Olfactory Inputs Activate the Medial Entorhinal Cortex Via the Hippocampus

GERARDO BIELLA AND MARCO DE CURTIS
Department of Experimental Neurophysiology, Istituto Nazionale Neurologico, 20133 Milan, Italy

Biella, Gerardo and Marco de Curtis. Olfactory inputs activate the medial entorhinal cortex via the hippocampus. J. Neurophysiol. 83: 1924–1931, 2000. The lateral and medial regions of the entorhinal cortex differ substantially in terms of connectivity and pattern of activation. With regard to olfactory input, a detailed and extensive physiological map of the olfactory projection to the entorhinal cortex is missing, even if anatomic studies suggest that the olfactory afferents are confined to the lateral and rostral entorhinal region. We studied the contribution of the medial and lateral entorhinal areas to olfactory processing by analyzing the responses induced by lateral olfactory tract stimulation in different entorhinal subfields of the in vitro isolated guinea pig brain. The pattern of synaptic activation of the medial and lateral entorhinal regions was reconstructed either by performing simultaneous multisite recordings or by applying current source density analysis on field potential laminar profiles obtained with 16-channel silicon probes. Current source density analysis demonstrated the existence of a direct monosynaptic olfactory input into the superficial 300 μm of the most rostral part of the lateral entorhinal cortex exclusively, whereas disynaptic sinks mediated by associative fibers arising from the piriform cortex were observed at 100–350 μm depth in the entire lateral aspect of the cortex. No local field responses were recorded in the medial entorhinal region unless a large population spike was generated in the hippocampus (dentate gyrus and CA1 region) by a stimulus 3–5 ms intensity necessary to obtain a maximal monosynaptic response in the piriform cortex. In these conditions, a late sink was recorded at a depth of 600–1000 μm in the medial entorhinal area (layers III–V) 10.6 ± 0.9 (SD) msec after a population spike was simultaneously recorded in CA1. Diffuse activation of the medial entorhinal region was also obtained by repetitive low-intensity stimulation of the lateral olfactory tract at 2–8 Hz. Higher or lower stimulation frequencies did not induce hippocampal-medial entorhinal cortex activation. These results suggest that the medial and the lateral entorhinal regions have substantially different roles in processing olfactory sensory inputs.

INTRODUCTION

Several pieces of evidence indicate that the lateral entorhinal cortex (LERC) and the medial entorhinal cortex (MERC) can be distinguished according to their general cytoarchitectonic features (Insausti et al. 1997), their connectivity (Deadwyler et al. 1975; Hjorth-Simonsen and Jeune 1972; Kosel et al. 1982; Liu and Bilkey 1997; Shipley 1975; Swanson and Köhler 1986; Wu et al. 1998), and their pattern of activation (van der Linden et al. 1999). The possibility that these two cortical regions represent functionally independent structures will be further tested here by analyzing their functional activation in response to olfactory input. The olfactory projection to the limbic cortex has been extensively studied using anatomic techniques (Krettek and Price 1978; Luskin and Price 1983; Room et al. 1984; Schwerdtfeger et al. 1990; Wilson and Steward 1978). The fibers of the lateral olfactory tract (LOT) rise from the olfactory bulbs and project to the piriform cortex (PC) and the rostral part of the entorhinal cortex (ERC). Both LOT fibers and corticocortical associative fibers that originate in the PC terminate principally in the superficial layers of the LERC. Electrophysiological studies have confirmed the selective olfactory projection to the rostrolateral ERC (Boeijinga and Van Groen 1984; Chapman and Racine 1997; Deadwyler et al. 1975; Liu and Bilkey 1997; Moulty et al. 1998; Van Groen et al. 1990). These reports suggested that olfactory inputs do not project directly to the MERC. Moreover, olfactory afferents cannot be transmitted to the MERC via the LERC because the lateral and medial ERC are not interconnected (M. de Curtis, G. Biella, and T. Iijima, unpublished observations; Dolorfo and Amaral 1998). The LERC projects via the lateral perforant path to the hippocampus (Canning and Leung 1997; Hjorth-Simonsen and Jeune 1972; Leung et al. 1995), from which a diffusely distributed projection returns to the ERC (Lopes da Silva et al. 1990; Witter 1993).

