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Research Paper

# Cortical electroconvulsive stimulation alleviates breeding-induced prepulse inhibition deficit in rats

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ABSTRACT

In patients with medical-refractory schizophrenia electroconvulsive therapy (ECT), i.e., the induction of therapeutic seizures via cortical surface electrodes, is effectively used. Electroconvulsive stimulation (ECS) in rodents simulates ECT in humans and is applied to investigate the mechanisms underlying this treatment. Experimentally-induced reduced prepulse inhibition (PPI) of the acoustic startle response (ASR), i.e., the reduction of the startle response to an intense acoustic stimulus when this stimulus is shortly preceded by a weaker not-startling stimulus, serves as an endophenotype for neuropsychiatric disorders that are accompanied by disturbed sensorimotor gating, such as schizophrenia. Here we used rats selectively bred for *high* and *low* PPI to evaluate whether bifrontal cortical ECS would affect PPI.

For this purpose, cortical screw electrodes were stereotactically implanted above the frontal cortex. After recovery ECS was applied for five consecutive days with stimuli of 1 ms pulse-width, 100 pulses/s, 1 s duration, ranging from 5.5 mA to 10 mA. PPI of ASR was measured one day before ECS, and on days 1, 7, and 14 after the last ECS. In rats with breeding-induced *low* PPI ECS increased PPI one week after stimulation. In contrast, ECS decreased PPI in rats with *high* PPI on the first day after stimulation. The reaction to the startle impulse was reduced by ECS without difference between groups. This work provides evidence that rats with breeding-induced *high* or *low* PPI could be used to further investigate the underlying mechanisms of ECT in neuropsychiatric disorders with disturbed sensorimotor gating like schizophrenia.



## 1. Introduction

Electroconvulsive therapy (ECT) is an established treatment for mood disorders such as bipolar disorder and major depression. In schizophrenia ECT is effectively used in cases accompanied by catatonia, severe depression, mania or other affective components (Pompili et al., 2013), as well as in patients who are refractory to medical treatment (Lehnhardt et al., 2012; Petrides et al., 2015). Although ECT is applied since almost 80 years, its therapeutic relevant effects on molecular level or on neuronal network activity are not yet understood.

Electroconvulsive stimulation (ECS) in rodents has been used to elucidate the therapeutic mechanisms of ECT (Ferraro et al., 1990; Fochtmann, 1994). While ECS mostly uses electrical stimulation via auricular or, less often, corneal electrodes, ECT in the clinical setting is performed via extracranial electrodes above the frontal cortex or temporal lobe. Recently it was reported that the clinical and cognitive effects of bifrontal ECT in schizophrenia would be superior to bitemporal ECT both with regard to efficacy and cognitive adverse effects (Phutane et al., 2013). One recent experimental study (Theilmann et al., 2014) used cortical screw electrodes placed above the rat frontal cortex thus simulating the bifrontal ECT in humans more closely than the traditional auricular ECS. In this study, cortical ECS reduced immobility in the forced swim test more effectively than auricular ECS. Importantly, in contrast to auricular stimulation cortical ECS has no negative influence on general health condition of the rats, and the animals do not show any signs of fear during the stimulation sessions (Theilmann et al., 2014).

In healthy individuals sensorimotor gating processes allow to act in a context-dependent and goal-oriented manner. Certain neuropsychiatric disorders, such as schizophrenia, Tourette's syndrome, and obsessive compulsive disorder are accompanied by disturbed sensorimotor gating, which is linked to the inability of these patients to filter or "gate" irrelevant or inferring information of motor, cognitive and emotional domains with consecutive sensory overload and respective clinical symptoms (Kohl et al., 2013). A paradigm often used to investigate sensorimotor gating across species is the prepulse inhibition (PPI) of the

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acoustic startle response (ASR), i.e. the reduction of the startle response to an intense acoustic stimulus, when this stimulus is preceded by a weaker stimulus. Experimentally-induced deficient PPI in rodents is used as an endophenotype to study the pathophysiology and therapeutic strategies of these disorders (Braff et al., 2001).

In previous studies we have shown that selective breeding in Wistar rats for high and low PPI leads to a segregation of two rat lines with either *high* or *low* PPI (Schwabe et al., 2007). Behavioral deficits, neuronal activity and epigenetic findings in PPI *low* rats corroborate a number of clinical findings in neuropsychiatric disorders with reduced sensorimotor gating (Alam et al., 2014; Dieckmann et al., 2007; Freudenberg et al., 2007; Rhein et al., 2013). Further, the dopamine receptor agonist haloperidol enhanced PPI in PPI *low* rats (Hadamitzky et al., 2007), thus indicating a predictive value of this model for the treatment used in schizophrenia. To examine whether these rats would be suitable for investigating the underlying mechanisms of ECS, we here tested whether repeated ECS applied via cortical frontal electrodes would affect PPI in rats with breeding-induced *high* or *low* PPI. The postictal suppression index (PSI) is clinically used as a predictor for therapeutic outcome (Azuma et al., 2007a,b; Krystal et al., 1993, 1995; Nobler et al., 1993; Suppes et al., 1996), but seldom used in experimental models to evaluate the outcome of ECS. We calculated the PSI in order to determine whether the PSI would predict the effect of ECS on PPI in our experimental setting. According to the clinical efficacy of ECS in patients, we hypothesize that ECS positively affect the reduced PPI in PPI *low* rats and that the therapeutic effect correlates with the PSI.

