-regulated phosphoprotein
DMSO	dimethyl sulfoxide
EPSP	excitatory postsynaptic potential
EPSC	excitatory postsynaptic current
fEPSP	field excitatory postsynaptic potential
GABA	gamma-aminobutyric acid
LTP	long-term potentiation
NMDA	*N*-methyl-d-aspartate
PKA	protein kinase A
PP1	protein phosphatase 1

INTRODUCTION

Learning and memory are essential processes that regulate adaptive behaviours and support cognition in animals and humans. To further our understanding of the principles that govern these processes, neuroscientists have focused on determining which brain areas support these functions, and which distinct cellular mechanisms underlie them. Early studies have highlighted the crucial role of the hippocampal formation in learning and memory functions, but recently, a growing literature has started to emphasize the critical role played by the parahippocampal areas in these processes (Leonard, Amaral, Squire, & Zola-Morgan, 1995; Squire & Zola, 1996; van Strien, Cappaert, & Witter, 2009). As such, the entorhinal cortex, a major parahippocampal region, has been suggested to play a pivotal role in learning and mnemonic functions, given its location as an interface between the cortical mantle and the hippocampus (e.g. Witter et al., 1989; Fyhn et al., 2004). Neuroanatomical tracing studies have highlighted the critical role of the entorhinal cortex in information processing by demonstrating that the superficial layers of the entorhinal cortex receive widespread, converging inputs from all sensory modalities, both through direct and indirect projections (Burwell & Amaral, 1998). This multimodal sensory information was then shown to be conveyed to the dentate gyrus and CA3 regions of the hippocampus via the perforant path, and to the CA1 region of the hippocampus via the temporoammonic pathway, thus providing the hippocampus with most of its cortical sensory input (Van Hoesen & Pandya, 1975; Amaral & Witter, 1989; Burwell, 2000). Specific layers of the entorhinal cortex were shown to have different projections to the hippocampus, wherein layer II entorhinal neurons project to the dentate gyrus and the CA3

region (Ruth et al., 1988) and layer III neurons project primarily to the CA1 region and the subiculum (Witter et al., 1989). In turn, the CA1 region and subiculum project back to the deep layers of the entorhinal cortex (Amaral & Witter, 1989), providing a pathway through which the hippocampal formation sends a large part of its output back to neocortical areas via the entorhinal cortex (Amaral & Witter, 1989). Therefore, the entorhinal cortex can be described as the primary interface that mediates synaptic transmission between the neocortex and the hippocampus, both by mediating cortical sensory inputs *to* the hippocampus, and by mediating the output *from* the hippocampus back to neocortical areas. This degree of connectivity thus suggests that the entorhinal cortex plays a unique, pivotal role in the sensory and mnemonic functions of the temporal lobe (Sirota, Csicsvari, Buhl, & Buzsáki, 2003). Given its central anatomical placement and its suggested involvement in learning and memory processes, the present thesis has examined how synaptic transmission in the superficial layers of the lateral entorhinal cortex may be modulated, with a focus on how the neurotransmitter dopamine can affect the strength of evoked synaptic responses.

In addition to the anatomical evidence suggesting that a great amount of information travels between the neocortical areas and the hippocampus via the entorhinal cortex, behavioural evidence indicates that the entorhinal cortex plays a critical role in sensory and cognitive processing (Otto & Eichenbaum, 1992). Lesion studies in both animals and humans have indicated that interactions between cortical, parahippocampal and hippocampal areas contribute to networks that allow the coding, storage, and retrieval of declarative memories (Scoville & Milner, 1957; Zola-Morgan, Squire, & Mishkin, 1982; Squire & Zola-Morgan, 1991; Squire & Zola, 1996; Eichenbaum, 1999) and the

entorhinal cortex itself is believed to play a significant role in processes of attention, motivation, and memory (Swanson & Kohler; 1986; Galani et al., 1997; Yaniv et al., 2003). Given that the entorhinal cortex provides the hippocampus with the majority of its cortical sensory input, it can be difficult to discern whether memory deficits observed following lesions of the entorhinal cortex are due to disruption of entorhinal versus hippocampal function. Furthermore, previous lesion studies have often combined damage to both the perirhinal, entorhinal cortices and parahippocampal cortices. Combined lesions to the perirhinal and entorhinal areas in monkeys resulted in severe impairment on delayed nonmatching-to-sample tasks, although the impairment produced by damage to the entorhinal cortex alone was milder (Meunier, Bachevalier, Mishkin & Murray, 1993; Leonard, Amaral, Squire, & Zola-Morgan, 1995), suggesting that although the entorhinal cortex may not be *essential* for learning and performance on the task, it participates in the normal processes of learning mediated by the medial temporal lobe memory system. In fact, lesioning of the entorhinal cortex *in addition* to the perirhinal cortex significantly increases impairment on the task, compared to perirhinal lesions alone (Meunier et al., 1993). The entorhinal cortex is also the first brain structure to show neuronal degeneration in the early stages of Alzheimer's disease, suggesting that deficits associated with memory loss in the initial phases of the disease are due to localized cell loss and neurofibrillary tangles that first appear in superficial layers of the entorhinal cortex (van Hoesen, Hyman & Damasio, 2000). Furthermore, the degeneration of the entorhinal cortex progresses with the disease, resulting in a significant decrease in the volume of layer II of the entorhinal cortex (Kordower et al., 2001).

In his cytoarchitectural descriptions, Brodmann (1909) described and classified the entorhinal cortex in a variety of mammalian species, and suggested that the entorhinal cortex should be subdivided into two distinct domains, namely the lateral and the medial divisions (areas 28a and 28b, respectively). These two divisions of the entorhinal cortex can be distinguished in a number of ways. Foremost, perhaps, is the differentiation of their input signals. Previous studies have demonstrated that medial entorhinal cortex neurons fire in response to location-specific spatial inputs (Hargreaves, Rao, Lee, & Knierim, 2005) and that the medial entorhinal cortex incorporates specialized place cells, referred to as "grid cells", that are thought to contribute to spatial navigation (Young, Otto, Fox, & Eichenbaum, 1997) and to localization of position within the environment (Fynn et al., 2004; Hafting et al., 2005). In contrast, the lateral entorhinal cortex does not appear to be modulated by spatial information inputs (Hargreaves et al., 2005). Instead, lateral entorhinal cortex neurons fire in response to sensory stimulation from all cortices, although the strongest inputs arrive from olfactory regions (Young et al.,1997; Petrulis, Alvarez, & Eichenbaum, 2005). Based on these findings, it has been suggested that the lateral entorhinal cortex plays an overarching role in the formation of non-spatial memories (Hargreaves et al., 2005; Steffenach, Witter, Moser, & Moser, 2005; Kniermin, Lee, & Hargreaves, 2006; Kerr, Agster, Furtak, & Burwell, 2007). This conclusion is not surprising given that the lateral entorhinal cortex receives strong inputs of highly processed information from the primary olfactory (piriform) cortex (Boeijinga & Van Groen, 1984; Van Groen et al., 1987; Biella & de Curtis, 1995; Burwell, 2000), the insular regions, and the amygdala (Kerr et al., 2007), suggesting that it may provide the hippocampus with

nonspatial information about the situational context (Kerr et al., 2007). In contrast, the medial entorhinal cortex receives its densest innervations from the hippocampal and parahippocamal structures, as well as from the dorsal thalamus, visual, posterior parietal, and cingulate cortices, areas that are implicated in spatial learning and memory processes (Mizumori & Williams, 1993; Taube, 1995; Burwell, Witter, & Amaral, 1995; Amaral & Witter, 1995; Kerr et al., 2007). Moreover, the lateral and the medial entorhinal areas give rise to two distinct divisions of the perforant path: the lateral and the medial perforant paths. Both projections terminate in the hippocampal formation, but they innervate distinctly different parts of the dentate gyrus, CA3 and CA1 regions (Hjorth-Simonsen & Jeune, 1972; Witter, Groenewegen, Lopes da Silva & Lohman, 1989; Amaral & Witter, 1995; Dolorfo & Amaral, 1998) and their cells of origin have a different peptide content (McNaughton, 1980;Gauthier, Destrade, & Soumireu-Mourat, 1983), suggesting different functions. Based on these noticeable input-output connections, it has been previously suggested that the medial and lateral divisions of the entorhinal cortex are likely to play different roles in sensory processing, learning, and memory; the lateral entorhinal cortex was postulated to provide sensory, situational-contextual "what" information, whereas the medial entorhinal cortex was postulated to provide spatial and navigational "where" information (Kerr et al., 2007).

At the cellular level, the dissimilar organization of the medial and lateral divisions of the entorhinal cortex also supports a difference in function of these two regions. The principle cell types in layer II of the medial and lateral entorhinal cortex do not share the same morphological and electrophysiological characteristics (Tahvildari & Alonso, 2005).