To verify the existence of selective projection of olfactory afferents to ERC subregions, we used in vitro isolated guinea pig brain preparation to map the responses evoked by LOT stimulation in the medial and lateral portions of the entorhinal region. Isolated brain preparation is an ideal preparation with which to perform such a study because the position of the recording electrodes can be easily and rapidly moved under direct visual control in different sites of the exposed ERC (Biella and de Curtis 1995; Biella et al. 1996; de Curtis et al. 1991; Muhlethaler et al. 1993). We show that the most lateral aspect of the rostral ERC receives monosynaptic olfactory input whereas an associative polysynaptic response is observed in the entire LERC. The deep layers of the MERC were exclusively activated polysynaptically via the hippocampus when a large population spike was generated in the CA1 region by increased stimulation intensity. Preliminary results were reported in abstract form (Biella and de Curtis 1999).

METHODS

Adult guinea pigs (150–250 g) were anesthetized with sodium pentothal (20 mg/kg i.p.). During anesthesia, an intracardiac perfusion with cold, oxygenated saline solution was performed before the brain isolation procedure was started (for details see de Curtis et al. 1991, 1998; Muhlethaler et al. 1993). The brain was perfused through the basilar artery with a complex saline solution composed of 126 mM NaCl, 3 mM KCl, 1.2 mM KH₂PO₄, 1.3 mM MgSO₄, 2.4 mM CaCl₂,
26 mM NaHCO₃, 15 mM glucose, 2.1 mM HEPES, and 3% dextran M. W. 70.000 (SIFRA, Isola della Scala, Italy) and was saturated with a 95% O₂-5% CO₂ gas mixture. The brain was maintained in vitro in an incubation chamber at 15°C during the dissection and the temperature of the chamber was slowly increased (0.2°C/min) to 32°C before the experiment was started. The experimental protocol was reviewed and approved by the Committee on Animal Care and Use and by the Ethical Committee of the Istituto Nazionale Neurologico.

Extracellular recordings were performed with tungsten electrodes, glass micropipettes filled with 1 M NaCl, stainless steel electrodes, and multichannel silicon probes featuring 16 iridium recording sites 100 μm apart and vertically assembled in a single shaft (obtained from the Center of Neural Communication Technology, University of Michigan, Ann Arbor, MI). These probes have been proven to be ideal for recording laminar profiles in cortical structures (Bragin et al. 1995). The input resistance of the extracellular recording electrodes varied between 2 and 4 MOhm. The multichannel electrodes were positioned perpendicular to the cortical lamination at different sites in the medial and lateral parts of the ERC. The placement of the electrodes was performed under direct visual control via a stereoscopic microscope. LOT-evoked responses were used to verify that all recording sites along the shaft of the silicon probe were inserted in the cortex; the most superficial contact was positioned at the pial surface. The positions of the recording electrodes were verified by identifying the lesions by passing a 20 μAmp current between the two deepest iridium contacts for 10–20 s at a depth of 1500–1600 μm. Histological controls were performed on 100-μm coronal sections cut from brains fixed with 4% paraformaldehyde. Stimulating bipolar electrodes (custom-made twisted silver wires or tungsten electrodes, FHC, Bowdoinham, ME) were positioned either on the LOT or in the molecular layer of the posterior PC.

Averages of 5–7 responses were used to build field potential laminar profiles recorded with the 16-channel silicon probes. Current source density (CSD) analysis was performed on 100-μm per step profiles with a 400-μm separation grid, as previously described (Biella and de Curtis 1995; de Curtis et al. 1994). The data were recorded with a 16-channel extracellular amplifier (Biomedical Engineering, Thornwood, NY) and were stored on a digital tape recorder (Biologic, Clai, France). Online and offline analyses were performed with CLAMPVIEW (SIDeA, Milan, Italy).

Specific subroutines for CSD data analysis were developed in our laboratory by G. Biella in collaboration with SIDeA.

