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Brown Norway rats, a putative schizophrenia model, show increased electroencephalographic activity at rest and decreased event-related potential amplitude, power, and coherence in the auditory sensory gating paradigm

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ABSTRACT

In recent schizophrenia clinical research, electroencephalographic (EEG) oscillatory activities induced by a sensory stimulus or behavioral tasks have gained considerable interest as functional and pathophysiological biomarkers. The Brown Norway (BN) rat is a putative schizophrenia model that shows naturally low sensorimotor gating and deficits in cognitive performance, although other phenotypes have not been studied. The present study aimed to investigate the neurophysiological features of BN rats, particularly EEG/event-related potential (ERP). EEG activity was recorded at rest and during the auditory sensory gating paradigm under an awake, freely moving condition. Frequency and ERP analysis were performed along with time-frequency analysis of evoked power and intertrial coherence. Compared with Wistar–Kyoto rats, a well-documented control line, BN rats showed increased EEG power at rest, particularly in the theta and gamma ranges. In ERP analysis, BN rats showed reduced N40-P20 amplitude but normal sensory gating. The rats also showed reduced evoked power and intertrial coherence against auditory stimuli. These results suggest that BN rats show features of EEG/ERP measures clinically relevant to schizophrenia and may provide additional opportunities for translational research.

1. Introduction

Schizophrenia is a chronic disabling mental disorder characterized by positive symptoms, negative symptoms, and cognitive deficits (Freedman, 2003). To date, a number of large-scale genome-wide investigations of schizophrenia have indicated the involvement of multiple and heterogeneous genetic factors (International Schizophrenia Consortium, 2008; International Schizophrenia Consortium et al., 2009; Ripke et al., 2013). Apart from the influence of risk genes, environmental factors may also affect the disease course of schizophrenia. These facts indicate that in addition to the preclinical models of schizophrenia such as single gene modification and psychotomimetic drug injection, models that show penetrant phenotype will also be useful. The Brown Norway (BN) rat is known to show naturally low sensorimotor gating and deficits in cognitive performance that are well-documented in patients with schizophrenia (Palmer et al., 2000; Conti et al., 2001; Swerdlow et al., 2006a; Feifel et al., 2011). The risk loci of BN rats for behavioral deficits have been identified in the homologous region of the human risk loci for schizophrenia (Palmer et al., 2003). From these observations, we speculated that BN rats may show a broader range of clinically relevant deficits than observed to date and serve as a better model of schizophrenia.

Electroencephalographic (EEG) activities provide objective, quantitative, and real-time data that are useful for studying brain functions in healthy and disease conditions (Uhlhaas and Singer, 2006). In the schizophrenia research field, EEG activities at rest or event-related EEG changes have been investigated for searching biological processes related to the symptoms (Light et al., 2006; van der Stelt and Belger, 2007; Korostenskaja and Kahkonen, 2009; Hasey and Kiang, 2013). For example, increased EEG theta power at rest has been reported as one of the key marker for the deficits in processing information (Hanslmayr et al., 2013; Lakatos et al., 2013). Also it has been reported that increased EEG gamma power at rest is associated with positive symptoms (Baldeweg et al., 1998; Lee et al., 2008), and reduced oscillatory activity during cognitive tasks is associated with cognitive decline (Schmiedt et al., 2005; Cho et al., 2006; Haenschel et al., 2009). Schizophrenia-related deficits in EEG are also observed even during simple tasks, such as the sensory stimulation paradigm. In the auditory stimulation paradigm, it has been reported that event-related potential

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(ERP) amplitude and its gating in patients with schizophrenia are smaller than those in healthy controls (Adler et al., 1982; Hu et al., 2012; Smith et al., 2013). Reduced response amplitude and intertrial coherence (ITC) in patients have been demonstrated using time-frequency analysis (Shin et al., 2010). Furthermore, a smaller difference in brain oscillatory activities between resting and performing tasks (reduced signal-to-noise ratio) has been argued to be a fundamental biomarker of schizophrenia (Winterer et al., 2000; Winterer and Weinberger, 2004). These findings suggest that electrophysiology-based phenotype analysis of schizophrenia models will provide more information than other behavioral analyses.

In this study, the electrophysiological phenotypes of BN rats were characterized to test the potential usefulness of BN rats in studying the neurophysiological deficits related to schizophrenia. In the auditory sensory gating paradigm, 4 EEG analytical measures were chosen: (i) baseline power; (ii) ERP P20/N40 amplitude and its gating; (iii) event-related spectral perturbation (ERSP), a baseline-corrected event-related change in the power spectrum; and (iv) ITC, a measure of event-related phase synchronization across multiple trials. Wistar–Kyoto (WKY) rats were selected as a control line, similar to previous studies (Palmer et al., 2000; Conti et al., 2001).

2. Methods

All animal protocols were approved by the Institutional Animal Care and Use Committee of the Pharmaceutical Research Division, Takeda Pharmaceutical Company Ltd. (Permit Number: 2318). Animal care was in compliance with the Guide for Care and Use of Laboratory Animals. All efforts were made to minimize suffering and reduce the number of animals to be used.

