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SENSORY RESPONSES IN THE MEDIAL PREFRONTAL CORTEX OF ANESTHETIZED RATS. IMPLICATIONS FOR SENSORY PROCESSING

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Abstract—The medial prefrontal cortex (mPFC) plays a key role in higher functions such as memory and attention. In order to demonstrate sensory responses in the mPFC, we used electrophysiological recordings of urethane-anesthetized rats to record somatosensory-evoked potentials (SEPs) or auditory-evoked potentials (AEPs) elicited by whisker deflections and click stimulation, respectively. Contralateral whisker stimulation or auditory stimuli were also applied to study sensory interference in the mPFC. Interference with other sensory stimuli or recent stimulation history reduced whisker responses in the infralimbic and prelimbic cortices of the ventral mPFC. This effect could be mediated by activation of parvalbumin (PV) interneurons since the effect was blocked by the P/Q calcium channel antagonist ω-agatoxin. In contrast, sensory interference or the recent stimulation history was not detected by the dorsal mPFC or the primary somatosensory cortex. Results obtained from retrograde tracer injections in the dorsal and ventral regions of the mPFC indicated that somatosensory and auditory sensory inputs may arrive at the dorsal mPFC through secondary sensory cortical areas, and through the insular and temporal cortical areas. The ventral mPFC may receive sensory information through the strong anatomical connections between the dorsal and ventral mPFC areas. In conclusion, results suggest mPFC plays an important role in sensory processing, which may have important implications in attentional and memory processes.

Key words: prelimbic cortex, infralimbic cortex, sensory interference, evoked potentials.

INTRODUCTION

In both humans and rodents, the medial prefrontal cortex (mPFC) plays a key role in many higher executive functions including working memory, attention, decision-making, goal-directed behavior and emotion (Groenewegen and Uylings, 2000; Miller and Cohen, 2001; Dalley et al., 2004; Wise, 2008). The mPFC is a heterogeneous area comprised of four main divisions, which are the medial agranular (AGm) cortex, the anterior cingulate (AC) cortex, the prelimbic (PL) and infralimbic (IL) cortices. Although the prefrontal cortex regulates cognitive functions, it is not known to contain representational maps of sensory space that are necessary to perform all functions listed above.

Both deep and superficial mPFC layers receive long-range inputs from cortical and subcortical regions and project to other structures (Gabbott et al., 1997, 2005; Groenewegen et al., 1997; Hoover and Vertes, 2007). The main sources of afferent projections shift along the mPFC from predominantly sensorimotor inputs to the dorsal mPFC (AGm and dorsal AC) to primarily limbic inputs to the ventral mPFC (PL and IL). Major long-range inputs to layer 5 mPFC neurons originate from other prefrontal areas, including agranular insula, dorsal polymodal thalamic nuclei, contralateral mPFC and motor cortical areas (DeNardo et al., 2015). There is a marked reduction in cortical projections, mainly involving sensory (all sensory modalities), motor or associational regions of the cortex, to the ventral mPFC when compared to the dorsal mPFC (Hoover and Vertes, 2007). Its many connections with other cortical and subcortical areas could allow the mPFC to act as a control station, integrating information it receives from numerous input structures and converging updated information to output structures (Miller and Cohen, 2001). In contrast to the barrel cortex, a neuronal circuit dedicated to processing somatosensory information, mPFC circuits integrate information from diverse brain regions that are involved in a wide variety of cognitive functions.

It has been indicated that the mPFC in rats is required for working-memory tasks (Yang et al., 2014). Therefore, lesions to the mPFC impair the rat's performance on delayed alternation tasks (Dunnett et al., 1999). The participation of the mPFC in attention functions has also been well-characterized using serial reaction time tasks and excitotoxic lesions. Rats with mPFC lesions are impaired over a wide range of spatial tasks, especially those requiring working memory (Kolb et al., 1983, 1994), reversal learning (De Bruin et al., 2000) or attentional shift (Birrell and Brown, 2000; Passetti et al., 2002; Pezze et al., 2014). Lesions restricted to the IL cortex increase impulsive responses (Chudasama et al., 2003), and infusion of the $GABA_A$ receptor antagonist

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Abbreviations: AC, anterior cingulate; AEPs, auditory-evoked potentials; AGm, medial agranular; EP, evoked potential; FlGo, Fluoro-Gold; IL, infralimbic; mPFC, medial prefrontal cortex; PL, prelimbic; POm, posterior medial; PV, parvalbumin; SEPs, somatosensory-evoked potentials; VPM, ventral posterior medial.

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bicuculline into the PL/IL cortex impairs their attentional performance (for a review, see Cassaday et al., 2014). Attentional deficits following peripubertal stress are accompanied by a reduction in the expression of the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD), across the different layers in the mPFC and in the medial and ventral orbitofrontal cortex. This suggests that alterations in the function of GABAergic transmission in the mPFC may be a relevant mechanism underlying attentional deficits (Tzanoulinou et al., 2016).