**RESULTS**

This study was performed on 31 isolated guinea pig brains. In the first set of experiments, the responses evoked by LOT stimulation in the olfactory-limbic region were characterized. Responses were mapped by making recordings, within the same session, from 10–20 sites in the piriform, entorhinal, insular, and perirhinal cortices and in the amygdala, with simultaneous recordings from as many as seven electrodes. One electrode was permanently positioned in the anterior piriform cortex to monitor the stability of the LOT-evoked response when the other recording electrodes were moved around during the experiment. Figure 1 illustrates the typical pattern activated by a LOT stimulus (70% of the intensity necessary to induce a maximal monosynaptic response in electrodes 1 and 2 from the anterior PC). The positions of the cortical recording electrodes, shown in the ventral view of a guinea pig brain in Fig. 1, left, were reproduced between experiments by using surface brain structures as reference points. A monosynaptic response was observed in the piriform cortex (electrodes 1, 2, and 3), the periamygdaloid cortex (electrode 4), the basolateral amygdala (electrode 5), and the rostral part of the LERC (electrode 9). A large-amplitude polysynaptic response was recorded in all cortical sites analyzed, with the exception of the MERC (electrodes 12 and 13) and the caudal perirhinal cortex (electrode 8), where small-amplitude, possibly volume-conducted, responses were observed (see Fig. 3). The latencies of the monosynaptic peak amplitude potentials in the posterior piriform cortex (PPC) and the LERC were 12.38 ± 1.92 (SD) and 15.59 ± 1.52 ms, respectively (n = 12). The polysynaptic responses in the PPC, the rostral LERC (electrode 9), and the caudal LERC (electrode 11) peaked at 21.45 ± 2.93, 29.18 ± 2.47, and 37.33 ± 2.66 ms, respectively (n = 11). Current source density analysis

![Field responses evoked by lateral olfactory tract (LOT) stimulation in the olfactory cortex and limbic structures of isolated guinea pig brain preparation. Positions of electrodes (left) used to record responses (right) are shown on a schematic ventral view of the guinea pig brain. Cortical recordings were performed at depths 500–700 μm from the pial surface. Arrowheads, LOT stimulus. SE, stimulation electrode.](http://jn.physiology.org/)
performed on laminar field responses recorded with multichannel silicon probes demonstrated that the mono- and disynaptic responses in the PPC \((n = 22)\) and the LERC \((n = 20)\) were generated by current sinks located in the superficial layers (Fig. 2; see also Biella and de Curtis 1995; Biella et al. 1996; Boeijinga and Van Groen 1984; de Curtis et al. 1991); no locally generated sinks were observed in the perirhinal cortex (PRC) \((n = 7)\;\text{not shown}\) and MERC (Fig. 3; \(n = 6)\). We
could not detect a monosynaptic sink in the caudal two-thirds of the LERC, where the field profile in Fig. 2 was performed. Two electrode tracks determined by the 16-channel silicon probes in the LERC and the MERC are illustrated in Fig. 3C. As in other mammals, MERC in the guinea pig was characterized by six distinct layers whereas the LERC cytoarchitecture featured 1) clusters or islands of neurons in layer II, 2) a thinner lamina dissecans (layer IV), and 3) a less well-defined distinction between deep layers V and VI (Insausti et al. 1997). The CSD results confirmed that the small-amplitude field responses recorded in the MERC and the PRC represent far fields generated in the LERC and passively volume-conducted through the tissue. In nine of 13 experiments, the MERC was activated when

FIG. 4. Field responses recorded simultaneously in limbic cortices and evoked by 2 intensities of LOT stimulation (7 and 20 μA, 0.1 ms). Site 1, anterior piriform cortex (APC); sites 2 and 3, rostral and caudal LERC; sites 4 and 5, rostral and caudal MERC; sites 6 and 7, dentate gyrus (DG) and CA1. Arrowhead, stimulus delay; arrows, DG and CA1 population spikes; asterisk, late response in the MERC evoked by high-intensity LOT stimulation. Piriform cortex (PC) and entorhinal cortex (ERC) recordings were performed at depths of 500–700 μm.
the intensity of LOT stimulation was increased above the threshold for induction of a large and synchronous population spike in the hippocampus. In Fig. 4, simultaneous recordings were performed in the anterior piriform cortex (APC) (electrode 1), at two sites in the LERC (electrodes 2 and 3) and the MERC (electrodes 4 and 5), in the dentate gyrus (DG) (electrode 6), and in the CA1 region of the hippocampus (electrode 7). The positions of the recording electrodes are illustrated in Fig. 4, left. The location of the hippocampal electrodes was confirmed histologically by identifying the electrolytic lesions formed by stainless steel electrodes at the end of electrophysiological recording (not shown). A large field response was observed in the MERC when the stimulus intensity was increased to generate a population spike in the DG and CA1 recording sites (arrows in Fig. 4, right). A population spike was consistently observed in the MERC response (asterisk in Fig. 4, right). The latency between the CA1 spike and the population spike in the MERC response was 10.6 ± 0.9 ms (n = 11). As illustrated in Fig. 5, late posthippocampal responses (asterisks) were observed in the caudal and medial parts of the entorhinal region (dark gray area) whereas no late responses were recorded in the lateral and rostral parts (light gray area), in which

