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Research report

Rewarding stimulation of the lateral hypothalamus induces a dopamine-dependent suppression of synaptic responses in the entorhinal cortex

Juliana A. Hutter, Allison Martel, Lianne Trigiani, Shawnna G. Barrett, C. Andrew Chapman*

Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montréal, Québec H4B 1R6, Canada

HIGHLIGHTS

- The lateral entorhinal cortex receives inputs from midbrain dopamine neurons.
- Rewarding lateral hypothalamic (LH) stimulation suppresses evoked Field excitatory postsynaptic potential (fEPSPs) in entorhinal cortex.
- The suppression is dependent upon activation of dopamine D_2 receptors.
- The suppression occurs when LH stimulation is initiated by the animal, or delivered at a fixed interval.
- Both tonic and phasic dopamine release may contribute to the suppression of fEPSPs.

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ABSTRACT

The entorhinal cortex receives inputs from sensory and associational cortices, as well as a substantial input from midbrain dopaminergic neurons. Dopamine is likely to modulate the responsiveness of entorhinal cortex neurons to sensory inputs, and excitatory synaptic responses in layers I/II of the entorhinal cortex *in vitro* can be either facilitated or suppressed by dopamine depending upon the concentration applied. Rewarding stimulation of the lateral hypothalamus leads to activation of dopamine neurons, and the present study evaluated the effect of rewarding stimulation on synaptic responses in the lateral entorhinal cortex evoked by stimulation of the primary olfactory (piriform) cortex in behaving rats. Rewarding brain stimulation reduced the amplitude of synaptic responses in the entorhinal cortex evoked by single pulses delivered to the piriform cortex at intervals of 100–500 ms following the train. Synaptic responses were suppressed when stimulation trains were delivered at a fixed interval, or when trains were initiated by the animal pressing a bar. The suppression depended on the strength of stimulation trains; delivery of higher frequency trains that were sufficient to induce maximal, or 50% of maximal, rates of bar-pressing resulted in significant suppression effects, but lower frequency trains did not. Systemic administration of the dopamine D_2 receptor antagonist eticlopride, but not the D_1 receptor antagonist SCH23390 or the muscarinic antagonist scopolamine, blocked the suppression of synaptic responses. Results suggest that rewarding brain stimulation leads to a phasic increase in dopamine in the entorhinal cortex resulting in a D_2 receptor-dependent suppression of excitatory synaptic responses, and that a similar synaptic modulation may be induced by stimuli associated with appetitive motivation and reward.

The entorhinal cortex is a structure within the medial temporal lobe that is thought to play roles in sensory processing and integration, memory formation, and spatial navigation [1–3]. Neurons in the superficial layers of the entorhinal cortex receive converging projections from primary sensory and associational cortices and also provide the hippocampus with a majority of its cortical sensory input; excitatory transmission within the entorhinal cortex is, therefore, likely to play an important role in sensory integration and mnemonic function [1,4,5]. The entorhinal cortex also receives one of the largest cortical projections of midbrain dopamine

Abbreviations: LH, Lateral hypothalamus; fEPSP, Field excitatory postsynaptic potential.

* Corresponding author at: Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, 7141 Sherbrooke Street W., Rm. SP-244, Montréal, Québec H4B 1R6, Canada. Tel.: +1 514 848 2424x2220; fax: +1 514 848 2817.
E-mail address: andrew.chapman@concordia.ca (C.A. Chapman).

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neurons [6–11]. It is well-established that dopamine release within the prefrontal cortex can alter synaptic transmission and contribute to working memory function during motivated behaviors [12–15], but the effects of dopamine on entorhinal cortex synapses during motivated behaviors, and how this may modulate sensory and mnemonic function, is not clear see [16].

Recordings in behaving animals and in brain slices have shown that dopamine can result in either a facilitation or suppression of evoked excitatory synaptic responses in the entorhinal cortex depending upon the concentration of dopamine applied [17–20] see also [21]. The dopamine reuptake blocker GBR12909 increases dopamine in the entorhinal cortex and facilitates entorhinal synaptic responses to stimulation of the piriform cortex *in vivo* [19], and application of a low concentration (i.e., 1 μmol/l) of dopamine in brain slices also induces a D_1 receptor-mediated facilitation of synaptic responses in layer II of the entorhinal cortex [20]. In contrast, tests of the effects of higher concentrations of dopamine in brain slices have consistently resulted in a suppression of EPSPs in layers II, III, and V of the medial entorhinal cortex [17,18] and in layer II of the lateral entorhinal cortex where the suppression is mediated primarily by D_2 receptors [19,20].

