Dopamine Has Bidirectional Effects on Synaptic Responses to Cortical Inputs in Layer II of the Lateral Entorhinal Cortex

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Submitted 30 May 2006; accepted in final form 25 September 2006

INTRODUCTION

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 and Amaral 2000; Schwarz and Witter 2002; Squire and Zola 1996; Squire et al. 2004). 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 et al. 1995; Bouras and Chapman 2003; Burwell 2000; Caruana and Chapman 2004; Chapman and Racine 1997; Lavenex and 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 and 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, 2000; Goldman-Rakic 1999). Regional depletion of dopamine (Brozoski et al. 1979) and disruption of dopamine receptor function in the prefrontal cortex (Sawaguchi and 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 et al. 1986; Bjorklund and Lindvall 1984; Fallon and Loughlin 1987; Oades and 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 and 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 and Lindvall 1984; Fallon and Loughlin 1987) and the two divisions differ markedly in electroresponsiveness of principal cells (Alonso and Klink 1993; Tahvildari and Alonso 2005; Wang and Lambert 2003) and connectivity with cortical inputs (Hargreaves et al. 2005; Sowards and Sowards 2003). Although dopamine does not directly suppress excitatory postsynaptic potentials (EPSPs) in layer V neurons of lateral entorhinal cortex, it does reduce temporal summation of EPSPs by enhancing the hyperpolarization-activated current ($I_h$) (Roskranz and 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 (fEPSPs) evoked by stimulation of the piriform (primary olfactory) cortex were recorded before and after systemic administration of the dopamine reuptake inhibitor GBR12909 {1-(2-bis(4-fluorophenyl)-[methoxy]ethyl)-4-(3-phenylpropyl) piperazine} 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.

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M E T H O D S

Microdialysis and high-performance liquid chromatography

SURGERY. Male Long–Evans hooded rats (9 to 11 wk old; 300–320 g) were anesthetized with a 5% isoflurane and 95% oxygen mixture and placed in a stereotaxic apparatus with bregma and lambda leveled. A 20-gauge stainless steel guide cannula (Plastics One) was lowered to a position approximately 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 skull and the screws and cannula were embedded in dental cement. A 24-gauge obturator (Plastics One) was inserted into the guide cannula. Buprenorphine (0.02 mg/kg, administered subcutaneously [sc]) was used as a postsurgical analgesic. Animals were housed individually and tested after a ≥10-day recovery period during the lights-off phase of a 12-h light–dark schedule.

APPARATUS AND MICRODIALYSIS PROBES. During microdialysis sampling, animals were housed in a 42 × 39 × 34-cm Plexiglas chamber with a stainless steel grid floor. Each chamber was housed within a 65 × 65 × 75-cm sound-attenuating wooden cubicle. Two pairs of photocells were positioned 2.5 cm above the floor 10 cm apart and the number of photobeam breaks made by animals during 20-min sampling periods was recorded. Food was removed from chambers immediately using high-performance liquid chromatography (HPLC)–valued 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, 7.5–8.5 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 anteroverentral portion of the lateral entorhinal cortex (Bjoerklund and Lindvall 1984; Fallon and Loughlin 1987; Oades and 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 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 nine-pin connector. The assembly was embedded in dental cement and anchored to the skull with jeweler’s screws. Buprenorphine (0.02 mg/kg, sc) was administered after surgery. Animals were housed individually and tested during the lights-on phase of a 12-h light–dark schedule.

MICRODIALYSIS SAMPLING AND HPLC ANALYSIS. Probes were inserted into guide cannulae 1 day before microdialysis testing. To prevent occlusion of probes, artificial cerebrospinal fluid (ACSF) consisting of (in mM) 145 Na+, 2.7 K+, 1.2 Ca2+, 1 Mg2+, 150 Cl−, 0.2 ascorbate, and 2 Na2HPO4 (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 (about 14 μl/sample) and measures of locomotion (number of photocell 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 <10% variation over three consecutive samples. Rats were then injected with either saline (0.9%, 1 ml/kg) or the selective dopamine reuptake inhibitor GBR12909 (10 mg/kg, administered intraperitoneally [ip]; Nakachi et al. 1995) and dialysate samples and activity measures were collected at 20-min intervals for 120 min. Differences in postinjection locomotion and dopamine levels after 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 (Model 7125, 20-μl loop; Rhodyne) and the redox current for dopamine was measured with a dual-channel coulometric detector (ESA Biosciences, Coulchem III with a Model 5011 analytical cell). The mobile phase (20% acetonitrile, 0.076 M SDS, 0.1 M EDTA, 0.058 M Na2PO4, 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 using an EZChrom Chromatography Data System (Scientific Software).