The medial entorhinal division is characterized primarily by stellate cells and, to a lesser extent, pyramidal cells. In contrast, three subtypes of neurons are observed within layer II of the lateral division: 'fan'-like cells (which are similar to stellate cells), pyramidal cells, and multiform cells (as previously described by Tahvildari & Alonso, 2005). Fan cells are characterized by a sag in their voltage response to strong hyperpolarizing current injection, and are known to fire action potentials in intermittent clusters, paced by theta-frequency membrane potential oscillations. In contrast, pyramidal neurons fail to show a sag response, and fire regularly. Multiform cells are characterized by a combination of these electrophysiological properties (Tahvildari & Alonso, 2005). Although all three cell types project to the dentate gyrus, they receive slightly different synaptic inputs via layers II and III, with greater inputs in layer III for pyramidal and multiform neurons (Tahvildari & Alonso, 2005), and their differences in cellular properties suggest that they may serve different computational functions in the entorhinal cortex. It is currently unknown whether the activity of these distinct cell types is modulated differently by dopamine. However, a differential modulation of synaptic activity by dopamine between cell types could suggest different roles for the cell types in cortical functions associated with release of dopamine.

The neurotransmitter dopamine is known to be involved in a variety of cognitive processes including motivation, reward, and stress (Schultz, 2005; Hyman et al., 2006; Wise, 2006; Berridge, 2007), and there is strong evidence that dopamine modulates cellular processing related to working memory (Sawaguchi & Goldman-Rakic, 1991; Seamans & Yang, 2004). For example, there is evidence that dopamine can facilitate working memory-related functions in the prefrontal cortex, a key brain area involved in learning and

memory processes (Fuster, 2000), and that the observed facilitation is consistent across species, notably in rodents (Sakurai & Sugimoto, 1985), primates (Passingham, 1975), and humans (Müller, von Cramon, & Pollmann, 1998). A large body of evidence suggests that dopamine may also contribute to working memory function by facilitating spontaneous activity and firing rates of neurons in the prefrontal cortex (e.g. Sawaguchi, Matsumure, & Kubota, 1990; Collins et al., 1998; Seamans, Floresco, & Phillips, 1998). This dopaminergic modulation of neuronal activity appears to be concentration-specific, wherein low doses of dopamine promote learning and memory, and high doses of dopamine inhibit theses same processes, according to a dose-depended, inverted bell-shape (e.g. Seamans, Gorelova, Durstewitz, & Yang, 2001).

Furthermore, the manner in which dopamine modulates synaptic activity and learning and memory processes appears to be receptor-subtype specific. Dopamine receptors may be divided into two major subclasses, namely D_1-like (D_1, D_5) and D_2-like (D_2 - D_4) receptors (Stoof & Kebabian, 1984; Sibley & Monsma, 1992; O'Dowd, 1993). These two classes of receptors are coupled to different G-proteins and have distinct electrophysiological and pharmacological properties (Cooper, Bloom, & Roth, 1991), thus they are likely to serve different modulatory roles. Previous research has shown that D_1-like receptors may modulate behavior (Malloy & Waddington, 1984), affect behavioral arousal (Ongini, Caporali, & Massotti, 1985) and modulate memory processes (Sawaguchi & Goldman-Rakic, 1991) by facilitating synaptic transmission. For example, Sawaguchi et al. (1988) suggested that D_1-like receptors alone may modulate neuronal responses, after observing that the typical facilitation of responses typically seen in the prefrontal cortex

during a working memory task in monkeys was abolished in the presence of a D_1-like receptor antagonist, but that it was unaltered by application of the D_2-like antagonist sulpiride. These findings are supported by a consistent body of literature indicating that dopaminergic facilitation of synaptic responses and increased firing induced by dopamine are largely dependent on D_1-receptor-mediated mechanisms; in turn, D_1-like receptors may act by enhancing glutamate-mediated synaptic responses (Sawaguchi & Goldman-Rakic, 1991; Funahashi, Bruce & Goldman-Rakic, 1993; Puig & Miller, 2012). To illustrate, dopamine has been shown to enhance isolated AMPA-mediated currents in layers II and II of the prefrontal cortex via a postsynaptic D_1-receptor mediated mechanism (Gonzalez-Islas & Hablitz, 2003; Bandyopadhyay, Gonzalez-Islas, & Hablitz, 2005), possibly by increased phosphorlylation of AMPA receptors (Prince et al., 1995, Snyder et al., 2000). Activation of D_1-like receptors during behavioural states in which dopamine is released, such as appetitive motivation, may therefore contribute to working memory function by enhancing synaptic responses and facilitating maintained neuronal firing which may help maintain memory representations.

However, although the role of dopamine has been studied extensively in the prefrontal cortex, and, to a lesser degree, in the hippocampus, the mechanisms of action of dopamine in the entorhinal cortex remain poorly understood. It is likely, however, that dopamine release in the lateral entorhinal cortex may have substantial modulatory effects on neurons that mediate sensory and/or mnemonic functions, especially as it relates to processing of olfactory information. Previous anatomical studies have demonstrated that layers II and III of the lateral entorhinal cortex receive robust dopaminergic projections,

with strong terminations within the 'cell islands' typically observed in superficial layers II and III (Lindvall, Björklund, Moore, & Stenevi, 1974; Fallon & Loughlin, 1987; Oads & Halliday, 1987; Lingenhöhl & Finch, 1991). Although both the medial and lateral entorhinal cortex receive dopaminergic inputs, dopaminergic inputs are much stronger to that lateral entorhinal region (Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1987). This suggests that dopamine is likely to be a stronger modulator of the sensory and mnemonic functions of cells within the lateral entorhinal division compared to the medial division.

Previous research investigating the effects of dopamine in the entorhinal cortex has suggested that high doses of dopamine *suppress* the amplitude of glutamate-mediated synaptic responses in layers II, III and V of the medial entorhinal cortex (Pralong & Jones, 1993; Stenkamp, Heinemann, & Schmitz, 1998). However, in contrast to these findings, more recent studies in the lateral entorhinal cortex demonstrated dose-dependent, bidirectional effects, wherein dopamine can either suppress or *facilitate* synaptic transmission in vitro (Caruana, Sorge, Stewart, & Chapman, 2006). Application of a high concentration of dopamine (50 - 100 μM) resulted in a strong suppression of synaptic transmission, mediated via a D_2 receptor-mediated suppression of transmitter release, and a D_1 receptor-mediated reduction in postsynaptic input resistance (Caruana et al., 2006). In contrast, application of a lower dose of dopamine (10 μM) dopamine resulted in a D_1-like receptor-mediated *facilitation* of synaptic responses (Caruana et al., 2006). These results are consistent with previous findings in the prefrontal cortex, where dopamine also induced a bidirectional, dose-dependent modulation of synaptic transmission (e.g. Seamans et al., 2001). Thus, these findings indicate that dopamine may have powerful effects on cognitive

functions mediated by the entorhinal cortex during behavioral states associated with release of dopamine. However, the exact cellular mechanisms of action through which the neuromodulator dopamine regulates the functioning of the lateral entorhinal cortex are not fully understood.

Here, we have used visually-guided whole-cell recordings to characterize the dopaminergic facilitation of intracellular excitatory postsynaptic potentials (EPSPs) among the major cell types within the lateral entorhinal cortex. Current clamp recordings were first used to assess the effect of a low concentration of dopamine on firing properties and to assess changes in excitatory and inhibitory synaptic potentials in electrophysiologically differentiated fan and pyramidal neurons. Voltage clamp recordings were then used to evaluate the dopamine receptors that mediate the facilitation of excitatory postsynaptic currents (EPSCs), and the signaling mechanisms that mediate the facilitation of excitatory synaptic responses. Activation of D_1-receptors can increase the production of cyclic AMP by adenylyl cyclase through activation of G_s proteins, and the increased cyclic AMP can lead to activation of protein kinase A (Young & Yang, 2004; Wang & O'Donnell, 2001; Gonzalez-Islas & Hablitz, 2003; Kruse, Premont, Krebs, & Jay, 2009). In the prefrontal cortex, dopamine D_1 receptor-mediated increases in synaptic responses have been linked to activation of PKA that can result in increased AMPA and NMDA receptor-mediated responses (Gonzales-Islas & Hablitz, 2003; Sun, Zhao & Wolf, 2005). Furthermore, previous electrophysiological studies have also suggested that the activity of PKA may indirectly gate synaptic changes by modifying AMPA-mediated responses (Blitzer et al., 1998). In the presence of low levels of PKA, the PP1 inhibitor, “inhibitor 1”, is rendered

inactive. This inactivation of inhibitor 1 results in an increase in PP1 activation, that, in turn, inhibits increases in synaptic strength. Conversely, high levels of cAMP and PKA activate inhibitor 1, thus inhibiting PP1 and promoting synaptic strengthening (Silva, 2003). Further, in the hippocampus, the application of D_1 receptor agonists, working through activation of adenylyl cyclase, can induce a long-term potentiation of field excitatory postsynaptic potentials (fEPSPs) that can last for up to 6 hours (Huang & Kandel, 1995). Previous research has demonstrated that protein kinase A mediates the phosphorylation of AMPA receptors (reviewed by Song & Huganir, 2002). For example, in the hippocampus, both intracellular and/or extracellular application of PKA activity-modifiers results in a significant potentiation of AMPA and kainate currents (Wang et al., 1991) and PKA activation increases the opening frequency *and* mean opening duration of these receptor channels to enhance the amplitude of excitatory postsynaptic currents (Greengard et al., 1991). These findings show that increases in PKA activation result in an overall enhancement of EPSC amplitudes in hippocampal cells. Thus, the present study also investigated the potential role of the cAMP-PKA signaling pathway in mediating the facilitation of synaptic currents in the superficial layers of the entorhinal cortex. The role of PKA was investigated by inhibiting its activation in recorded neurons with the PKA inhibitor H-89 included in the intracellular recording solution.