The dose-dependent, bidirectional effects of dopamine on synaptic transmission in the entorhinal cortex suggest that the endogenous effects of dopamine depend strongly on the extent to which dopamine neurons are activated. The number of dopamine neurons engaged in steady low-frequency firing sets the extent of tonic dopamine release, and phasic bursts of firing in dopamine neurons in response to reward-relevant cues can also drive transient increases in cortical dopamine levels [22–24]. However, the manner in which endogenous dopamine release may modulate glutamatergic synaptic transmission in the entorhinal cortex, and how such modulation may depend on D_1 and D_2 receptor-mediated facilitation and suppression effects [19,20] is not known.

The present study has assessed the effect of rewarding electrical stimulation of the lateral hypothalamus on the strength of synaptic inputs from the piriform cortex to the lateral entorhinal cortex *in vivo*. Animals are highly motivated to obtain stimulation of the lateral hypothalamus, which results in strong indirect activation of dopamine neurons [25–29]. Rewarding brain stimulation results in large sustained increases in dopamine in nucleus accumbens and prefrontal cortex [30,31], and is also likely to result in dopamine release in the entorhinal cortex [7,10]. Rewarding stimulation may, therefore, provide a method to assess the effects of tonic increases in endogenous dopamine in the entorhinal cortex. The discrete, controlled timing of rewarding stimulation also provides a method to assess the time-course of possible phasic effects on synaptic transmission [23,32]. Trains of rewarding stimulation were, therefore, delivered at short intervals prior to delivering single pulses to the piriform cortex to evoke synaptic responses in the entorhinal cortex. The resulting suppression of synaptic responses was investigated with respect to its dependence on the number, duration, and frequency of stimulation trains, delivery of trains using a fixed-interval schedule versus following an operant response, and the nature of the dopamine receptors that mediate the effect.

1. Material and methods

1.1. Patients and surgery

Male Long-Evans rats (8–10 weeks old; Charles-River) were housed individually and had free access to food and water. For chronic implantation of stimulating and recording electrodes, animals were anesthetized with isoflurane (1.5–2% in O_2) and placed in a stereotaxic frame. Bipolar, twisted wire, Teflon-coated stainless-steel stimulating electrodes (125 μm exposed tips) were lowered into the right piriform cortex (P, 3.6 mm; L, 6.5 mm; V, 9.0 mm relative to bregma; tip separation 1.0 mm), superficial layers of the lateral entorhinal cortex (P, 6.5 mm; L, 6.5 mm; V, 7.5–8.5 mm; tip separation 0.8 mm), and the lateral hypothalamus (P, −2.8 mm; L, 1.7 mm; V, −8.8 mm; tip separation 0.5 mm). Coordinates in the entorhinal cortex were chosen based on the distribution of dopaminergic afferents from the ventral tegmental area and substantia nigra to layers II and III of the anteroventral entorhinal cortex [6,8]. The vertical positions of the stimulating and recording electrodes were adjusted to maximize the current threshold and amplitude of evoked field excitatory postsynaptic potentials (fEPSP). One of four jeweler's screws was placed in the contralateral frontal bone to serve as a reference electrode, and a screw in the left parietal bone served as the ground electrode. Gold-plated Amphenol pins connected to electrode leads were mounted in a plastic 9-pin connector. The assembly was fixed to the skull by embedding the jeweler's screws, electrodes, and connector in dental cement. The analgesic buprenorphine (0.02 mg/kg, s.c.) was administered after surgery, and there was a 2–3 week recovery period before experimental testing.