HISTOLOGY. Animals were deeply anesthetized with sodium pentobarbital (65 mg/kg, ip) 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 1 day before sectioning with a cryostat and coronal sections (40 μm thick) were stained with formol-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 wk old; 300–350 g) were treated with atropine methylnitrite (0.1 mg/kg, ip), anesthetized with sodium pentobarbital (65 mg/kg, ip), 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, 7.5–8.5 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 anteroverentral portion of the lateral entorhinal cortex (Bjoerklund and Lindvall 1984; Fallon and Loughlin 1987; Oades and 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 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 nine-pin connector. The assembly was embedded in dental cement and anchored to the skull with jeweler’s screws. Buprenorphine (0.02 mg/kg, sc) was administered after surgery. Animals were housed individually and tested during the lights-on phase of a 12-h light–dark schedule.

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

Animals were placed in a 40 × 40 × 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, ten responses to stimulation of the piriform cortex were recorded and averaged at each of ten intensities (100 to 1,000 μA) using a 10-s intertrial 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 and Regehr 2002). During these tests, pairs of stimulation pulses, separated by interpulse...
intervals of 10, 30, 100, and 1,000 ms, were delivered to the piriform cortex using pulse intensities that evoked responses roughly 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 artifact 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 after the last baseline input–output test, animals received an injection of physiological saline (0.9%, 1 ml/kg; ip; 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; ip) for each of the stimulation intensities or each interval of at least 1 h before recordings.

**Synaptic responses in vitro**

**SLICE PREPARATION.** Slices were obtained from male Long–Evans hooded rats (3.5–6 wk old) as described previously (Chapman et al. 1998). Briefly, animals were anesthetized with halothane and brains were rapidly removed and cooled (4°C) in oxygenated ACSF, which consisted of (in mM): 124 NaCl, 5 KCl, 1.25 NaHPO₄, 2 MgSO₄, 2 CaCl₂, 26 NaHCO₃, and 10 dextrose. Horizontal slices (400 μm thick) were cut using a vibratome (Vibroslice; WPI) and placed on a nylon net in a humidified 95% O₂-5% CO₂ atmosphere. There was a recovery period of at least 1 h before 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 – 8 M) and positioned with the aid of a dissecting microscope (M55; Leica) 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 (0.8 MΩ; FHC) was positioned to span the layer I–II border, approximately 1.0–2.0 mm rostral to the recording electrode. Constant-current pulses (0.1 ms) were delivered using a stimulus generator (Model A300; WPI) and a stimulus isolation unit (Model A360; WPI). Evoked field potentials were filtered (DC, 3 kHz) and amplified with an AxoClamp 2B amplifier (Axon Instruments) in bridge mode and responses were digitized at 20 kHz (Digidata 1322A; Axon Instruments) for storage on computer hard disk using the software package Clampfit 8.1 (Axon Instruments).

Responses to test pulses were monitored every 20 s using an intensity adjusted to evoke fEPSPs with an amplitude of roughly 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% baseline variability. 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 artifact 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.

**RESULTS**

**In vivo microdialysis**

Histological analysis confirmed that dialysis probes were on target in the lateral entorhinal cortex (Fig. 1A). In most cases (eight of ten), 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 about 300 μm below the cortical surface in upper layer III.

**MEASURES OF ENDOCRINE ACTIVITY**

Measurements of basal levels of extracellular dopamine in the lateral entorhinal cortex typically stabilized within about 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. 1B; F(1,14) = 4.7, P < 0.05]. Dopamine concentrations increased to 254.9 ± 73.2% of baseline levels 2 min after GBR12909 administration and peaked 80 min postinjection at 305.9 ± 79.9% (0.45 ± 0.08 and 0.51 ± 0.10 nM, respectively). Dopamine remained elevated for ≥2 h after GBR12909 administration. In contrast, dopamine levels were stable after saline injections.

**Locomotor activity during baseline dialysis sampling**

Resulted in an average of only 13.1 ± 4.9 photobeams every 20 min. The number of photobeam breaks increased significantly after injections of GBR12909 and peaked at 201.1 ± 21.4 breaks after 1 h [Fig. 1B; F(1,14) = 60.9, P < 0.001]. Locomotor activity in treated rats remained elevated...
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, A and B). Field potentials in the medial entorhinal cortex evoked by piriform cortex stimulation result from activation in layers I and II (Chapman and Racine 1997). The lateral entorhinal cortex also receives monosynaptic afferents from the piriform cortex (Burwell 2000; Kohler 1988), which evoke synaptic responses in layer II (Biella and de Curtis 2000; Boeijinga and Van Groen 1984). The major component of all 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. 2C). In some cases (six of 11) the major synaptic component was followed by a late-positive deflection (e.g., Fig. 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, C and D) and responses returned to baseline levels when examined 24 h 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 1,000 ms ($n = 11$). If transmitter release after 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 and Chapman 2003; Chapman and Racine 1997).

