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Research report

Assessment of auditory sensory processing in a neurodevelopmental animal model of schizophrenia—Gating of auditory-evoked potentials and prepulse inhibition

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ABSTRACT

The use of translational approaches to validate animal models is needed for the development of treatments that can effectively alleviate cognitive impairments associated with schizophrenia, which are unsuccessfully treated by the current available therapies. Deficits in pre-attentive stages of sensory information processing seen in schizophrenia patients, can be assessed by highly homologues methods in both humans and rodents, evident by the prepulse inhibition (PPI) of the auditory startle response and the P50 (termed P1 here) suppression paradigms. Treatment with the NMDA receptor antagonist PCP on postnatal days 7, 9, and 11 reliably induce cognitive impairments resembling those presented by schizophrenia patients. Here we evaluate the potential of early postnatal PCP (20 mg/kg) treatment in Lister Hooded rats to induce post-pubertal deficits in PPI and changes, such as reduced gating, in the P1 suppression paradigm in the EEG. The results indicate that early postnatal PCP treatment to rats leads to a reduction in PPI of the acoustic startle response. Furthermore, treated animals were assessed in the P1 suppression paradigm and produced significant changes in auditory-evoked potentials (AEP), specifically by an increased P1 amplitude and reduced P2 (P200 in humans) gating. However, the treatment neither disrupted normal P1 gating nor reduced N1 (N100 in humans) amplitude, representing two phenomena that are usually found to be disturbed in schizophrenia. In conclusion, the current findings confirm measures of early information processing to show high resemblance between rodents and humans, and indicate that early postnatal PCP-treated rats show deficits in pre-attentional processing, which are distinct from those observed in schizophrenia patients.

1. Introduction

Early clinical observations suggested that schizophrenia patients are unable to correctly filter irrelevant sensory stimuli. These observations led to the formulation of the "filter hypothesis" for schizophrenia [1,2], which states that brain pathological changes cause the decrease in selective and inhibitory functions of attention seen in schizophrenia. There are several available relevant paradigms to assess deficits in information processing, including prepulse inhibition (PPI) of the startle response, or EEG paradigms such as mismatch negativity, P300 or P50 (termed P1 here) gating. To better understand the genetic contribution in schizophrenia recent translational research has focused on using these measurements to characterize so-called endophenotypes of schizophrenia. Endophenotypes are quantifiable markers of pathophysiological processes that reflect the primary effects of susceptibility genes more closely than the clinical symptoms [3]. Among the suggested candidate endophenotypes are PPI and auditory-evoked potential suppression (specifically P50 suppression). The observation that abnormalities in these paradigms are present in both schizophrenia patients and their unaffected family members support this candidature [4–7]. PPI reflects sensorimotor gating and refers to the normal reduction of an individual's startle response that occurs when a startling stimulus is preceded 30–300 ms by a weak prestimulus [8,9]. P1 suppression is thought to reflect the brain's inhibitory mechanisms of its response to repeated sensory input [2] and is usually assessed by an auditory paired-click paradigm. In humans, the P50 wave (positive amplitude occurring after approximately 50 ms in the EEG following stimulus presentation) exhibits reduced amplitude, or suppression, to the second click when presented 500 ms after the initial click [10]. The rodent correlate of the human P50 wave is still debated. While some groups consider the N40 wave

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(negative amplitude occurring approximately 40 ms after stimulus presentation) as the rodent equivalent to the human P50 [e.g. 9] others claim the rodent P20 to represent the human P50 waveform [e.g. 11]. For the purpose of the present study the term P1 will be used to indicate both the human P50 as well as the rodent P20.

Valid animal models are important to optimize the search for preventive or curative treatments for schizophrenia. Both PPI and auditory P1 gating have been studied widely in humans (especially in healthy controls and schizophrenia patients) and rodents [12–14] and thus allow for a translational approach on the neurophysiological level. Some studies report a correlation between these neurophysiological measures and deficits in selective cognitive domains seen in schizophrenia, e.g. working memory [15,16].

We have previously demonstrated that early postnatal administration of the psychotomimetic non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine (PCP) to rats induces deficits in executive functioning [17] that phenotypically resemble observations in first-episode schizophrenia patients [18,19].

Consequently, the goal of our study was to further characterize the value of this disease model and to test if deficits also include the pre-attentional domain measures by assessment of gating of auditory-evoked potentials (AEP) and PPI. Specifically, we hypothesize that early postnatal treatment with PCP would cause both a lowering of PPI as well as gating of AEPs when tested at a post-pubertal stage.