1.2. Rewarding brain stimulation

Patients were tested in a 40 × 60 × 40 cm Plexiglas chamber surrounded by a Faraday cage during the lights-on phase of a 12-h light-dark cycle. Animals were trained to press a bar to obtain trains of stimulation pulses delivered to the lateral hypothalamus (LH). A 2.5 cm-diameter opening in the door of the testing chamber allowed access to a small metal bar (2.5 cm long, 0.5 cm wide) that, when pressed, resulted in an audible click, activation of three green LED lights placed above the lever, and delivery of a train of stimulation pulses to the LH. The 0.5 s-duration stimulation trains were composed of 0.1 ms-duration biphasic, square-wave, constant-current pulses delivered using a pulse generator (A-M Systems, Model 2100), and stimulus isolation unit (A-M systems, Model 2200).

Beginning with low currents (80 μA) and frequencies (60 Hz), stimulus parameters were adjusted for each animal to maximize bar-pressing behavior. Animals were shaped to press the lever by delivering stimulation trains when they showed investigatory behavior directed toward the bar, and touched or pressed the lever. Stimulus intensity was gradually increased until investigatory behavior was shown, and then stimulation frequency was increased in order to obtain reliable bar pressing behavior. After determining the highest stimulus intensity that resulted in robust and consistent bar-pressing in the absence of aversive reactions (150–400 μA), and after allowing the animal to learn to bar press for at least one 10-min period, a 'frequency sweep' was performed to determine how bar pressing rates varied as a function of train frequency. The number of bar presses within 1 min was recorded for frequencies ranging between 25 and 125 Hz beginning with the high frequency established in initial training. After each 1-min period the bar was retracted for 20 s, a new stimulation frequency was chosen, and the lever was reintroduced for a period of 60 s. An initial stimulation was delivered to facilitate bar-pressing, and the number of presses within the 60 s period was recorded. The frequency at which the maximal rate of bar-pressing was observed was used in subsequent tests of the effects of rewarding stimulation on evoked synaptic responses, and responses were fit to a sigmoid function (SigmaPlot 11.0; Systat Software Inc.) to determine stimulation frequencies resulting in 50 and 20% of maximal bar pressing rates. Animals were allowed to bar-press for LH stimulation for at least two 10 min sessions on different days prior to assessing the effects of LH stimulation on evoked synaptic responses.

1.3. Evoked synaptic responses

A computer digital-analog channel was used to deliver 0.1 ms biphasic constant-current square-wave pulses to the piriform cortex via a stimulus isolation unit (A-M Systems, Model 2200). Evoked field potentials were analog filtered (0.1–5 kHz passband), amplified (A-M Systems, Model 1700), and digitized at 10 kHz (12-bit) for storage on computer hard disk using the software package Sciworks (Datawave Tech.). A piriform cortex stimulus intensity that evoked a synaptic response of 60 to 75% of the maximal response amplitude was determined for each animal, and this intensity was used in subsequent tests of the effects of LH stimulation.

1.4. Effects of rewarding brain stimulation on synaptic responses

After determining parameters for rewarding electrical brain stimulation, each animal underwent testing to determine how rewarding brain stimulation delivered to the lateral hypothalamus could modulate the amplitude of responses evoked in the entorhinal cortex by stimulation of the piriform cortex. Initial tests were conducted on separate days to determine if delivery of LH stimulation trains (0.5 s duration, maximal frequency) with a fixed interval could modulate evoked synaptic responses in the entorhinal cortex. For each test there was an initial 10 or 15 min baseline period during which synaptic responses were evoked in the entorhinal cortex (typically once every 15 s) without prior LH stimulation, a 10 min LH-stimulation period in which stimulation trains were delivered to the LH at a set interval prior to each evoked synaptic response in the entorhinal cortex, and a follow-up period in which synaptic responses were evoked in the entorhinal cortex without prior LH stimulation. In the first tests, the trains were delivered once every 15 s, for a total of 40 trains during the 10 min LH stimulation period, and the piriform cortex stimulation pulse was delivered 0.5 s after each train. To determine if modulation of evoked responses depends on the number of stimulation trains, an additional test used a 4 s interval between trials throughout testing for a total of 150 trains (versus only 40) during the 10 min LH stimulation period. A further test was conducted (using a 15 sec inter-trial interval), to determine if the modulation of synaptic responses