Systemic administration of GBR12909 enhanced the amplitude of synaptic responses at all interpulse intervals tested, but the paired-pulse ratio was not reduced (Fig. 3, 30-ms interpulse interval; 159.8 ± 21.0% after saline vs. 164.0 ± 19.1% after 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 and Chapman 2003; Stenkamp et al. 1998; Yun et al. 2002). A short-latency presynaptic fiber volley preceded the major component of all field potential responses evoked in layer II by 10.2 ± 3.6 ms on November 3, 2016 http://jn.physiology.org/ Downloaded from
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 D1-receptor antagonist SCH23390 (50 μM) alone had no significant effect on synaptic responses (101.9 ± 1.9% of baseline), but attenuated the dopamine-induced increase to 119.3 ± 2.5% of baseline (Fig. 4). Synaptic responses increased to only 106.0 ± 2.3% of control levels during coapplication of SCH23390 and dopamine.

Bath application of the D2-receptor antagonist sulpiride (50 μM) had no significant effect on baseline synaptic responses (97.9 ± 2.5% of baseline) and did not significantly affect the peak facilitation induced by dopamine (Fig. 4B; n = 5). Responses increased significantly to 113.0 ± 1.8% of control levels during coapplication 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 D1 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 (n = 8) or 100 μM (n = 6) dopamine caused a significant, dose-dependent reduction in the amplitude of synaptic responses [Figs. 5A and 6A; F(3,22) = 28.09, P < 0.001 for the interaction of dose × time; 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).

Coapplication of the D1-receptor antagonist SCH23390 with either 50 μM (n = 6) or 100 μM (n = 8) dopamine reduced the time course of the dopamine-induced suppression, but did not significantly affect the peak reduction in synaptic responses (Figs. 5B and 6B). 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 after 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 after the coapplication of SCH23390 with 50 or 100 μM of dopamine.

**FIG. 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 field excitatory postsynaptic potentials (fEPSPs) from a representative animal after 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 min 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.
In contrast, coapplication of the D₂-receptor antagonist sulpiride blocked the reduction in synaptic responses induced by 50 (n = 11005) or 100 (n = 9262) M dopamine (Figs. 5B and 6B). Responses were not affected by application of sulpiride alone and subsequent coapplication of either 50 or 100 M 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 at the number of slices tested here.

**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 used 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 by 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 by D₁ receptors and higher concentrations (50 to 100 μM) reduced synaptic responses by D₂ receptors. Reductions in synaptic responses were previously observed in the medial entorhinal cortex (Pralong and Jones 1993; Stenkamp et al. 1998) and we observed similar reductions in fEPSPs with high concentrations of dopamine. We demonstrate here, however, 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 D₁ 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). Such facilitation could have resulted, in part, from indirect effects of enhanced dopamine release in other brain areas. Systemic dopamine can enhance firing of raphe neurons (Martín-Ruiz et al. 2001) and the superficial layers of the entorhinal cortex receive serotonergic inputs (Kohler et al. 1980), although serotonin inhibits synaptic transmission in superficial layer neurons in both the medial (Schmitz et al. 1998, 1999; Sizer et al. 1992) and lateral (Grunschlag et al. 1997) divisions in vitro. The facilitation of synaptic responses observed here after GBR12909 is therefore unlikely to reflect actions of dopamine on serotonin inputs to the entorhinal cortex. The findings from microdialysis demonstrated that systemic GBR12909 enhanced extracellular dopamine in the entorhinal cortex (Fig. 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 (0.21–0.51 nM) was substantially lower than the smallest concentration used in vivo experiments (10 μM), dopamine levels fall off extremely rapidly with distance from the release site (Cragg and Rice 2004), are affected by flow rate, and significantly 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 subiculum are much less dense than those to the entorhinal cortex (Gasbarri et al. 1994, 1996, 1997) and probes clearly included layer II where dopamine afferents surround principal cell islands (Bjorklund and 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. 1B₂; Nakachi et al. 1995). Forebrain cholinergic neurons are active during movement (Bland and Oddie 2001) and cholinergic activation can suppress EPSPs in hippocampus and medial entorhinal cortex (Caruana et al. 2003; Cheong et al. 2001; Kremin et al. 2006). Cholinergic and dopaminergic systems are likely to be coactivated 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 and 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 D1-receptor–dependent facilitation that mirrored our findings in awake rats (Fig. 4). Responses were also facilitated during washout of higher doses as bath concentrations of dopamine fell (Figs. 5 and 6). This rebound facilitation could be a result of the lower concentration of dopamine present and activation of dopamine receptors, but could also reflect interactions between D1- and D2-receptor activation or a more persistent dopamine-mediated potentiation effect (Huang and Kandel 1995). The effects of the lower dose of 10 μM dopamine suggest that the most common role of dopamine is to enhance synaptic responses to cortical afferents by a D1-receptor–mediated mechanism. This is consistent with the higher affinity of D1 versus D2 receptors (Seeman and Van Tol 1993) and with the high density of D1 receptors in layer II of the entorhinal cortex (Huang et al. 1992; Kohler et al. 1991; Weiner et al. 1991).