METHOD

In Vitro Slice Preparation

The preparation of brain slices for whole cell recordings was conducted in accordance with the guidelines of the Canadian Council on Animal Care and the Concordia

University Animal Research Ethics Committee as described previously (e.g. Caruana et al., 2006). Briefly, acute brain slices were collected from 4-6 week-old male Long-Evans rats (Charles River). The animals were deeply anesthetized with halothane prior to decapitation, and the brains were quickly extracted and submerged into an ice-cold, high-sucrose artificial cerebrospinal fluid solution (ACSF; bubbled with 95% O_2 and 5% CO_2 , pH 7.4) containing (in mM) 2 KCl, 1.25 NaH_2PO_4, 7 $MgCl_2$, 26 $NaHCO_3$, 250 sucrose and 10 D-(+)-glucose. Slices (300 μM thick) containing both the hippocampal and entorhinal regions were obtained using a vibratome (WPI, Vibroslice, Sarasota, USA) and were allowed to recover for a period of an hour at room temperature (~22C), in ACSF 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). Slices were then transferred to the recording chamber, where they were kept submerged in ACSF by a nylon net. Individual slices were visualized using an upright microscope (Leica, DM-LFS) equipped with a 40x water immersion objective and differential interference contrast optics. The flow rate of the ACSF in the chamber was regulated at a rate of 2 mL/min at all times. Layer II of the lateral entorhinal cortex was identified visually, based on the characteristic presence of clusters of cells in 'islands' which is unique as compared to the organization of layers I and III (Blackstad, 1956; Carboni & Lavelle, 2000).

Stimulation and Recording

Intracellular evoked EPSPs and EPSCs were recorded in fan, pyramidal, and multiform cells in layer II of the lateral entorhinal cortex. Neurons were classified based on their electrophysiological profiles, as previously described by Tahvildari and Alonso

(2005). Recording pipettes used for whole-cell recordings were pulled from borosilicate glass (1.0 mm OD, 3 to 6 MΩ) and were filled with a standard recording 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 7.2-7.3 with KOH). Pipettes were placed onto the soma of the target entorhinal cells, and gentle negative pressure was applied to the neuron under voltage-clamp conditions to form a tight seal (1-2 GΩ). Stronger pressure was used to rupture the cell membrane and cells were allowed to stabilize for 3-5 minutes prior to the start of current-clamp experiments, and 10-15 minutes prior to the start of voltage-clamp experiments. Synaptic responses were evoked using a bipolar stimulating electrode made from two tungsten electrodes (~1 MΩ, FHC Inc.) placed in layer I of the entorhinal cortex, about .1 to .2 mm rostral to the recording electrode. Synaptic responses were evoked through 0.1 ms-duration constant current pulses delivered using a stimulus timer and isolation unit (WPI, Models A300 and A360). Current and voltage clamp recordings were obtained using an Axopatch 200B amplifier, filtered at 10 kHz, and digitized at 20 kHz (Axon Instruments, Digidata 1322A) for storage on computer hard drive.

Series resistance was estimated in current clamp recordings by compensating for the discontinuity in the voltage response to -50 pA current pulses, and recordings were accepted if series resistance was < 20 MΩ (M: 14.3 ± 1.3 MΩ). Input resistance was monitored regularly, and was determined from the peak voltage response to a 500 ms, -100 pA current pulse from a holding level of -60 mV. Membrane potential responses to 500 ms duration hyperpolarizing and depolarizing steps (range -200 to +60 pA) was used to

characterize input resistances and firing properties of all neurons. Inward rectification was quantified as the ratio between peak and steady-state input resistances in response to a -200 pA hyperpolarizing current pulse (rectification ratio). In voltage clamp recordings, series resistance was monitored from the transient response to at the onset of 100 ms 3 mV voltage steps (mean: 27 ± 2.6 MΩ), and input resistance was measured based on the current response at the end of the voltage steps. Recordings were discontinued if either value changed by >15%.

Electrophysiological characteristics of entorhinal neurons were analyzed using the Clampfit 8.2 software package (Axon Instr.). Spike properties were measured from the first action potential evoked in response to a 500 ms duration positive current injection, and action potential amplitude was calculated from resting membrane potential. Action potential duration and afterhyperpolarization were measured from action potential threshold. Fan cells displayed a sag in the voltage response to strong hyperpolarizing current injection, and fired action potentials in intermittent clusters that were paced by theta-frequency membrane potential oscillations, while pyramidal neurons showed a regular firing pattern and no sag response. Remaining neurons that did not fit these profiles were classified as multiform neurons, as per previous records (Tahvildari & Alonso, 2005).

Excitatory Postsynaptic Potentials

The effect of dopamine of EPSPs was assessed by comparing EPSPs recorded in normal ACSF to those recorded following constant bath application of 1 μM dopamine for a period of 5 min, and following 20 min washout of dopamine in normal ACSF. Paired-pulse stimuli, in which two pulses were delivered separated by a 20 ms interval,

were used to evoke EPSPs, and 5 paired-pulse stimuli, separated by intervals of 10 sec, were obtained during each recording condition.

Excitatory Postsynaptic Currents

Recordings of EPSCs were obtained in order to determine if dopamine has a facilitatory effect on AMPA receptor-mediated synaptic currents. The EPSCs were evoked by stimulation of layer I inputs at a hyperpolarized holding potential of -70 mV in order to eliminate the contribution of NMDA receptor-mediated currents. Experiments in 5 cells verified that EPSCs were completely blocked by the AMPA receptor blocker 7-nitro-2,3-dioxo-1,4- dihydroquinoxaline-6-carbonitrile (CNQX, 50 μM; data not shown). Ten EPSCs evoked by single pulses were evoked during initial baseline recordings in normal ACSF, following 5 min application of dopamine, and following wash in normal ACSF.

To determine whether the facilitation of responses by dopamine is dependent on D_1- or D_2-like receptors, the effects of dopamine on EPSCs were assessed during constant bath application of selective dopamine-receptor antagonists. Following a baseline period in normal ACSF, responses were recorded in the presence of either the D_1 receptor antagonist SCH23390 (10 μM) or the D_2 receptor antagonist sulpiride (50 μM; Caruana et al., 2006), and then following application of 1 μM dopamine for 5 min.

For experiments testing the role of protein kinase A in the facilitation of synaptic currents by dopamine, the PKA inhibitor H-89 (10 μM) was included in the intracellular recording pipette solution, and evoked responses were recorded prior to and following application of 1 μM dopamine.

Drugs

All drugs, except H-89 (Ascent) were obtained from Sigma-Aldrich. Drugs were stored as concentrated stock solutions at –20ºC until needed except for sulpiride which was dissolved daily in 6% DMSO in ACSF titrated with 0.1 N HCl, and diluted to a final concentration of 50 μM in ACSF with 0.1% DMSO. Dopamine HCl was also dissolved just prior to bath application.

Since dopamine oxidizes rapidly and is light sensitive, sodium metabisulfite was also applied to slow the degradation of dopamine (Stenkamp Heinemann, & Schmitz, 1998; Yang & Seamans, 1996).

Data Analysis

Changes in synaptic responses recorded from entorhinal cortex neurons were analyzed using Clampfit 8.2 software (Axon Instr.). The amplitudes of ten consecutive synaptic responses were measured relative to the pre-stimulus baseline and averaged for each phase of recordings. The slopes of EPSPs and EPSCs were also measured, and changes were congruent with shifts in the amplitude of the evoked responses. Paired-pulse facilitation ratio was determined by expressing the amplitude of the response to the second pulse as a percentage of the response to the initial pulse. Data were analyzed using ANOVAs followed by Newman-Keuls *post hoc* comparisons to determine the statistical significance of changes in cellular properties and synaptic responses in response to drug application. Summary data were presented as group means ± standard error of the mean. Significance level was set to $p < 0.05$.

RESULTS

Dopaminergic Effects on Cellular Properties and EPSPs

Recordings were obtained from 23 neurons in the layer II of lateral entorhinal cortex, and neurons were classified into three categories based on their electrophysiological properties (Tahvildari & Alonso, 2005; Figure 1). As representative of the cell populations in the lateral entorhinal cortex, most cells were either fan (n = 16) or pyramidal cells (n = 10). Fan cells (n = 16) were characterized by a marked sag in the voltage response to strong depolarizing current injection, and the presence of theta-frequency voltage-dependent membrane potential oscillations that paced the clustering firing of action potentials. Pyramidal neurons fired more regularly, and showed no substantial sag response (n = 10). Multiform neurons had shared properties, such that they showed a sag but also had regular firing, or vice-versa (n = 3).

Dopamine had similar effects on the electrophysiological properties of fan and pyramidal neurons (Table 1). Similar to previous findings with higher concentrations of dopamine (Caruana et al., 2006, Uchimura, Higashi & Nishi, 1986, Benardo & Prince, 1982) there was a statistically significant hyperpolarization of the resting membrane potential induced by 1 μM dopamine in both fan (t_8 = 3.44, p < .01) and pyramidal neurons (t_8 = 2.74, p < .05). In addition, the medium afterhyperpolarization showed a small but significant reduction in amplitude in fan cells (t_8 = 8.20, p < .01), but did not show a significant change in amplitude in pyramidal neurons (t_8 = .13, p = .89). Although previous studies have linked a change in medium afterhyperpolarization to changes in the excitability of neurons (Jonge, Black, Deyo, & Disterhoft, 1990; Storm, 1989) there was no

observed significant change in the number of spikes evoked in fan cells by a 500 ms duration, 20 pA current pulse delivered in cells held near -65 mV using constant current injection (Table 1). Other cellular properties, including input resistance, rectification ratio, and action potential waveform were not affected by 1 μM dopamine.