2. Methods and materials

All experiments were performed in accordance with Danish legislation, and animals were treated in adherence to guidelines for the care of experimental animals.

2.1. Animals

All animals were housed under controlled conditions (12 h of light starting at 06:00; 20 ± 2 °C; 30–70% humidity) in Macrolon (type III) cages with standard sawdust bedding and environmental enrichment (plastic house and wooden chew blocks). Food (Altromin 1324) and water was available ad libitum.

The early postnatal PCP paradigm as applied in the current study has been previously described in [17]. Briefly, timed pregnant Lister Hooded (LH) rats were obtained at gestational day 15 from Charles River (Germany), and were housed individually until delivery. The day of parturition was counted as postnatal day (PND) 0, on PND 7 pups were cross-fostered and randomly assigned to a lactating dam. Furthermore, on PND 7, 9 and 11 pups were treated subcutaneously (s.c.) with either vehicle (0.9% isotonic saline) or PCP (MW: 243.4 g/mol, H. Lundbeck A/S) 20 mg/kg in a 10 ml/kg dose volume, groups called VEH and PCP henceforth. Treatment with PCP within the first two weeks of a rat's postnatal life corresponds to the second late trimester in human pregnancy in terms of neurodevelopmental changes [20,21], and fits with the hypothesis that exposure to PCP during this trimester increases the probability for the progeny to develop schizophrenia [22,23]. Pups were weaned on PND 25 and housed two rats per cage until surgery or testing, which always occurred in the post-pubertal period [24].

Rats underwent surgery during week 10 after birth, and were acclimatized to auditory gating test environment during week 12, before testing in week 13 after birth. Acclimatization to PPI test environment and testing was carried out in week 14 after birth.

2.2. Surgery

Animals were anesthetized with a mixture of, on part Hypnorm® (0.315 mg/ml fentanyl and 10 mg/ml fluanisone, Janssen-Cilag Inc., USA) and one part Dormicum® (5 mg/ml midazolam, F. Hoffman-La Roche Ltd., Switzerland) in two parts of isotonic water. After ensuring deep animal anaesthesia level the hair was clipped from neck to nose and the animal was immobilised in a stereotaxic frame (Kopf, Germany). Before opening the skin over the skull a 1:1 mixture of Marcain® (5 mg/ml bupivacain, AstraZeneca, UK) and xylocain® (10 mg/ml lidocain, AstraZeneca, UK) was injected. Next small burr holes were drilled in the skull for placement of a hippocampal deep electrode (Plastic One, Virginia, USA), reference surface screw electrode (Plastic One, Virginia, USA), and two for anchor screws (Plastic One, Virginia, USA). Target area for the electrode and coordinates relative to bregma were the following: ventral hippocampus CA3 (5.2 mm posterior to bregma, 5.0 mm lateral, 4.8 mm ventral to dura), reference (8.0 mm anterior to bregma, 1.0 mm lateral). Additionally, a ground electrode was inserted in the subcutaneous (s.c.) area not covered by dental cement. Finally, electrodes were placed in a miniature 6-channel pedestal (Plastic One, Virginia, USA), which was fixated with dental cement and skin was sutured. For immediate pain relief and to prevent dehydration animals were given 0.05 mg/kg s.c. Temgesic® (buprenorphin, Schering-Plough, USA) and 3 ml of saline s.c., respectively. Animals were treated once daily for five days post surgery with 5 mg/kg s.c. Baytril vet® (50 mg/ml enrofloxacin, Bayer, Germany) and 1.5 mg/kg s.c. Rimadyl® (50 mg/ml carprofen, Pfizer, USA), and allowed to recover for two weeks.

2.3. Paradigms

2.3.1. Auditory-evoked potentials

EEG was recorded in a plastic chamber (*L*: 40 cm, *W*: 40 cm, *H*: 50 cm) shielded with a copper net, to avoid interference of external electrical installations. Auditory stimuli were generated with a programmable tone generator (Med Associates, Vermount, USA) connected via a custom made amplifier to four loudspeakers (placed two in box ceiling and two in box rear wall) in the test boxes (Ellegaard Systems, Denmark). STAR software (Ellegaard Systems, Denmark) was used to control the auditory stimuli. For generation of AEPs, two consecutive clicks of 85 db white noise (5 ms duration and a 1 ms rise/fall-time) were presented with inter-stimulus interval (ISI) of 500 ms and an inter-pair interval of 10 s. No background noise was used. For EEG recording the pedestal mounted on the animal's head was connected to a 6-channel cable and commutator (Plastic One, Virginia, USA) allowing the animal free range of movement. On the day of testing animals were placed in the recording box and allowed to habituate for one hour. Following the habituation period, a test session was started where EEG was recorded during the presentation of one hundred click pairs.