To perform these functions one would expect the PL/IL cortex to receive sensory information either from the sensory cortical areas or the thalamus. However, most of the sensory inputs arrive at the mPFC through the AGm cortex or the AC cortex (Hoover and Vertes, 2007). As a result, the manner in which the mPFC integrates this diversity of information is not fully understood. The goal of the present study is to compare tactile responses in the mPFC to those in the S1 cortex. To achieve this we used electrophysiological recordings in anesthetized rats and whisker and/or auditory stimuli were applied to determine sensory interactions in the mPFC. The mPFC and S1 cortex displayed tactile responses that were reduced when the stimulation frequency increased. Moreover, mPFC responses displayed more important changes during sensory interference processes or according to the recent whisker stimulation history than S1 cortex. Retrograde tracers were injected into the dorsal and ventral regions of the mPFC to establish the origin of sensory inputs. Our results implicate the mPFC as a key center in modifying cortical responses according to the context in which sensory stimuli appear.

EXPERIMENTAL PROCEDURES

Electrophysiological experiments

Experiments were performed on 95 (46 males and 49 females) adult Sprague–Dawley rats, both sexes weighing 250–300 g. The animals were housed under standard colony conditions and food and water were supplied *ad libitum*. All animal procedures were approved by the Ethics Committee of the Autonomous University of Madrid, in accordance with Council Directive 2010/63 of the European Community. Efforts were made to minimize the number of animals used and their level of discomfort.

Animals were anesthetized with urethane (1.6 g/kg i.p) and placed in the stereotaxic device where surgical procedures and recordings were performed. Their body temperature was maintained at 37 °C; the level of anesthesia was monitored by the absence of whisker movements and pinch withdrawal reflex and kept constant using supplemental doses of urethane (0.5 g/kg i.p.). A midline skin incision was made, and the periosteum was removed to expose the skull. Then, the dura mater membrane was removed and the cortex was covered with mineral oil to prevent drying.

Tungsten microelectrodes (1–2 MΩ) were lowered vertically in order to obtain EEG recordings in the barrel field of the primary somatosensory (S1) cortex (*AP*: −0.3 mm, *L*: 4–6 mm, *D*: 1.5 mm) and mPFC (*AP*: +3 mm, *L*: 0.2–0.8 mm, *D*: 1–5 mm), according to the Paxinos and Watson Atlas (2007). Electrocortical recordings were filtered (0.3–300 Hz) and amplified using a P15 preamplifier (Grass, West Warwick, USA). These data were recorded continuously, sampled at 1 kHz via an analog-to-digital converter built into the Power 1401 data acquisition unit, and fed into a PC computer for off-line analysis with Spike 2 software (Cambridge Electronic Design, Cambridge, UK). Multiunit recordings were performed (filters: 0.3–3KHz) to ensure the correct location of the electrode in the barrel field of the S1 cortex. After testing the largest response to whisker stimulation, the signal was filtered for EEG recording (0.3–300 Hz). Somatosensory-evoked potentials (SEPs) were extracted in the mPFC and S1 cortices by calculating the average of 60 whisker stimuli; auditory-evoked potentials (AEPs) were recorded in the mPFC and A1 cortices using 60 click stimuli. Grand averages from different animals were also calculated in order to depict SEPs and AEPs (Fig. 1A, B, respectively).

Sensory stimulation and protocols

Whisker deflections were generated by a pneumatic pressure pump (Picospritzer) that delivered a brief air pulse (20 ms duration) through a 1-mm-inner diameter polyethylene tube. All whiskers were trimmed to 5 mm in length, to ensure that reproducible responses were evoked from one to three whiskers. The pressure was set at 1–2 kg/cm^2, resulting in whisker deflections of ≈15°. AEPs were elicited by trains of clicks delivered at 1 Hz (1-ms duration, 50 dB SPL) through Sony earphones which were located 5 cm from the animal's head.

The response of the mPFC neurons to a stimulus delivered to the whiskers was studied using different experimental designs. The first one, depicted in Fig. 4A, consisted of two 1 Hz whisker stimulation trains (first protocol; control). They were interrupted by a 5-Hz whisker stimulation train or a white noise train (50 dB, frequency band of 10–160 kHz), lasting 10 s (second and third protocols, respectively). The SEP from the second stimulation train was compared to the first control train, which was normalized to 100%.

The second experimental design, depicted in Fig. 5A, consisted of a 5-Hz air puff train that was applied twice to whiskers (60 s between them) to compare the SEP response from the first train (test stimulus) in respect to the second train (first protocol; control). During the time lag between them, whisker stimulation (pulses of 20-ms duration at 1 Hz) or click stimulation (1 ms duration at 1 Hz) was applied for 60 s (second and third protocols, respectively). The SEP from the first stimulation train was compared to the second (control) stimulation train, which was normalized to 100%.

Two different experimental designs were also performed to test sensory interference in the mPFC. The *somatosensory interference protocol* consisted of three 60-s periods of 1-Hz whisker stimulation (see Fig. 6A, upper trace) or 1-Hz click stimulation (see Fig. 7, upper trace). In the second, a distractor stimulus was presented as a contralateral multi-whisker