Application of 1 μM dopamine caused an increase in the amplitude of postsynaptic potentials evoked by stimulation of layer I in the layer II entorhinal cortex neurons recorded. Baseline synaptic responses had a mean amplitude of 2.88 ± 0.45 mV, and bath application of dopamine for 5 min significantly facilitated the synaptic responses to 3.38 ± 0.60 mV, or 117.5 ± 10.4 % of baseline values ($F_{2, 36} = 3.94$, $p < .05$; N.-K., $p < .05$). After a washout period in normal ACSF for a period of 20 min, responses returned to baseline. The facilitation was also found to be dependent on the type of cell recorded (Figure 2). Fan cells showed a moderate but consistent facilitation in the amplitude of EPSPs to 117.9 ± 5.4 % of baseline ($n = 16$; $F_{2, 15} = 7.99$, $p < .01$; N.-K., $p < .01$), whereas changes in EPSPs in pyramidal cells were more variable, such that individual pyramidal cells could show either a suppression or a facilitation of EPSPs, and there was a larger, but not statistically significant increase in the mean amplitudes of EPSPs to 144.20 ± 21.78 % of baseline levels, (range: 30.33 to 308.85 % of baseline; $n = 10$; $F_{2, 9} = 3.04$, $p = .078$). The EPSPs recorded in the small group of multiform cells did not appear to be modulated by dopamine as the average amplitude of the responses did not change significantly with application of dopamine ($n = 3$; 103.08 ± 4.34 % +of baseline; $F_{2, 2} = 1.18$, $p = .39$).

The increased EPSPs in fan and pyramidal neurons were not associated with any significant changes in paired-pulse facilitation ratio. Baseline paired-pulse facilitation

tended to be larger in pyramidal neurons than in fan neurons, but there was no significant change in paired-pulse ratio induced by dopamine in either cell type (216 ± 36 % vs. 235 ± 37 % for pyramidal cells, and 172 ± 12 % vs. 182 ± 12 % in fan cells; Figure 2). Increases in neurotransmitter release can be associated with a reduction in paired-pulse facilitation effects because of a reduction in the pool of readily releasable transmitter (Zucker, 1999), and the stability of paired-pulse facilitation ratios observed here suggest that the synaptic enhancement induced by dopamine is expressed post-synaptically.

Dopaminergic Modulation of EPSCs

Voltage-clamp recordings of EPSCs were used to characterize the synaptic currents evoked in layer II entorhinal neurons by stimulation of layer I inputs, and to investigate the transmitter receptors and postsynaptic signals involved in the dopamine-induced facilitation of synaptic responses. The same intracellular recording solution was used for recordings of EPSPs and EPSCs. In order to isolate the AMPA receptor-mediated component of responses, voltage clamp recordings of evoked EPSCs were obtained at a holding potential of -70 mV. Recordings in a group of cells showed that application of the AMPA receptor blocker CNQX (50 μM) entirely blocked the synaptic response, indicating that it is mediated almost exclusively by AMPA receptors, with little or no contribution of NMDA receptors in these recording conditions ($n = 5$, data not shown). Application of 1 μM dopamine caused the amplitude of EPSCs to increase significantly to 138.07 ± 8.7 % of baseline values ($n = 7$; $F_{2,6} = 7.23$, $p < .01$; N.-K., $p < .01$). Responses returned towards baseline values in 4 of 7 slices, but responses in remaining slices remained elevated, indicating only a partial washout of the effect of dopamine on EPSCs after 10 minutes

(Figure 3) .

The dopamine receptors that mediate the facilitation of EPSCs were investigated by co-applying dopamine in the presence of D_1 or D_2 receptor blockers. Application of the specific D_2 receptor antagonist sulpiride (50 μM) had no significant effect on the amplitude of baseline EPSCs. Further, application sulpiride did not significantly affect the size of the facilitation of EPSCs induced by dopamine, and application of dopamine in the presence of sulpiride caused a reversible increase in EPSC amplitude to 203.5 ± 65.1 % of baseline values ($n = 6$; $F_{3, 5} = 3.41$, $p = .05$; Figure 5). Bath application of the D_1 receptor antagonist SCH 23390 (50 μM) alone had no significant effect on baseline synaptic responses ($t_4 = .06$, $p > .05$). However, the presence of SCH 23390 significantly blocked the facilitation of evoked responses induced by dopamine, and EPSCs remained close to baseline values (97.2 ± 12.2 mV), such that the blocking of D_1-like receptors completely prevented the facilitation of evoked currents by dopamine ($n = 5$; $F_{3, 4} = 2.87$, $p = .09$; Figure 4). Therefore, a D_1-like receptor-mediated mechanism appears to mediate the facilitation of evoked synaptic currents in the lateral entorhinal cortex.

The PKA inhibitor H-89 was added to the intracellular recording solution in order to test the potential role of PKA activation for dopaminergic modulation of evoked EPSCs in layer II neurons. The amplitude of EPSCs did not vary significantly between the baseline period, following dopamine application, or following washout in normal ACSF. In the presence of 1 μM dopamine, EPSC amplitude remained at 102.5 ± 6.3 % of baseline values, indicating that inhibiting PKA activation blocks the facilitation of EPSCs induced by dopamine ($n = 6$; $F_{2, 5} = .47$, $p = .64$; Figure 5). The activation of D_1 receptors by

dopamine is therefore likely to lead to the facilitation of AMPA-mediated EPSCs through a signaling pathway involving activation of PKA.

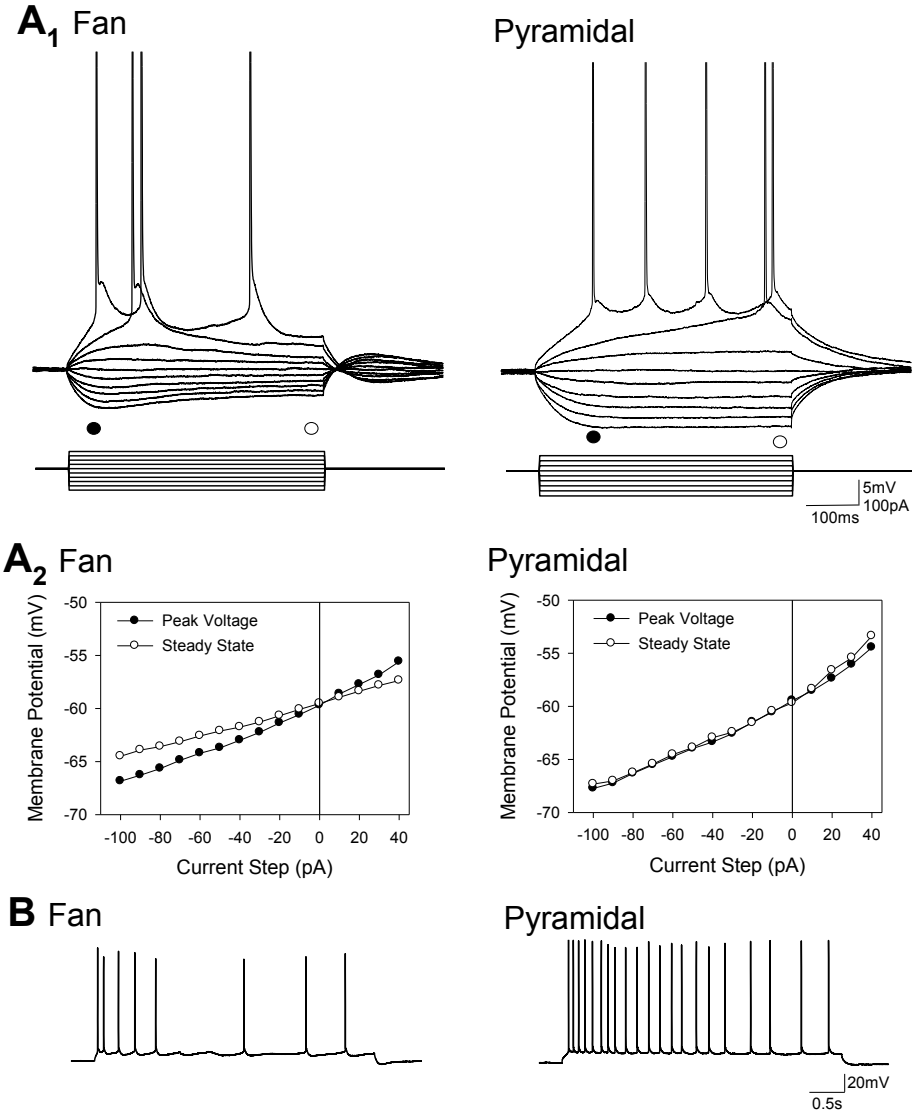
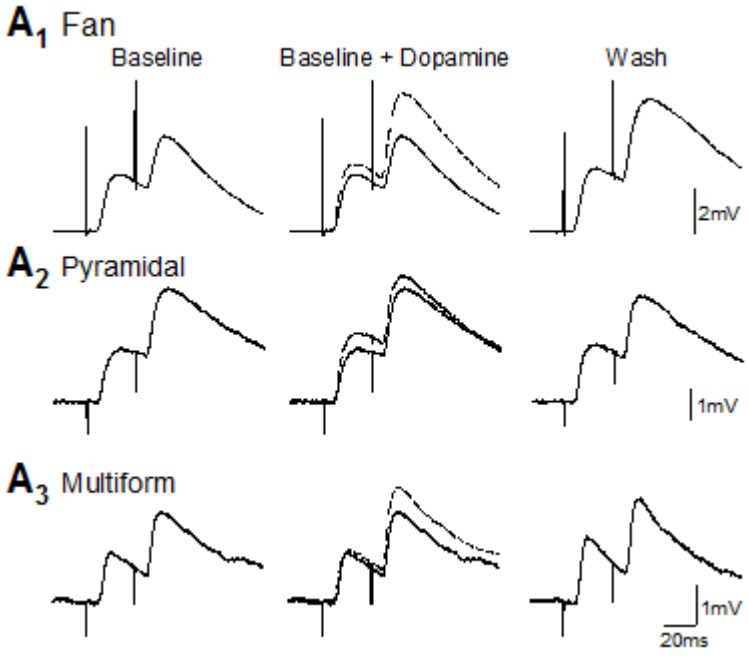