## 2. Methods

### 2.1. Animals

Rats were kept in groups of four in Macrolon Type IVS cages under controlled ambient conditions (22 °C, 14 h light/10 h dark cycle) with water and rat chow ad libitum. The experiments were carried out in accordance with the EU directive 2010/63/EU and were approved by the local animal ethic committee (LAVES). All efforts were made to minimize pain or discomfort of the animals used.

### 2.2. PPI measurement and selective breeding

Selective breeding for PPI *high* and *low* was initiated with 23 male and 27 female rats (outbred adult Hannover-strain Wistar rats from Harlan-Winkelmann, Germany). For testing of PPI rats were placed inside a cylinder connected to a motion sensitive platform. Testing started with an acclimation phase of 5 min (background noise of 60 dB white noise), followed by 5 pulse-alone trials (20 ms of 105 dB white noise sound pressure level (SPL)). Subsequently 4 types of trials were presented that all last 500 ms with a continuous background of 60 dB: (1) pulse-alone trials, (2) prepulse-pulse trials, i.e., 80 dB SPL, 10 kHz pure tone pulse of 20 ms duration followed by pulse 100 ms after prepulse onset, (3) prepulse-alone trials, and (4) control trials, in which only the background noise was presented. At the end of each test session 5 pulse-alone trials were presented. Each trial type was presented 10 times in a random order, separated by an intertrial interval ranging randomly between 20 s and 30 s. PPI was calculated as the percent decrease of the startle response in pulse-alone compared to the startle response in prepulse-pulse trials in a TSE Startle Response System™. 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 the present study adult males of the 19th generation (230–430 g) were used, i.e. 13 PPI *low* and 15 PPI *high* rats with a mean PPI of 19.47% ± 7.2 SEM and 60.36% ± 3.5 SEM, respectively ($P < 0.05$), and an ASR of 5694 arbitrary units (AU) ± 490 SEM and 1619 AU ± 299.6 SEM ($P < 0.05$).

### 2.3. Implantation of screw electrodes

Rats were anesthetized with chloral hydrate (360 mg/kg i.p.), fixated in a stereotaxic frame, and the area of surgery was anesthetized with Xylocain 2%. Afterwards screw electrodes were implanted bilaterally above the frontal cortex with the following coordinates in mm relative to bregma (Paxinos and Watson, 1998): anterior–posterior: +2.7, mediolateral: ±4.0. For EEG recordings, a reference electrode was placed above the right parietal cortex (anterior–posterior: −2, lateral: −2). A head stage molded from dental acrylic cement (Paladur®, Heraus Kulzer GmBH, Germany) served for plug-in connection.

### 2.4. Electroconvulsive stimulation

ECS was applied once daily for five consecutive days in freely moving rats with the following stimulation settings: alternating positive and negative square wave pulses 1 ms pulse-width, 100 pulses/s, 1 s duration (A310 Accupulser, WPI, USA). This setting is analog to the Thymatron®System (Somatics LLC), which is clinically used for ECT. The current was individually adapted ranging from 5.5 mA to 10 mA to induce a generalized seizure lasting ≥15 s in the electroencephalogram (EEG) recorded by a one-channel amplifier and an analog–digital converter (PowerLab/800 s; both ADInstruments Ltd., Australia). The sampling rate of the EEG recording was 200 Hz. A high-pass filter of 0.1 Hz and a low-pass filter of 60 Hz were used. If seizure duration was less than 15 s, the current was increased in 0.5 mA steps on the following days.

For calculation of PSI a method adapted to Nobler et al. (2000) was used. The processing of the EEG data was obtained using Spike2 (version 6, Cambridge Electronic Devices). Direct current (DC) shifts were removed and total EEG power spectra were calculated within the 0.78–50 Hz frequency range by fast Fourier transformation (FFT = 256, Hanning window, frequency resolution 0.78 Hz). The PSI was defined as the percentage reduction of the total spectral power from the whole ictal phase to the post ictal phase (first 15 s after seizure termination) (Fig. 1A). The neuronal activity recorded after stimulation on the first and last day of the ECS course was used to calculate the PSI.

### 2.5. Study design and statistical analyses

Two weeks after electrode implantation PPI was measured. Thereafter ECS was applied once daily for five days; PPI was retested on days 1, 7, and 14 after the ECS course. Calculation of PSI was done in a blinded fashion. Blinded testing of PPI, however, was not possible, since rats of the PPI *high* and *low* strains differ according to social behavior (Dieckmann et al., 2007) and body weight (Schwabe et al., 2007). Therefore, in most cases it is obvious for the experimenter whether a PPI *low* or *high* rat is tested. However, the procedure of ECS is standardized and does not depend on the personal assessment of the experimenter. Also, testing in the Startle-Response-System and analyzation of the data is an automated procedure.

PPI and ASR were analyzed by two-way analysis of variance for repeated measurements (ANOVA) with group (PPI *high* and *low*) and test day (pre; 1, 7, and 14 days after ECS) as factors, followed by Tukey's test for pairwise post-hoc comparison. The *t*-test was used to compare measures between groups during the ECS phase, i.e., current used to induce seizures, seizure duration in EEG as well as motoric seizure duration, and PSI on days 1 and 5 of ECS. When an effect of ECS on PPI was found, the correlation between PSI and PPI was analyzed by Pearson's correlation for that day. All tests were performed two-sided with $P < 0.05$ considered significant.