In the prefrontal cortex, although dopamine increases the excitability of pyramidal neurons (e.g., Gorelova and Yang 2000; Petit-Soria 1987), dopamine most commonly results in a reduction of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) synaptic responses through a D1-receptor–mediated reduction in transmitter release (Gao et al. 2001; Law-Tho et al. 1994; Seamans et al. 2001b; Urban et al. 2002; Zheng et al. 1999). However, there are reports of increased AMPA responses in prefrontal cortex layer V (Chen et al. 2004; Onn et al. 2006; Seamans et al. 2001b) and low concentrations (<20 μM) of dopamine can lead to a D1-mediated increase in AMPA EPSCs by a postsynaptic mechanism in layers II/III (Bandyopadhyay et al. 2005; Gonzalez-Islas and Hablitz 2003). In the hippocampus, activation of D1 receptors in CA1 pyramidal cells with a selective agonist can also lead to a sustained enhancement of AMPA-mediated EPSCs (Yang 1999, 2000). Similarly, increases in N-methyl-d-aspartate (NMDA)–mediated responses induced by dopamine are also commonly observed in the prefrontal cortex (Chen et al. 2004; Gonzalez-Islas and Hablitz 2003; Seamans et al. 2001b; Zheng et al. 1999) and hippocampus (Yang 1999, 2000) and are consistent with the D1-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 by D1 receptors is consistent with these reports. In the prefrontal cortex, previous studies reported both a facilitation of NMDA responses at low doses by D1 receptors and a suppression of responses at high concentrations by D2 receptors (Seeman et al. 2001b; Zheng et al. 1999). Activation of D1 and D2 receptors has parallel bidirectional effects on evoked inhibitory postsynaptic potentials (IPSCs) in layer V (Seeman et al. 2001a; Trantham-Davidson et al. 2004). D2-receptor activation was also shown to suppress synaptic responses in the CA1 region of the hippocampus (Gribkoff and Ashe 1984; Huang and Kandel 1995) while also leading to a lasting D1-receptor–mediated facilitation. In the medial entorhinal cortex both D2 and D1 receptors contribute to the suppression of EPSPs (Pralong and Jones 1993; Stenkamp et al. 1998) and evoked field responses in the CA1 region of the hippocampus are also suppressed by activation of either D1 or D2 receptors (Otmakhova and Lisman 1998). Here, although D1-receptor antagonism did not affect the peak suppression of fEPSPs induced by dopamine, it did reduce the time course of the effect (Figs. 5B1 and 6B1). This suggests that strong activation of D1-like receptors could contribute to the reduction of synaptic responses. A D1-mediated suppression of responses at high concentrations of dopamine may also account for why a facilitation was not revealed when D2 receptors were blocked with sulpiride (Figs. 5B2 and 6B2). Thus similar to findings in prefrontal cortex that have suggested an inverted U-shaped curve for the effects of D1-receptor activation (Arnsten 1998; Goldman-Rakic et al. 2000), a facilitation or a suppression of glutamatergic responses may result, respectively, from low versus high levels of D1-receptor activation.

Changes in intrinsic conductances that affect postsynaptic excitability may also contribute to dopaminergic modulation of evoked responses. Indeed, previous reports showed that dopamine can reduce input resistance in layer II of the entorhinal cortex, likely by an increased K+ conductance (Pralong and 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 and Johnston 2006). Input resistance was reduced by ≤30% when high concentrations of dopamine (≥500 μM) were applied in layer II (Pralong and 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 and Johnston 2006). Dopamine at this concentration (10 μM) did not significantly depress responses to single-stimulation pulses (Rosenkranz and Johnston 2006), so although changes in I_h may contribute to the 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 D1- and D2-receptor activation has bidirectional effects on IPSCs in prefrontal cortex (Seeman et al. 2001a; 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 γ-aminobutyric acid transmission in lateral entorhinal cortex—this remains to be investigated more closely.

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 D1 receptors (Arnsten 1998; Goldman-Rakic et al. 2000; Seamans and Yang 2004). Few behavioral studies bear directly on the function of dopaminergic inputs to the entorhinal cortex


