Progress in Neuro-Psychopharmacology & Biological Psychiatry 56 (2015) 174–184

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Neuronal activity of the prefrontal cortex is reduced in rats selectively bred for deficient sensorimotor gating

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ARTICLE INFO

Article history:
Received 1 October 2013
Received in revised form 8 August 2014
Accepted 15 August 2014
Available online 16 September 2014

Keywords:
Entopeduncular nucleus
Local field potentials
Neuropsychiatric disorders
Nucleus accumbens
Prepulse inhibition

ABSTRACT

Rats selectively bred for deficient prepulse inhibition (PPI), an operant measure of sensorimotor gating in which a weak prepulse stimulus attenuates the response to a subsequent startling stimulus, may be used to study certain pathophysiological mechanisms and therapeutic strategies for neuropsychiatric disorders with abnormalities in information processing, such as schizophrenia and Tourette's syndrome (TS). Little is known about neuronal activity in the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAC), which are involved in the modulation of PPI. Here, we examined neuronal activity in these structures, and also in the entopeduncular nucleus (EPN), since lesions of this region alleviate the PPI deficit.
Male rats with breeding-induced *high* and *low* expression of PPI ($n = 7$, each) were anesthetized with urethane (1.4 mg/kg). Single-unit activity and local field potentials were recorded in the mPFC, the NAC and in the EPN. In the mPFC discharge rate, measures of irregularity and burst activity were significantly reduced in PPI *low* compared to PPI *high* rats ($P < 0.05$), while analysis in the NAC showed approximately inverse behavior. In the EPN no difference between groups was found. Additionally, the oscillatory theta band activity (4–8 Hz) was enhanced and the beta band (13–30 Hz) and gamma band (30–100 Hz) activity was reduced in the NAC in PPI *low* rats. Reduced neuronal activity in the mPFC and enhanced activity in the NAC of PPI *low* rats, together with altered oscillatory behavior are clearly associated with reduced PPI. PPI *low* rats may thus be used to study the pathophysiology and therapeutic strategies for neuropsychiatric disorders accompanied by deficient sensorimotor gating.

1. Introduction

Neuropsychiatric disorders are increasingly recognized as network disorders with abnormal neuronal activity in cortico-subcortical loops. Understanding the abnormalities in the firing patterns and synchrony of neuronal activity that underlie specific behavioural disturbances would be useful to develop and improve therapeutics to attenuate such pathological processes (Carlson et al., 2006; Kopell and Greenberg, 2008).

Sensorimotor gating mechanisms, which allow the nervous system to suppress or "gate" responding to external stimuli and internally generated signals or impulses, are disturbed in certain neuropsychiatric disorders (Swerdlow and Geyer, 1998; Braff et al., 2001). Such gating mechanisms have been operationalized in measures of prepulse inhibition (PPI) of the acoustic startle response (ASR), i.e., the reduction of the ASR when the startling noise pulse is shortly preceded by a weak prepulse (Koch et al., 2000; Swerdlow et al., 2001). Deficient PPI has been demonstrated in schizophrenia, Tourette's syndrome (TS), and obsessive compulsive disorder (Swerdlow and Sutherland, 2006; Braff et al., 2001; Kohl et al., 2013), and experimentally-induced PPI deficits in rodents are used as a common endophenotype to model this basic deficiency in these disorders (Cadenhead et al., 2002; Braff and Light, 2005).

Selective breeding in Wistar rats for *high* and *low* PPI leads to a segregation of two rat lines with significantly different PPI (Schwabe et al., 2007). The antipsychotic dopamine (DA) receptor antagonist haloperidol alleviated the breeding-induced PPI-deficit (Hadamitzky et al., 2007). Additionally, behavioral deficits and epigenetic factors in PPI *low* rats corroborate clinical findings seen in clinical practice (Dieckmann et al., 2007; Freudenberg et al., 2007; Rhein et al., 2013).

