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Neuronal coding of auditory sensorimotor gating in medial prefrontal cortex

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ABSTRACT

The medial prefrontal cortex (mPFC) is thought to be an essential brain region for sensorimotor gating. The exact neuronal mechanisms, however, have not been extensively investigated yet by delicate single unit recording methods Prepulse inhibition (PPI) of the startle response is a broadly used important tool to investigate the inhibitory processes of sensorimotor gating. The present study was designed to examine the neuronal mechanisms of sensorimotor gating in the mPFC in freely moving rats. In these experiments, the animals were subjected to both pulse alone and prepulse + pulse stimulations. Head acceleration and the neuronal activity of the mPFC were simultaneously recorded. To adequately measure the startle reflex, a new headstage with 3D-accelerometer was created. The duration of head acceleration was longer in pulse alone trials than in prepulse + pulse trial conditions, and the amplitude of head movements was significantly larger during the pulse alone than during the prepulse + pulse situations. Single unit activities in the mPFC were recorded by means of chronically implanted tetrodes during acoustic stimulation evoked startle response and PPI. High proportion of medial prefrontal cortical neurons responded to these stimulations by characteristic firing patterns: short duration equal and unequal excitatory, medium duration excitatory, and long duration excitatory and inhibitory responses were recorded. The present findings, first time in the literature, demonstrated the startle and PPI elicited neuronal activity changes of the mPFC, and thus, provided evidence for a key role of this limbic forebrain area in sensorimotor gating process.

1. Introduction

Sensorimotor gating is an essential mechanism of information processing to screen and filter out irrelevant sensory stimuli [1,2]. Sensorimotor gating deficits were observed in neuropsychiatric disorders such as schizophrenia [3,1] or e.g. Gilles de la Tourette-syndrome [4]. The employment of repetitive stimulation is crucial to measure sensory gating processes and to reveal the neuronal networks involved in the filtering mechanism [5]. Prepulse inhibition (PPI) refers to a sensorimotor gating processing sequence of actions in which the prior presentation of a weak stimulus inhibits the motor response to the startle response [6]. This phenomenon has been reported to exist in a large number of species [7–9]. In humans, in pathological conditions, PPI deficits are recorded with examining different kinds of sensory modalities. However, in the majority of cases of animal studies specifically auditory stimuli have usually been used. PPI is thought to be regulated by the prefrontocortico-limbic-striato-pallidal circuit that connects to the primary startle reflex pathway through mesopontine and nigral projections [9]. The medial prefrontal cortex (mPFC) plays an important role in the operation of this complex network. Manipulations that decrease mPFC dopaminergic [10,11] or GABAergic transmission [12] are known to disrupt PPI. The role of mPFC in the modulation of PPI has also been shown by lesioning and inactivation studies. PPI is reduced by infusion of 6-hydroxydopamine (6-OHDA) [11] or intra-

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mPFC infusion of D_1 or D_2 DA antagonists [10]. Such PPI disruptive effects can also be due to disinhibition of descending glutamatergic fibers of mPFC, which can thus modulate the subcortical DA transmission at the level of the nucleus accumbens. Other reports proved that the GABA antagonist picrotoxin infusion into the mPFC also disrupts PPI [12]. On the other hand, PPI was increased after neonatal mPFC lesions [13]. Neonatal lesions of the ventral mPFC led to decreased number of parvalbumin-positive GABAergic interneurons in all mPFC subregions. Nevertheless, the invasive methods mentioned above cannot fully explain the mechanisms of the physiological functioning of the mPFC in these behavioral conditions. Since to date electrophysiological methods were scarcely used to elucidate the role of mPFC in sensorimotor gating mechanisms, recorded by means of the tetrode technique in relation to the startle alone and the startle associated PPI paradigms. It is also important to note that exact measuring of the magnitude of startle response in chronic freely moving animals was technically unresolved yet. Rohleder and colleges [14] identified two counterbalancing modulation network, that modulate PPI. The medial network closely linked to the startle (facilitate-, motor site- of the startle pathway). The lateral network involves prepulse processing and increase PPI. The associated protective and orienting behaviors require more precise measurements than those allowed by the classical startle measuring methods. The 3D head accelerometer invented by us appears to solve this problem getting especially important during reactions to startle stimuli to protect the head from blows and also during motor orienting responses for PPI decreasing startle reaction amplitudes. Thus, as one of the major part of this study, a 3D accelerometer has been developed to record head acceleration, i.e. to objectively measure startle response, in chronic freely moving rats.