EEG recordings were amplified (gain setting 5000) with a Brownlee Precision Model 440 amplifier (filter settings: High pass = 1 Hz; Low pass = 100 Hz; 50 Hz Notch) and digitized with a Micro1401 using Spike2 version 5.16 (Cambridge Electronic Design, Cambridge, UK) system at a rate of 1000 Hz. The raw EEG was recorded continuously, and stored on a computer hard disk along with time-locked digital stimulus tags.

Data processing was performed off-line in Spike2 software (version 5.16, Cambridge Electronic Design, Cambridge, UK). Following manual and visual artefact rejection of large amplitude artefacts (>3 μV), e.g. due to movement, epochs were averaged off-line for each animal separately. For the analyses of AEPs, epochs were constructed consisting of a 50 ms pre-stimulus baseline and a 900 ms post-stimulus interval. AEPs in rodents typically show a positive deflection between 10 and 30 ms, a negative deflection between 30 and 50 ms and a positive deflection between 50 and 100 ms (see Fig. 1A). This sequence of waveforms is near identical to humans, except for the fact that in humans they are spread out a little more in time, probably caused by a combination the size of the human skull compared to the rat, and the usage of in depth electrodes for most rat EEG recording. Therefore, the first positive amplitude has been considered by some authors as equivalent to the human P50 (called P1 here), the first negative deflection as equivalent of the human N100 (called N1 here) and the second positive deflection as equivalent of the human P200 (called P2 here) [25,26].

Based on this and the inspection of the grand average AEPs the individual waveforms were identified as the maximum amplitudes in the following time windows: P1: between 10 and 30 ms; N1: between 30 and 50 ms; P2: between 50 and 100 ms (see Fig. 1). Amplitudes were assessed according to Oranje et al. [27] by measuring P1: preceding trough to peak; N1: baseline to peak; P2: baseline to peak. The baseline was calculated as the average baseline 50 ms prior to stimulus presentation. For each animal two sets of amplitudes were assessed: one from conditioning (C) stimuli and one from test (T) stimuli. For the C-stimuli the amplitude with the largest amplitude within the above specified time windows were scored. For the T-stimuli, amplitude scoring was limited to those peaks having their maximum within 4 ms (±) of C-stimulus latency. T/C ratios were calculated from the amplitude data, as a measure of gating. In one case, no AEP was observed following the C-stimulus and this rat was excluded from subsequent analysis.

2.3.2. PPI

All PPI testing was performed with the Startle Monitor System (Kinder Scientific), which consisted of 8 sound-attenuated startle chambers and custom designed computer software. During testing, animals were placed in an adjustable holder positioned directly above the sensing platform, providing limited restraint but still allowing for individual adjustments. The PPI regime consisted of a 5-min acclimation period with only background white noise (62 dB), followed by 4 types of trials presented in a randomized manner: no pulse, pulse alone, prepulse + pulse, or finally highest prepulse intensity (77 dB) alone. Prepulse intensities varied between 5, 10 and 15 db above background noise. Inter-trial interval (ITI) varied between 9 and 21 s (average ITI 15 s) and inter-stimulus interval (ISI) was set to 100 ms with prepulse length of 20 ms. Although trials were presented in a randomized manner, each trial session started and ended with 8 startle pulses of 105 db to estimate habituation within trials. PPI was calculated as % PPI for each prepulse intensity as: $100 - ((\text{prepulse} + \text{pulse}/\text{pulse alone}) \times 100)$, i.e. a lower percentage score indicates deficient PPI (decrease in gating capacity). In order to ensure that the prepulses did not induce a startle reaction by themselves, 11 prepulse alone trials were included in the trial protocol, but were not used for calculating PPI. Startle magnitude was calculated as an average of pulse alone trials and habituation as percent change from first 8 pulse alone and last 8 pulse alone trials. Startle amplitude was recorded in a window of 100 ms starting from the onset of the stimulus.