Within the neuronal circuitry that regulates PPI, the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAC) play key roles (Swerdlow et al., 2001; Pothuizen et al., 2005). Abnormal neurofunctional coupling of the mPFC and NAC has been found in different animal models for deficient sensorimotor gating (Miller et al., 2010; Arime et al., 2012; Li et al., 2013; Swerdlow et al., 2013). Lesions or deep

Abbreviations: ASR, acoustic startle response; AP, anterior-posterior; AU, arbitrary units; DA, dopamine; ECG, electrocardiographic; EEG, electroencephalogram; EPN, entopeduncular nucleus; LFPs, local field potentials; mPFC, medial prefrontal cortex; ML, mediolateral; NAC, nucleus accumbens; PPTg, pedunculopontine tegmental nucleus; PPI, prepulse inhibition; SU, single unit; SPL, sound pressure level; TS, Tourette's syndrome; V, ventral.

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http://dx.doi.org/10.1016/j.pnpbp.2014.08.017
0278-5846/

brain stimulation of the entopeduncular nucleus (EPN), *i.e.*, the equivalent to the human globus pallidus internus (GPi), alleviate breeding- or apomorphine-induced deficient PPI in rats, indicating that dysfunction of neuronal activity may be altered as well in this region (Schwabe et al., 2009; Lütjens et al., 2011; Posch et al., 2012). Notably, deep brain stimulation of the GPi is clinically used to improve tics in TS (Houeto et al., 2005; Shahed et al., 2007; Servello et al., 2008).

We hypothesize that specific neuronal firing and oscillatory activity in the mPFC, NAC and EPN is altered in sensory gating abnormalities. We therefore examined the spontaneous neuronal activity in these regions in PPI *high* and *low* rats.

2. Material and methods

2.1. Subjects

Rats with either breeding-induced reduced (PPI *low*) or increased PPI (PPI *high*) were housed in groups of four in standard Macrolon Type IVS cages (Tecniplast, Hohenpeissenberg, Germany) under a 14-h light/10-h dark cycle (on at 07:00 h) at a room temperature of 22 ± 2 °C, with food and water ad libitum. All experiments were carried out in accordance with the EU directive 2010/63/EU and were approved by the local animal ethic committee.

2.2. Breeding selection for PPI high or low

The parental generation for our PPI *high* and *low* lines consisted of 23 male and 27 female rats (outbred adult Hannover-strain Wistar rats from Harlan-Winkelmann, Borchen, Germany). The TSE Startle Response System™ (Bad Homburg, Germany) was used to test rats for PPI, *i.e.*, the percent decrease of the startle response in pulse-alone (20 ms white noise pulse at 105 dB sound pressure level (SPL)) compared to the startle response in prepulse-pulse trials (80 dB SPL, 10 kHz pure tone pulse, 20 ms duration followed by pulse 100 ms after prepulse onset). Two females and males with the highest and the lowest level of PPI, respectively, were chosen for selective breeding of two lines with either *high* or *low* level of PPI. After the 10th generation the startle response system of San Diego instruments was used for testing of PPI as described before, but with 68 dB white noise as prepulse. For this study we used PPI low and PPI high rats (n = 7 each) from the 11th and 12th generation, which differed in their PPI measures (normally distributed; 8.38 ± 3.9% vs. 66.7 ± 2.0%, Student's *t*-test, $P < 0.001$), but not in their ASR measures (not normally distributed; 1945 ± 304 AU vs. 1762 ± 344 AU, Mann Whitney *U* test, $P = 0.563$).

2.3. Single-unit and local field potential recording procedures

In vivo neuronal activity measurements were carried out on a total of 14 rats, i.e., high PPI (N = 7) and low PPI (N = 7), one to two weeks after PPI assessment.