A1 Fan
Pyramidal
5mV
100pA
100ms
A2 Fan
Pyramidal
Peak Voltage
Steady State
Membrane Potential (mV)
-50
-55
-60
-65
-70
-100 -80 -60 -40 -20 0 20 40
Current Step (pA)
B Fan
Pyramidal
20mV
0.5s

Figure 1. Electrophysiological profiles of fan and pyramidal cells in the lateral entorhinal cortex. **A.** Voltage responses to positive and negative current steps have been superimposed for a representative fan cell and pyramidal cell (A_1). Note the hyperpolarization-dependent inward rectification in the fan cell that is absent in the pyramidal cell. Current-voltage plots in A_2 show the peak and steady-state voltage responses to current steps measured at the time-points indicated by the open and closed circles in A_1. **B.** The pattern of action potential firing in response to prolonged strong positive current injection is shown for the fan and pyramidal cells. Fan cells tended to fire periodically compared to the more regular discharge in pyramidal cells.


A1 Fan
Baseline
Baseline + Dopamine
Wash
2mV
A2 Pyramidal
1mV
A3 Multiform
1mV
20ms

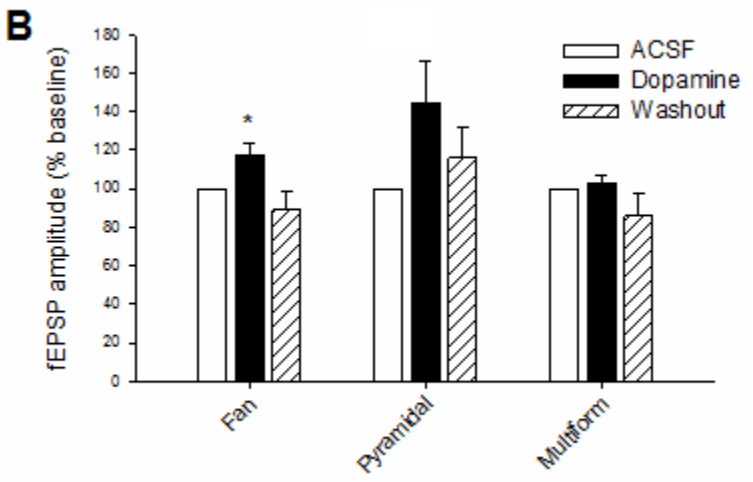

B
fEPSP amplitude (% baseline)
180
160
140
120
100
80
60
40
20
0
*
ACSF
Dopamine
Washout
Fan
Pyramidal
Multiform

Figure 2. Dopamine facilitates EPSPs in fan and pyramidal neurons of the lateral entorhinal cortex. **A.** Intracellular EPSPs evoked by paired-pulse stimulation (20 ms interval) are shown for representative fan, pyramidal, and mulitform neurons during baseline recordings, during addition of 1μM dopamine, and following 20 min washout in normal ACSF. Traces recorded in the presence of dopamine (dashed) have been superimposed with baseline traces for comparison. **B.** The mean amplitudes of EPSPs in fan cells (n = 16) were significantly and reversibly increased by dopamine (*). The facilitation of EPSPs in pyramidal cells (n = 10) did not reach statistical significance ($p <$ .078), and no reliable change was observed in a small group of multiform neurons (n = 3).

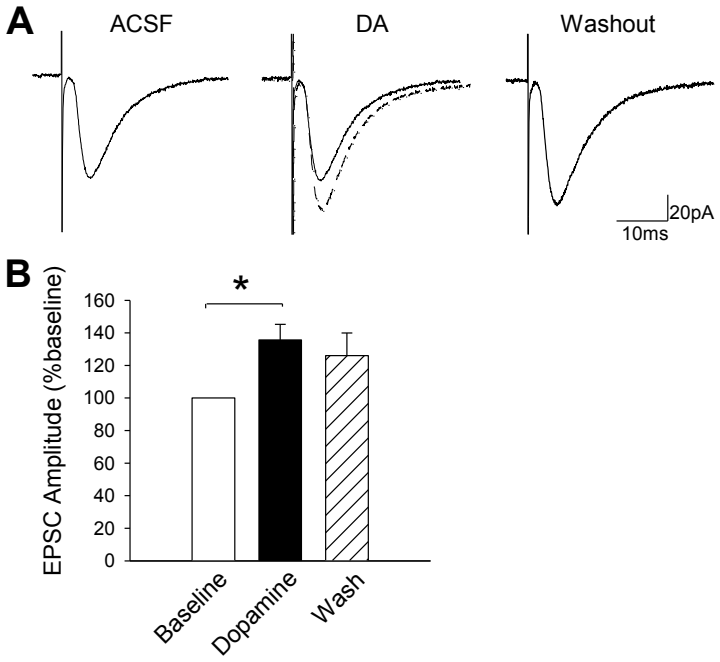
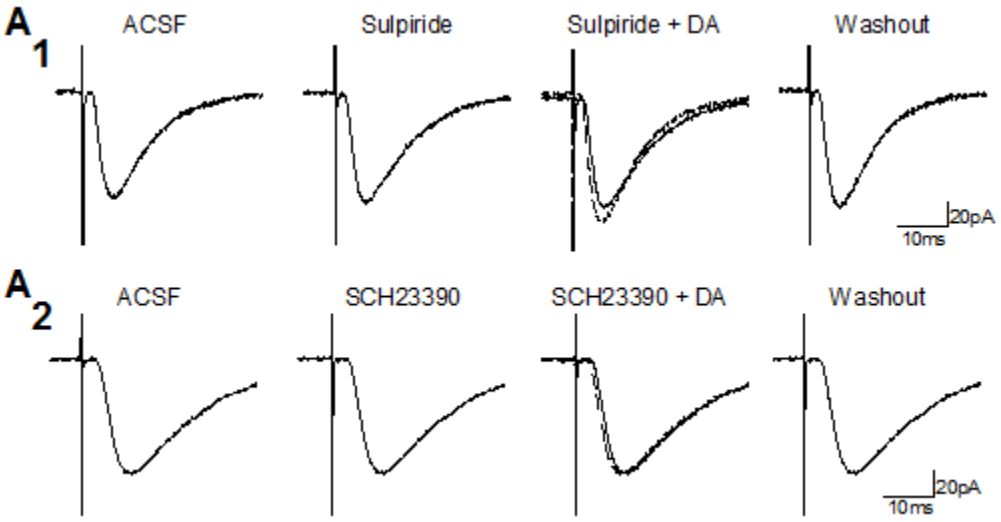

A
ACSF
DA
Washout
20pA
10ms
B
EPSC Amplitude (%baseline)
160
140
120
100
80
60
40
20
0
*
Baseline
Dopamine
Wash

Figure 3. Application of dopamine facilitates the amplitude of AMPA receptor mediated excitatory postsynaptic currents (EPSCs) in the lateral entorhinal cortex. **A.** Sample traces of averaged representative EPSCs recorded at a holding potential of -70 mV, are shown during baseline recordings in normal ACSF, after 5 min application of 1 μM dopamine, and after a 10 min washout period in normal ACSF. **B.** A histogram shows group averages of EPSC amplitudes recorded during baseline, dopamine application, and the washout period, and indicates a significant facilitation of synaptic currents during application of dopamine.