2.1. Subjects

Nine male BN rats and 7 WKY rats (BN/CrlCrlj and WKY/NCrlCrlj; Charles River Laboratories Japan, Inc., Kanagawa, Japan) were used for the experiments. The rats were kept under standard laboratory conditions (12:12 h light/dark cycle, illuminated from 07:00 to 19:00, with food and water available ad libitum). Surgery was performed 7–10 days after arrival.

2.2. EEG recording and paired auditory stimulation

2.2.1. Surgical preparations

The rats were anesthetized with pentobarbital (40 mg/kg, i.p. Somnopentyl ; Kyoritsu Seiyaku Corporation, Tokyo, Japan) and positioned in a David Kopf stereotaxic instrument (Tujunga, CA). A single midline incision was made over the scalp, and 8 holes were drilled through the skull for stereotaxic implantation of the recording screw electrodes. The electrode was positioned as follows: −4.5 mm anterior and 3.5 mm lateral to the bregma (active electrode); 10 mm anterior and 1 mm lateral to the bregma (ground electrode); 2 mm posterior and 2 mm lateral to the lambda (reference electrode); and 5 other screws (anchors) were used. The electrodes were screwed into the skull without breaching the dura, and dental cement (Repairsin ; GC Corporation, Tokyo, Japan) was applied to fix the electrodes, anchors, and socket in place. The rats were kept in separate cages for more than 1 week for recovery before the experiments.

2.2.2. Recording procedure

In the light phase of the testing day, the rats were individually placed into an electrically shielded chamber (48 cm × 45 cm × 70 cm), and the recording electrodes were attached to a cable suspended from the ceiling. EEG signals were filtered at 0.1–1 kHz, amplified using an AC amplifier (BBA-2208; Biotex Ltd., Kyoto, Japan), and digitized at 500 Hz using a MICRO3 interface system (Cambridge Electronic Design Ltd., Cambridge, UK). Auditory stimuli were generated using a custom-made stimulator (O'Hara & Co., Ltd, Tokyo, Japan). Each auditory stimulus session consisted of a total of 200 click pairs (pure tone, 10 kHz, 10-ms duration, 85 dB). In each click pair, the first stimulus (conditioning stimulus) was presented at 500 ms, and the second stimulus (test stimulus) was presented at 1000 ms after the TTL trigger pulse (0 ms) to MICRO3. Intervals between pairs were set at 5 s.

2.3. EEG analysis

The datasets obtained in the auditory sensory gating paradigm were analyzed 4 times by different analytical methods.

2.3.1. EEG power at rest

EEG power at rest was assessed during a 5-min period before the auditory sensory gating paradigm. Obtained EEG data were analyzed using a custom-made script in Spike2 software (Cambridge Electronic Design Ltd., Cambridge, UK). Fast Fourier transformation (FFT) under conditions of 0.98-Hz resolution with Hanning window was used to calculate the sum of power in the theta (5–8 Hz), alpha (9–12 Hz), beta (13–29 Hz), and gamma (30–80 Hz) frequencies.

2.3.2. ERPs

Spike2 software (CED, UK) was used to analyze and quantify grand average ERPs. P20/N40 was analyzed as an analog of human P50/N100 ERP (Adler et al., 1998; Maxwell et al., 2004a, 2004b; Phillips et al., 2007; Siegel et al., 2003). The N40 component was defined as the maximum negative waveform in the range of 20–60 ms, and the P20 component was defined as the maximum positive voltage in the range of 0–30 ms. To avoid the effect of different baseline EEG values, the N40-P20 amplitude was calculated, and then the test/conditioning (T/C) ratio was calculated by dividing the N40-P20 amplitude against the test stimulus by the N40-P20 amplitude against the conditioning stimulus.

2.3.3. ERSP and ITC

To analyze ERSP values and ITC, EEG data were exported from Spike2 into MATLAB (Version R2013a; Mathworks, Natick, Massachusetts). EEGLAB toolbox (Schwartz Center for Computational Neuroscience) was used to process the data and create time-frequency measures. Single-trial epochs of −500 ms and 1500 ms relative to the TTL trigger were extracted from the continuous EEG data. ERSP and ITC were calculated within each epoch using Morlet wavelets in 120 logarithmically spaced frequency bins between 4 and 100 Hz, with wavelet cycle numbers ranging from 2 (at low frequencies) to 10 (at high frequencies) (Delorme and Makeig, 2004). ERSPs were expressed in dB, and ITCs were expressed as a unitless ratio between 0 and 1, where 1 represents complete phase synchrony at a given frequency and time across trials. To create a single value for each measure, values in the theta range were averaged between 2 and 400 ms after the conditioning stimulus, and values in the alpha, beta, and gamma ranges were averaged between 2 and 154 ms after the conditioning stimulus. Because the T/C ratio of the ERSP and ITC was not the focus of this experiment, ERSP and ITC values were calculated only for the conditioning stimulus.

2.4. Statistical analysis

Data are presented as plot of individual data points with means ± S.E.Ms. To analyze the differences in the calculated values between BN rats and WKY rats, Bartlett's test followed by Student's t-test or Welch's test was used. Data were analyzed using the SAS preclinical package (Ver.5.0; SAS Institute Inc., Cary, NC, USA), and statistical significance was set at $p \leq 0.05$ in all cases.

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