**FIG. 5.** Map of late response in the ERC in a typical experiment. Histological identification of typical MERC and LERC cytoarchitectonic features of the region in which the different recordings were performed was verified. LERC and MERC are light gray and dark gray, respectively. Recordings in the MERC were performed at a depth of 200 μm and recordings in the LERC at a depth of 500 μm. Asterisks, late MERC responses. Amplitude calibration bars on the left and right refer to LERC and MERC potentials, respectively.

**FIG. 6.** CSD analysis of MERC field potential profile (A) performed with 16-channel probes. A: potentials from a representative experiment. Three-dimensional (B) and contour plots (C) of CSD profile (average of 6 profiles obtained in different experiments) are shown. Asterisk, large sink generated in deep cortical layers. Current values in B are mV/mm². Contour intervals in C are 0.12 V/mm².
large short-latency LOT responses were observed. Small-amplitude posthippocampal responses in the LERC were observed in nine experiments (see Fig. 7A). Such responses probably represent far fields because they were not associated with locally generated current sinks (not shown; n = 3). Predictions of the recording electrode location in the LERC or MERC on the basis of electrophysiological responses were consistently confirmed by morphological controls performed on Nissl-stained (100 μm) coronal sections after electrocoagulation of the recording electrode tip (see DISCUSSION).

CSD analysis of MERC field potential profiles confirmed the local origin of the late posthippocampal response. As illustrated in Fig. 6, B and C (the average of 6 CSD profiles), a fast, large-amplitude sink superimposed on a slower sink centered at 600–1000 μm (layers III–V in MERC) was observed. During paired LOT stimulation (10–50% of the intensity necessary for achieving the hippocampal activation threshold), the hippocampus-MERC circuit was activated in the second conditioned response for an interstimulus interval between 100 and 900 ms (Fig. 7B). As illustrated in in Fig. 7C, bottom trace, repeated low-intensity LOT stimulation at a frequency between 2 and 8 Hz determined MERC response activation. MERC activation via the hippocampus (Fig. 7C; arrows) showed a noncontinuous pattern.

**DISCUSSION**

The present study demonstrates that, in the guinea pig, 1) stimulation of LOT fibers originating in the olfactory bulbs induces short-latency, monosynaptic responses in the LERC but not in the MERC, 2) the MERC is polysynaptically activated exclusively after hippocampal activation, and 3) the MERC can be entrained by augmenting LOT stimulation to an intensity above the threshold for hippocampal activation or by repetitive low-intensity stimulation at 2–8 Hz.

Anatomic studies with retrograde and anterograde tracers in different animal species demonstrated that the olfactory bulb projects to the ERC via the LOT. In most of these studies, the olfactory fibers were reported to project almost exclusively to the LERC (Haberly and Price 1977; Kosel et al. 1981; Luskin...
MERC is stimulated, and vice versa (unpublished observations that confirm the absence of an LERC response when the 1998). This conclusion is further strengthened by preliminary results. The prevalent view that olfactory input projects to the LERC is supported by electrophysiological studies that demonstrate field responses in the LERC after LOT stimulation (Boeijinga and Van Groen 1984; Chapman and Racine 1997; Liu and Bilkey 1997; Moully et al. 1998; Van Groen et al. 1987) whereas there are no reports of olfactory-evoked responses in the MERC. A study that described the olfactory projections to the hippocampus via the ERC (Wilson and Steward 1978) demonstrated that the response evoked by LOT stimulation in the DG was abolished when the LERC was lesioned, which suggests that the olfactory path to the hippocampus does not pass through the MERC. Our data confirmed that the rostral portion of the LERC mediates a direct olfactory projection to the hippocampus that does not involve MERC significantly.