A1
ACSF
Sulpiride
Sulpiride + DA
Washout
20pA
10ms
A2
ACSF
SCH23390
SCH23390 + DA
Washout
20pA
10ms

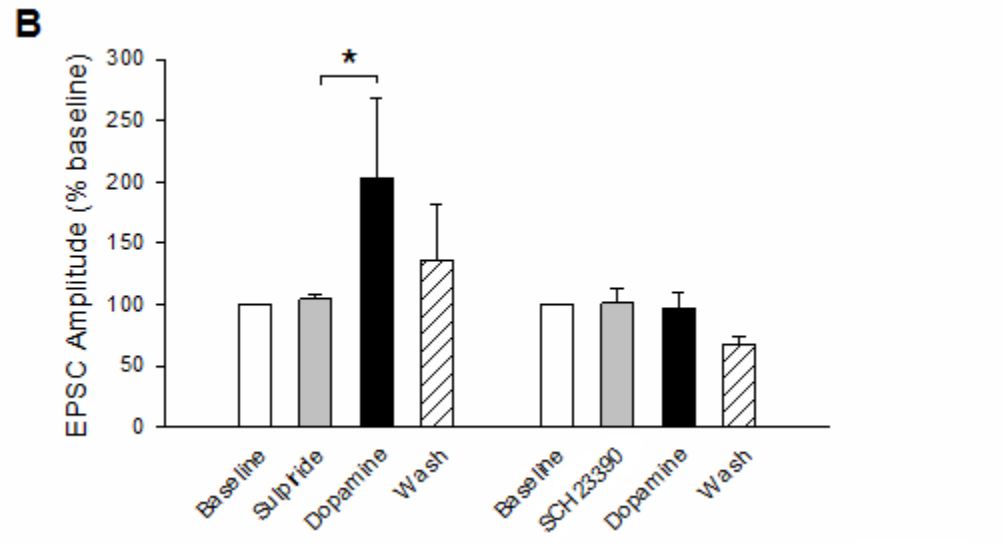

B
EPSC Amplitude (% baseline)
300
250
200
150
100
50
0
*
Baseline
Sulpride
Dopamine
Wash
Baseline
SCH23390
Dopamine
Wash

Figure 4. The dopaminergic facilitation of the amplitude of EPSCs in neurons in layer II of the entorhinal cortex depends on the activation of D_1-like, but not D_2-like receptors. **A.** Sample traces shown in A_1 are averaged representative EPSCs recorded during baseline recordings in normal ACSF, after 5 min application of the D_2-like receptor antagonist sulpiride (50 μM), after subsequent addition of dopamine (10 μM), and after a 20 min washout of dopamine in the presence of sulpiride. Sample traces shown in A_2 are averaged representative EPSCs recorded during recordings in normal ACSF, 5 min application of D_1-like receptor antagonist SCH23390 (10 μM), subsequent addition of dopamine (10 μM), and after a 20 min washout period. **B.** The histogram shows group averages of EPSC amplitudes recorded during each of the recording periods. The asterisk indicates a significant increase in EPSPs following application of dopamine in the presence of sulpiride.

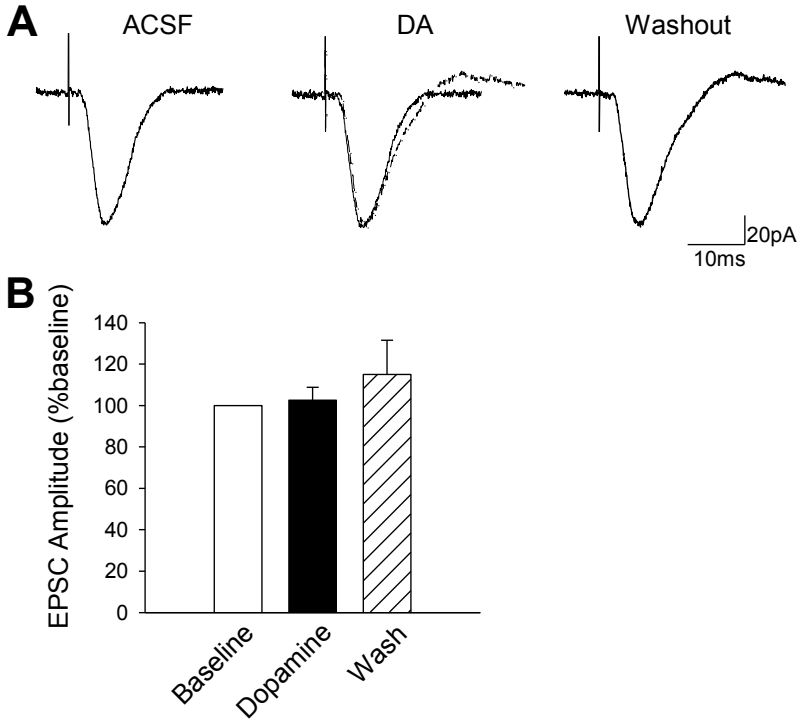

A
ACSF
DA
Washout
20pA
10ms
B
EPSC Amplitude (%baseline)
140
120
100
80
60
40
20
0
Baseline
Dopamine
Wash

Figure 5. Including the protein kinase A inhibitor H-89 (10 μM) in the intracellular recording solution blocks the dopaminergic facilitation of EPSCs in neurons in layer II of the lateral entorhinal cortex. **A.** Sample traces shown are averaged representative EPSCs recorded from H-89-filled cells during baseline recordings in normal ACSF, 5 min bath-application of dopamine (10 μM), and during a 20 min washout period in normal ACSF. **B.** The histogram shows group averages of EPSC amplitudes which remained stable during the treatment conditions.

Table 1

Summary Table of Means and Standard Errors of Cell Properties During Baseline and Dopamine Application.

	RMP	Rin (p)	Rin (e)	Rectification	AP threshold	AP height	AP duration	fAHP	mAHP	PPF Ratio
Fan (*n = 16*)										
Baseline	54.2 (+1.7)	100.6 (±6.3)	79.7 (+6.7)	1.3 (+ .06)	47.3 (+.7)	118.3 (+3.3)	3.82 (+.2)	-.20 (+.19)	-3.61 (+.42)	182.4 (+12)
DA	55.4 (+1.8)	103.3 (+5.6)	82.4 (+5.4)	1.3 (+.04)	48.3 (+1.1)	117.3 (+2.9)	4.44 (+.4)	-.42 (+.28)	-3.04 (+.48)	172.1 (+12.2)
Pyramidal (*n = 10*)										
Baseline	56.6 (+2.5)	115.6 (+14.3)	107.9 (+13.3)	1.06 (+.2)	46.9 (+.9)	114.5 (+3.8)	3.07 (+.2)	.07 (+.07)	-2.12 (+.45)	235.4 (+37.2)
DA	60.3 (+1.7)	114.8 (+16.3)	95.9 (+17.3)	1.07 (+.05)	48.5 (+.8)	115.2 (+3.7)	3.08 (+.2)	-.15 (+.11)	-3.37 (+.40)	216.5 (+35.8)

Note. RMP = resting membrane potential; Rin (p) = peak input resistance; Rin (e) = end input resistance; AP = action potential; fAHP = fast afterhyperpolarization; mAHP = medium afterhyperpolarization; PPF = paired-pulse facilitation. Application of dopamine did not markedly affect resting potential, input resistance, and action potential waveform in either fan or pyramidal neurons. The facilitation of EPSPs was also not accompanied by a significant change in paired-pulse facilitation ratio.

DISCUSSION

Previous work has shown that dopamine has dose-dependent, bidirectional effects on the strength of excitatory synaptic transmission in the superficial layers of the lateral entorhinal cortex, wherein high concentrations of dopamine suppress synaptic transmission, and lower concentrations of dopamine facilitate it (Caruana et al., 2006). It is likely that lower concentrations of dopamine reflect more physiologically realistic concentrations of dopamine present *in vivo* during activation of dopaminergic inputs to the entorhinal cortex and thus, the mechanisms underlying dopaminergic facilitation effects *in vitro* may be more representative of mechanisms at work in the cortex *in vivo*. Consequently, the present study has investigated the mechanisms through which application of low (1 μM) concentrations of dopamine produce a facilitation of synaptic transmission in layer II of the lateral entorhinal cortex. Findings indicate that the facilitation induced by a low concentration of dopamine is likely to be mediated primarily through activation of D_1-like receptors and intracellular mechanisms involving the cAMP-PKA pathway. Accordingly, the current findings provide further evidence that low doses of dopamine exert important modulatory effects within the layer II of the lateral entorhinal cortex, and that dopamine may have strong, overarching effects on multisensory and mnemonic processes mediated by the entorhinal area.

Dopaminergic Modulation of EPSPs is Dependent on Cell-Type

Compared to the medial entorhinal division, the lateral entorhinal cortex receives considerably larger dopaminergic inputs that target its superficial layers II and III (Lindvall et al., 1974; Fallon et al., 1987; Oads & Halliday, 1987). Layer II of the lateral entorhinal

division contains medium- to large-sized cells that are grouped into clusters, or cell "islands", and that can be subdivided into three morphologically and electrophysiologically distinct categories (Lindvall et al., 1974; Fallon et al., 1987; Oads & Halliday, 1987; Lingenhohl & Finch, 1991, Tahvildari & Alonso, 2005). The most common cell type in layer II is the 'fan' cell, that provides most of the sensory information to the dentate gyrus and CA3 region via the perforant path, but pyramidal and multiform cells also contribute to this projection (Amaral & Witter, 1989). Axon collaterals of layer II cells also innervate layer III, which provides temporo-ammonic inputs to the hippocampus, but layer II neurons do not project to the deep layers IV or V that primarily receive outputs from hippocampal structures (Kohler, 1985; Kohler, 1986; Lingenhohl & Finch, 1991). Interestingly, however, layer V neurons may project back to layers I and II of the lateral entorhinal cortex (Amaral & Witter, 1995) so that inputs from the deep layers may modulate activity within the superficial layers, but not vice-versa. Dopaminergic modulation of synaptic activity within the superficial layers of the entorhinal cortex is, therefore, most likely to modulate input pathways to superficial layers of the entorhinal cortex, while having a lesser effect on activity within the deeper entorhinal layers.