2. Materials and methods

2.1. Subjects

Three adult, 4 month old male Wistar rats (weighing 400–450 g) [15] were used in the present experiments. Individually caged animals were kept on 12-12-h light/dark cycle (light on at 6 a.m.) in a temperature and humidity controlled ($24 \pm 2\,^{\circ}C$) vivarium. Standard laboratory food pellets (CRLT/N, Charles River Laboratories, Budapest, Hungary) and tap water were available ad libitum. Body weights were measured daily. Rats were cared in accordance with institutional, national and international standards (BA02/2000-8/2012, Pécs University, Medical School; Law XXVIII, 1998, Hungary; 40/2013 Government Decree, 2013, Hungary; Community Council Directive 86/609/EEC, 1986, 2006; 2010; NIH Guidelines, 1997). All efforts were made to minimize the number of animals and to reduce their pain and suffering.

2.2. Electrode implantation

Prior to learning behavioral tasks, printed circuit board-based (PCB) microdrives loaded with eight tetrodes were implanted by stereotaxic guidance above the medial part of the PFC (details see later) [16,17]. To do so, rats were anesthetized with sodium-pentobarbital solution (60 mg/kg Nembutal, Phylaxia-Sanofi, Hungary) followed by i.p. atropine injection (2 mg/kg EGIS, Hungary). Skin was removed from the upper surface of the skull and anchor screws were inserted and driven into the bone. One of them was used as ground and another one as reference electrode. Burr holes were made and the PCB-microdrives were positioned by means of a micromanipulator above the mPFC (AP: 2.7 mm from bregma and ML: 0,8 mm according to the rat brain atlas of Paxinos and Watson (1996), Fig. 1) In this position thick silica tubes serving as guiding tubes for the tetrodes, reached the brain surface above the mPFC. All tetrodes were lowered approximately to the upper border of the prelimbic area.

2.3. Behavioral procedure and equipment

Recording of behavioral actions and multiple unit activity has been started after a recovery period of 2 weeks. Experiments were performed in a sound attenuated $40 \times 40 \times 40$ cm operant box during the light period. Auditory stimuli were presented via a dual piezo speaker (MPT-177) mounted on the roof of the chamber. The distance between the sound source and the rat head was 60 cm. We used two different trial types during the experiments. The first one was the startle/pulse alone trial condition (application of acoustic stimulus with 120 dB noise burst, presented for 40 ms), the second one was the prepulse + pulse trial condition (prepulse stimulus, 75 dB noise burst, presented for 20 ms, 160 ms before the startle stimulus, Fig. 2a). The sound pressure level was measured with validated sound pressure meter (ExTech EN300). Otherwise, the session began with a 5-min acclimatization (standard PPI paradigm) period during which broadband white noise was presented at 65 dB through a speaker. Next 48 stimulation were presented, each containing both trial types in pseudorandom order (24-24 startle/pulse alone and prepulse + pulse trials in pseudorandom order). For the analysis we used the first 24 trials of each stimulation types. Inter-trials intervals were at the pseudorandomly distributed range of 30–150 s. The whole experiment lasted for 9 days, in non-consecutive manner daily one session was performed. Behavioral testing, presentation of the different sound stimuli were controlled by the LabCommander software (Noted Bt., Pécs, Hungary). Sound stimulation was generated by playing of audio (.wav) files using the sound card of the computer.

2.4. Data recording and spike sorting

For electrophysiological recording of neuronal activity and head acceleration, a miniature 32-channel head stage amplifier (Noted Bt., Pécs, Hungary) with a homemade 3D-accelerometer (differential capacitive accelerometer: low power consumption, large output level, and fast response to motions) invented by A. T. was plugged into the socket at the interface board on the microdrive. Tetrodes were moved on after each session, obligatory noted for the subsequent reconstruction of recording sites. Wideband signals (0.1 Hz–18.75 kHz) from 8 tetrodes (Startle 28 + 4 channel preamplifier, Noted Bt., Pécs, Hungary), and events were recorded continuously by means of a 24-bit, 64-channel low voltage AD converter (LVC-64, Noted Bt., Pécs, Hungary). Spikes were extracted after automatic threshold detection (power higher than 5 times the standard deviation from the baseline), and reconstructed to 37.5 kHz by using the principles of the sampling theorem [18]. Employing a principal component analysis, a 12-dimensional feature vector was created for each spike (first three principal components for each channel from a tetrode) [18,19]. Spikes from putative individual neurons were segregated using automatic clustering software. Automatic clustering was performed by means of the KlustaKwik software [20]. Only units with clear symmetric gap in their autocorrelation, units with a clear refractory period (<3 ms, determined on the basis autocorrelograms) and well-defined oval shaped cluster boundaries were used for further analysis. Cross-correlation histograms of all possible pairs recorded from a given tetrode probe were calculated by means of the Klusters software [21]. In case of symmetrical gap in the center of bins indicated that the initial clusters represented activities of the same unit clusters, so these clusters were merged.