LOT stimulation induced prominent polysynaptic responses in the LERC. The results described here suggest that such a projection is functionally maintained by the contribution of associative cortical input from the piriform cortex, which is synchronously and massively activated by LOT stimulation. This conclusion is supported by 1) the demonstration of high-amplitude polysynaptic responses in the LERC during our experiments and in the rat in vivo (Moully et al. 1998) and 2) the observation of a larger response evoked by PPC stimulation in comparison with the LOT-evoked potential in the LERC (de Curtis et al. 1994; Habets et al. 1980; Wilson and Steward 1978). In our experiments, the monosynaptic response evoked by LOT stimulation indeed showed small amplitude and was restricted to the most rostral portion of the LERC whereas a large polysynaptic potential was found throughout the LERC (see also Moully et al. 1998). The presence of a strong associative connectivity between the PC and the LERC is suggested by the relative amplitudes of the mono- and disynaptic components in the PPC and the LERC (see also Boeijinga and Van Groen 1984). Although the monosynaptic potential amplitude decreased from rostral to caudal and virtually disappeared in the caudal two-thirds of the LERC, the disynaptic peak amplitude did not decline with the monosynaptic potential and was consistently observed throughout the LERC. The disynaptic potential in the LERC was abolished by interrupting the LOT and the associative fibers with a superficial coronal section at the PPC-ERC border (unpublished observations; Biella et al. 1996), suggesting that the disynaptic potential in the ERC is mediated by the activation of associative fibers originating in the PC.

The absence of an associative response in the MERC after LOT stimulation demonstrates that 1) the corticocortical projections arising from the entire PC do not project to the MERC and 2) the LERC and MERC are completely separate with regard to olfactory input. Our results not only confirm the pattern of distribution of the olfactory fibers in the ERC, but also corroborate anatomic observations that exclude the presence of a lateral-to-medial associative fiber system within the ERC and demonstrate intrinsic associative connections, predominantly in the rostrocaudal dimension (Dolorfo and Amaral 1998). This conclusion is further strengthened by preliminary results that confirm the absence of an LERC response when the MERC is stimulated, and vice versa (unpublished observations). Incidentally, our results show that there is no functionally active direct projection from the olfactory areas to the PRC whereas a polysynaptic response can be recorded in the rostral PRC and in the insular cortex located just lateral to the rhinal sulcus at the same rostrocaudal level of the piriform cortex. Based on the connectivity patterns shown here, it can be concluded that the LERC, but not the MERC, can be regarded as an associative olfactory area.

Our findings demonstrate that the hippocampus can be activated by olfactory stimulation. Hippocampal responses to LOT stimulation were previously reported in different animal species (Habets et al. 1980; Schwerdtfeger et al. 1990; Wilson and Steward 1978). When the hippocampal loop was activated by strong or repetitive LOT stimulation, an efferent signal reentered the MERC, as illustrated in Figs. 2, 3, and 6. The latencies between the hippocampal spike in CA1 and the MERC response were compatible with a single-synapse transmission. Even if anatomic studies show that all layers in the ERC receive fibers from the hippocampus (see Witter 1993), a large contingent of hippocampal efferents from the CA1/3 area and the subiculum has been shown to contact the deep layers in the MERC in rat and guinea pig (Hjorth-Simonsen and Jeune 1972; Swanson and Köhler 1986). According to our findings, the hippocampal efferent projection activated by olfactory stimulation generates a distinct sink 600–1000 μm deep in layers III–V of the MERC. Electrophysiological studies performed in vivo in the guinea pig demonstrated that stimulation of the dorsal psalterium induced activation of the contralateral hippocampus followed by a posthippocampal response generated in the contralateral ERC (Bartesaghi et al. 1989) that showed general features and latencies comparable to the MERC potential recorded in our experiments. In agreement with our findings, in these in vivo studies the posthippocampal potentials were 1) recorded in the medial part of the ERC, 2) observed diffusely in the rostrocaudal dimension of the ERC (Bartesaghi et al. 1994), and 3) generated between layers VI and III.

The hippocampus and the MERC were activated by repetitive LOT stimulation in a particular frequency range (2–8 Hz) close to olfactory theta activity (Freeman and Schneider 1982), an oscillatory pattern that has been linked to odor discrimination induced by sniffing in mammals (Macrides et al. 1982; Yougentob et al. 1987). It is tempting to speculate that repeated, rhythmic olfactory activation at a frequency that mimics “theta sniffing” might determine a condition that promotes associative interactions in the MERC between olfactory signals and nonolfactory cortical inputs. Further evaluation of such interactions in the MERC will help clarify the role of olfaction in memory formation and retrieval.

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Address for reprint requests: M. de Curtis, Dept. of Experimental Neurophysiology, Istituto Nazionale Neurologico, via Celoria 11, 20133 Milan, Italy.

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