In the present study, application of a low dose (1 μM) of dopamine resulted in an overall facilitation in the strength of synaptic activity evoked by layer I inputs to layer II neurons of the lateral entorhinal cortex. However, when electrophysiological criteria were used to differentiate the putative fan, pyramidal, and multiform neurons that were recorded from, it was found that constant bath-application of dopamine resulted in a differential modulation of the synaptic responses recorded in the three subtypes of neurons.

Fan cells are distinct from the other cell types due to the presence of a sag in their voltage response to strong hyperpolarizing current injection and the presence of membrane potential oscillations that likely contribute to clustered cell firing during cholinergically-induced theta activity associated with behavioural mobility (Tahvildari & Alonso, 2005; Hamam, Sinari, Poirier & Chapman, 2007). Cholinergic inputs to the entorhinal cortex are known to strongly inhibit synaptic transmission by suppressing presynaptic transmitter release (Hamam et al., 2007), and the concurrent activation of dopaminergic inputs to the entorhinal area may induce a facilitation of synaptic responses that may *counteract* the cholinergic suppression, and enhance the salience of cues associated with rewarding stimuli. Overall, fan cells make the largest contribution to the lateral perforant path, and the dopaminergic enhancement of synaptic inputs to these neurons may provide a powerful enhancement of the flow of reward-relevant information from sensory areas to the hippocampus.

All fan cells recorded from in the present study showed a consistent, moderate facilitation of EPSPs during bath-application of dopamine. Pyramidal cells, however, showed more variable changes in EPSPs in response to application of dopamine; whereas some pyramidal cells underwent a large facilitation of synaptic responses in the presence of dopamine, others showed minimal changes or a slight suppression of synaptic transmission. Thus, pyramidal cells appeared to express more erratic changes in synaptic responses in response to dopamine. This heterogeneity of responses in the group of pyramidal cells may be explained in part by recent findings using whole cell current-clamp recordings (Canto & Witter, 2012). Canto and Witter suggest that the pyramidal neuron

population in layer II of the lateral entorhinal cortex may be subdivided into two distinct subgroups. The first group, characterized by a vertically-oriented pyramidal shaped soma, spreads its dendritic tree within layer II and occasionally in layer III of the lateral entorhinal cortex. However, the second subgroup, characterized by a horizontally-oriented soma, branches mainly within layers I and II. These differences in physiology and connectivity may suggest a functional difference between the two subgroups that may, in part, explain the heterogeneous modulation by dopamine observed in the present study.

In contrast to fan and pyramidal neurons, the activity of the small group of multiform cells did not appear to be modulated by dopamine. Whereas this may be a true reflection of the physiological properties of these cells, the results may also be due to the small size of the group of cells recorded from (n = 3). Nevertheless, multiform cells are the least common cell type present in layer II of the lateral entorhinal cortex, and dopaminergic modulation of this cell group is likely to have a relatively minimal effect on overall neuronal processing in layer II of the lateral entorhinal cortex.

The present results are intriguing because they emphasize possible functional differences between the three cell types. All cell types appear to project to the dentate gyrus, as Schwartz and Coleman (1981) have found that no single cell type, but rather a diverse range of morphological types of neurons, project from the lateral entorhinal cortex to the dentate gyrus. However, it is possible that the variability in responses to dopamine may be related to possible differences in the incoming sensory information that is received by each cell type. In terms of incoming projections, fan cells' dendrites do not cover a wide area or extend into layer III of the lateral entorhinal cortex (Klink & Alonso, 1997). Instead,

they branch repeatedly within layers I and II, indicating that they are likely to receive sensory inputs primarily from these layers. Pyramidal cells also branch repeatedly within layers I and II of the lateral entorhinal cortex but may also reach into the upper third of layer III (Tahvildari & Alonso, 2005), indicating that that they may receive additional sensory inputs from layer III. Multiform cells, while branching within layers I and II, also branch extensively deep into layer III, moreso than pyramidal cells (Tahvildari & Alonso, 2005). Interestingly, hippocampal feedback fibers that reach the lateral entorhinal cortex do not terminate only in the deep layers, but may reach into layer III to some extent (Witter et al., 2000; Kloosterman et al., 2003), raising the possibility that feedback from the hippocampus may, perhaps, contribute to the modulation of synaptic transmission of pyramidal and multiform cells. The present results suggest that responses of multiform neurons to synaptic inputs are not markedly affected by dopamine. The previously outlined anatomical considerations suggest, however, that dopamine may have its most reliable effects on inputs from layers I and II to fan and pyramidal cells. In addition, it is possible that dopamine may have an additional modulatory effect on pyramidal cell responses to feedback information from the hippocampus. This hypothesis could be tested more directly by recording responses of pyramidal neurons to stimulation of hippocampal inputs to layer III.

Dopaminergic Modulation of AMPA Glutamate-Receptor Mediated EPSCs

Short-term and long-term changes in synaptic transmission can be modified by neuromodulators such as dopamine. Previous research (Caruana et al., 2006) has investigated how dopamine may facilitate glutamatergic transmission in mixed EPSPs that

contain both AMPA and NMDA receptor-mediated components. Therefore, it was difficult to determine the relative contribution of each of these receptor subtypes to the observed dopamine-induced facilitation effect. It has been previously shown that dopamine may modulate the activity of both AMPA or NMDA receptors in a variety of brain areas. Thus, in order to obtain a clear picture of the cellular mechanisms involved in the facilitation of synaptic transmission in the lateral entorhinal cortex, the present study investigated the contribution of each class of glutamatergic-receptors. The data shown here suggest that dopamine is likely to work primarily through enhancing AMPA-mediated synaptic responses, with a relatively minimal contribution of NMDA receptor-mediated responses. To our knowledge, there has been no previous study that has demonstrated the effect of low doses of dopamine on electrophysiologically isolated AMPA responses in the lateral entorhinal cortex.

Previous literature examining dopaminergic modulation of synaptic responses in the prefrontal cortex has often focused on how dopamine may enhance NMDA-receptor mediated responses in cortical areas, without substantial focus on AMPA-mediated responses, despite evidence that AMPA receptors mediate the majority of fast excitatory synaptic transmission (Hollmann & Heinemann, 1994; Dingledine, Borges, Bowie, & Traynelis, 1999). Recent in vitro studies have demonstrated that in the prefrontal cortex, activation of D_1-like receptors results in an increase in NMDA currents via a post-synaptic signalling cascade that involves Ca^{2+} and PKA (Seamans et al., 2001; Wang & O'Donnell, 2001; Gonzalez-Islas & Hablitz, 2003;Young & Yang, 2004; Kruse, Premont, Krebs, & Jay, 2009). Furthermore, studies have also shown a similar mechanism of modulation in

other cortical areas, wherein D_1-like receptors modulate NMDA-receptor currents in the striatum and hippocampus (Lee at al., 2002; Fiorentini et al., 2003; Pei et al., 2004). However, additional studies have observed a concurrent change in *both* AMPA- and NMDA-mediated responses in the presence of dopamine. For example, bath application of dopamine has been shown to significantly enhance both pharmacologically isolated AMPA- *and* NMDA-mediated EPSCs amplitudes in layer II/III cells of the prefrontal cortex (Gonzalez-Islas & Hablitz, 2003). Results in the prefrontal cortex also suggest that activation of D_1-like receptors can increase excitability of layer V neurons through an increase in AMPA-related synaptic activation (Yang & Seamans, 1996). Further, D_1-like receptors also appear to modulate the activity of isolated AMPA-mediated currents in layers II and II of the prefrontal cortex (Gonzalez-Islas & Hablitz, 2003; Bandyopadhyay, et al., 2005). Thus, dopamine can exert modulatory effects over both AMPA and NMDA glutamatergic transmission.

The results of the present study suggest that the dopaminergic facilitation of synaptic responses in the lateral entorhinal cortex is mediated primarily by modulation of the activity of AMPA glutamatergic receptors. It was found here that EPSCs recorded at -60 mV, using a standard intracellular solution, showed a negligible NMDA receptor-mediated current, such that EPSCs were completely blocked by the AMPA receptor blocker CNQX. These observations are consistent with previous studies that have shown that in the perforant path, mixed EPSCs recorded from neurons held at -60mV are mostly attributable to AMPA conductance, with NMDA components playing a minimal role in the mixed postsynaptic currents (Otmakhova et al., 2002). The present study has

shown that the dopaminergic modulation of EPSCs was still present even in recording conditions in which cells were hyperpolarized and held at -70 mV, a condition that accentuates the voltage block on NMDA receptors, suggesting a large contribution of AMPA receptor currents.

The present study used a standard K^+-gluconate-based intracellular recording solution, and it was not possible to definitively assess the role of NMDA receptor-mediated components in the facilitation of synaptic responses. However, it would be possible to use a Cs^+-based intracellular recording solution (which can enhance control over holding potential by closure of K^+ channels) in order to allow recording at more depolarized holding potentials to better quantify possible dopaminergic modulation of NMDA-mediated EPSCs (Otmakhova et al., 2002; Manabe, Wykllie, Perkel, & Nicoll, 1993; Bekkers & Stevens, 1993). Although results thus far suggest that the NMDA-mediated component is likely to be minimal, this procedure would allow for a more definitive evaluation of the potential contribution of NMDA receptors to the dopaminergic facilitation of synaptic responses.