Rats were anaesthetized with urethane (1.4 g/kg, i.p. ethyl carbamate, Sigma; with additional doses as needed, depth of anaesthesia was checked by the foot pinch), which has been widely used as an anesthetic in animal experiments because it can be administrated readily, produces a long-lasting steady level of anesthesia with minimal effects on autonomic and cardiovascular systems (Hara and Harris, 2002; Li et al., 2012). Body temperature was kept at 37.5 ± 0.5 °C with a heating pad. Electrocardiographic (ECG) activity was monitored constantly to ensure the animals' wellbeing. Rats were placed in a stereotaxic frame and craniotomies were made over the target coordinates, relative to bregma (flat skull position). For all regions we used two trajectories within the following coordinates in millimeter scale; for the mPFC: anterior-posterior (AP), +3.2 and +2.2; mediolateral (ML), ±0.5 and ±0.8; ventral (V), −3.2 and 4.5; for the NAC core: AP +1.7 and +1.2, ML ±1.5 and ±1.7, V 6.5 and 7.8, and for the EPN: AP −2.3 and −2.8, ML ±2.4 and ±2.6, V 7.4 and 8.0. At the end of all recordings the electrode tip was used to coagulate the tissue along each of the trajectories in 200 μm steps with bipolar current of 10 μA for 10 s to verify recordings in the targets after sacrifice of the animals (Fig. 1).

A single microelectrode for extracellular recordings (quartz coated electrode with a platinum-tungsten alloy core (95%–5%), diameter 80 μm, impedance 1–2 MΩ) was connected to the *Mini Matrix 2 channel version drives headstage* (Thomas Recording, Germany). The electrode was guided stereotaxically through the guide cannula towards the target coordinates in the mPFC, NAC or EPN, respectively. The microelectrode signal was passed through a headstage with unit gain and then split to separately extract the single unit (SU) and the local field potentials (LFPs) components. For SU recording signals were bandpass-filtered between 500 and 5000 Hz and amplified from × 9,500 to 19,000. The LFP signals were filtered to pass frequencies between 0.5 and 140 Hz, before being amplified and digitized at 1 kHz. Data were acquired using the CED 1401 A/D interface (Cambridge Electronic Design, Cambridge, UK).

2.4. Data analysis

Action potentials arising from a single neuron were discriminated by the template-matching function of the spike-sorting software (Spike2; Cambridge Electronic Design, Cambridge, UK). For analyses of spontaneous activity, one epoch of 300 s with simultaneously recorded spiking and LFP activities that was free of artefacts was used from every recorded neuron.

2.4.1. Firing rates and burst detection

The firing rate was calculated with the firing rate histograms produced in NeuroExplorer version 4 (NEX Technologies, NC). The term 'burst' is usually defined as a cluster of spikes from a single neuron that differs from other spikes in a particular way, usually being more closely spaced in time than neighboring spikes thus having a higher discharge rate than the surrounding spike trains (Lobb, 2014). One problem with any definition of a burst, however, is the comparison of two groups of spike trains in which there is a difference in the reference activity. Additionally, non-stationarities in spike trains may complicate burst analysis.

Basically, there are two different approaches to define and describe bursts, which both have certain advantages and disadvantages: a method based on specification of interspike interval (ISI) parameters, which depends on user-defined parameters (e.g., Harris et al., 2001 and Chiappalone et al., 2005). This method, however, does not allow identification of individual burst events and is strongly influenced by the background activity of the neuron. The second method, which is based on Poisson distribution of ISI, is able to identify individual burst events on the base of poissonian surprise (e.g. shown in Alam et al., 2014). Here, the choice of the threshold does not affect burst detection (Grace and Bunney, 1984; Legéndy and Salcman, 1985) and it also leads to less detection of artificial 'burst' as discussed by Chen et al. (2009). In the present study, the Poissonian surprise method of Legéndy and Salcman (1985) is used for bursts and burst related statistics. This method is implemented in NeuroExplorer and has been frequently used for analysis of spontaneous discharge of neurons by us and other groups (e.g., Jackson et al., 2004; Homayoun and Moghaddam, 2006; Rumpel et al., 2013).

2.4.2. Asymmetry index

Variations to the Gaussian distribution were evaluated by determining the asymmetry index, which is the ratio of the mode to the mean ISI. An asymmetry index close to 1 reveals a relatively regular firing pattern, whereas the more the index differs from unity, the more irregular the spike trains. A ratio of less than 1 reflects an asymmetrical shape, indicating a larger fraction of short interspike intervals (positively skewed), as is expected when there is bursting activity.