D_1-like Receptor Activity and the cAMP-PKA Pathway

Although the mechanisms through which dopamine may mediate NMDA responses has received substantial attention (Greengard, Nairn, & Stevens, 1991; Cepeda, Colwell, Itri, Chandler, & Levine, 1992; Cepeda, Buchwald, & Levine, 1993), the processes through which dopamine D_1 receptors may affect AMPA-mediated responses remain poorly understood. Previous studies have demonstrated that D_1-like receptors are functionally coupled to adenlylyl cyclase (Monsma, Mahan, McVittie, Gerfen, & Sibley, 1990) and that

dopamine, acting on the D_1-like dopamine receptors, stimulates adenylyl cyclase via G_s proteins to increase production of cyclic adenosine monophosphate (cAMP). Cyclic AMP was the first identified intracellular messenger (Sutherland & Rall, 1957), and it is known to serve as an intracellular second messenger for a large number of neurotransmitters, hormones, and signaling substances (Beebe, 1994; Skalhegg & Tasken, 2000). It has been shown that in mammalian cells, cAMP has three direct intracellular targets, but its main, and most extensively-investigated target is the cAMP-dependent protein kinase (PKA), which, in turn, can mediate further intracellular signal transmission (Lee et al., 2003).

Our results indicate that the *facilitation* of AMPA-currents in the lateral entorhinal cortex requires activation of PKA, since the PKA blocker H-89 blocked the facilitation of EPSCs (Figure 5). No increase in EPSCs was observed in H-89-filled cells during the baseline period, and there was a clear block of the facilitation of EPSCs during application of dopamine. However, although H-89 is a relatively selective PKA inhibitor that has been used extensively to investigate the roles of PKA (Kaneishi, Sakuma, & Kobayashi, 2002; Kim, Won, Mao, Jin, & Greenberg, 2005), it is somewhat non-selective and can affect multiple kinases including S6K1, MSK1, PKB and ROCK-II (Lochner & Moolman, 2006). Nevertheless, there are no apparent reports of these signaling mechanisms being activated by D_1-like receptors, and they are therefore less likely to play a significant role in the facilitation of synaptic responses observed here. Converging evidence for the role of PKA could be obtained using other inhibitors, but similarly potent PKA inhibitors, such as KT 5720, are also known to exert non-selective actions on several protein kinases (Lochner & Moolman, 2006).

Previous studies have identified the important role of the cAMP-PKA pathway in cellular processes related to learning and memory. The cAMP-PKA pathway can affect the short-term facilitation of synaptic responses by modulating the phosphorylation of ion channels, and can also affect long-term changes in synaptic transmission by modulating gene expression and protein synthesis (Kandel & Schwartz, 1982; Kandel, 2001). Previous studies have provided evidence suggesting that PKA may be able to closely regulate the activity of AMPA receptors through phosphorylation of serine 845, one of the two phosphorylation sites for GluR1, an AMPA-receptor subunit (Roche, O'Brien, Mammen, Bernhardt, & Huganir, 1996; Lee et al., 2003), and this provides a possible signaling cascade that could be responsible for the facilitatory effects of dopamine on AMPA-mediated synaptic transmission. Furthermore, Rosenmund et al. (1994) have demonstrated that displacement of PKA resulted in a significant reduction of AMPA and kainate receptor currents, suggesting that PKA plays a role in maintaining and enhancing AMPA-regulated synaptic transmission. This type of postsynaptic enhancement in AMPA receptor function is consistent with the present results; paired-pulse facilitation ratio was not affected in the present experiment, suggesting that the increased synaptic response is mediated by an enhancement in the response of postsynaptic receptors, rather than by an increase in transmitter release.

Previous studies suggest that dopamine D_1-like receptor activation may lead to increased AMPA responses by altering AMPA receptor trafficking in a manner similar to that which mediates long-term synaptic potentiation. Sun and colleagues (2005) found that incubating prefrontal cortex neurons in the D_1-like dopaminergic agonist SKF 81297 for 5

min significantly increased surface expression of glutamate receptor 1 (GluR1) -containing AMPA receptors, whereas application of the D_1-like antagonist SCH 23390 significantly attenuated this effect. This expression of GluR1 was mediated through a PKA-dependent mechanism, and required activation of NMDA glutamate receptors for insertion. Further, Stramiello and Wagner (2008) note that D_1-like receptor activity results in an increase in the persistence and early magnitude of long-term potentiation (LTP) that has been linked to the cAMP-PKA pathway. Thus, dopamine D_1-receptors are likely to facilitate mechanisms of learning and memory via PKA-dependent signaling that enhances AMPA receptor-mediated synaptic responses. The facilitatory effects of dopamine observed in the current study took several minutes to develop, and it is therefore possible that dopaminergic effects on AMPA receptor trafficking could contribute within that time-period (Sun et al., 2005).

Protein kinase A could also be linked to increases in AMPA receptor-mediated EPSCs through the involvement of dopamine- and cyclic AMP-regulated phosphoprotein (DARPP-32). Dopamine D_1-like receptors are known to be positively coupled to the cAMP-PKA pathway, and activation of PKA by D_1-like receptors results in phosphorylation of DARPP-32, a potent endogenous inhibitor of protein phosphatase 1 (PP-1; Hemmings, Williams, Konigsberg, & Greengard, 1984; Yan et al., 1999). Additionally, increases in dopamine have been previously linked to rapid and transient increases in DARPP-32 (Nishi et al., 2000), suggesting that DARPP-32 may contribute to the reversible and transient effects of dopamine on synaptic transmission that were observed in the present study. DARPP-32 inhibits protein phosphatase 1, a phosphatase

which can reduce synaptic responses by dephosphorylating AMPA receptors. Therefore, the inhibition of PP1 by DARPP-32 could result in an increase in AMPA-mediated EPSCs (Kemp & Bashin, 2001; Malenka & Bear, 2004). These suggested interactions between PKA, DARPP-32 and AMPA receptors could consequently mediate the relatively rapid increases in synaptic responses in the presence of dopamine that were observed here.

Additional evidence suggests that dopamine may also act through an intracellular process regulated by calcium. Previous studies have found that D_1-receptor-mediated increases in calcium signaling may enhance AMPA receptor-mediated responses or overall neuronal excitability. For example, Galarraga and colleagues (1997) have shown that, in neostriatal neurons, D_1-like receptors facilitate AMPA-mediated synaptic transmission during selective blockade of NMDA and GABA transmission, indicating that D_1 -like receptors are responsible for the observed facilitation of AMPA-mediated synaptic responses. In addition, other studies have demonstrated that postsynaptic calcium was necessary for a D_1-like related increase in AMPA and NMDA-mediated currents in the hippocampus (Yang, 2000), and in the prefrontal cortex (Gonzalez-Islas & Hablitz, 2003). This evidence highlights the need to investigate the role of calcium in mediating the relation between activation of D_1-like receptors and AMPA-mediated currents. The requirement of increases in postsynaptic calcium could be assessed in future experiments by including the calcium-chelator BAPTA in the intracellular recording solution.

Functional Significance

The present findings suggest that low doses of dopamine may act via D_1-like receptors to facilitate AMPA receptor-mediated synaptic transmission in the superficial

layers of the lateral entorhinal cortex. This facilitation may modulate information processing during behavioural states associated with motivation or reward. Similar processes have been shown to be at work in the prefrontal cortex, where dopamine acts on D_1-like receptors, cAMP and PKA, to impact learning and memory performance in tasks such as the nonmatching-to-position (delayed win-shift) task on the radial maze (Aujla & Beninger, 2001). This highlights the fact that activation of the cAMP-PKA pathway by dopamine via D_1-like receptors is important for working memory processes, and that similar cellular mechanisms may modulate the cognitive functions of the lateral entorhinal cortex.

Dopaminergic facilitation of synaptic responses could also contribute to longer-term changes in synaptic plasticity that are involved in processed of encoding or consolidation of reward-related memories, possibly through mechanisms related to LTP. A large amount of literature has characterized the molecular basis of LTP in the hippocampal region (e.g. Leonard, Amaral, Squire, & Zola-Morgan, 1995; Squire & Zola, 1996) and has focused on mechanisms mediated by NMDA receptor activation. Because postsynaptic depolarization enhances activation of NMDA receptors, the dopaminergic enhancement of AMPA receptor-mediated currents could also lead to an enhancement of the NMDA receptor-dependent induction of LTP in the entorhinal cortex (Chapman & Racine, 1997). Although the story is incomplete, previous studies suggest that PKA, DARPP-32 and Ca^{2+} may be involved in the intracellular pathway through which dopamine may modulate the induction of LTP (e.g. Stramiello & Wagner, 2008). Therefore, the current findings, indicating that dopamine enhances AMPA-mediated transmission suggest

that, in addition to possible enhancing effects on the salience of reward-related cures, dopamine may also serve to enhance cellular mechanisms in the entorhinal cortex that may contribute to both momentary and long-term changes in sensory and mnemonic processes in the temporal lobe.

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