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Research report

Electrophysiological alterations in a complex rat model of schizophrenia

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HIGHLIGHTS

- EEG phenotype characterization in a rat substrain related to schizophrenia/autism.
- ERPs showed significant changes in P2 latency and N1 amplitude.
- Acute ketamine treatment did not cause alterations in ERPs.
- Altered power of oscillations in different frequency bands was observed.
- Ketamine caused strain-dependent changes in the power of oscillations.

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ABSTRACT

Background: Psychiatric disorders are frequently accompanied by changes in brain electrical oscillations and abnormal auditory event related potentials. The goal of this study was to characterize these parameters of a new rat substrain showing several alterations related to schizophrenia.
Methods: Male rats of the new substrain, developed by selective breeding after combined subchronic ketamine treatment and postweaning social isolation, and naive Wistar ones group-housed without any interventions were involved in the present study. At the age of 3 months, animals were implanted with cortical electroencephalography electrodes. Auditory evoked potentials during paired-click stimuli and power of oscillation in different frequency bands were determined with and without acute ketamine (20 mg/kg) treatment.
Results: Regarding the auditory evoked potentials, the latency of P2 was delayed and the amplitude of N1 peak was lower in the new substrain. The new substrain showed increased power of oscillations in the theta, alpha and beta bands, while decreased power was detected in delta and gamma2 bands (52–70 Hz) compared with control animals. Acute ketamine treatment increased the gamma1 band (30–48 Hz) power in both groups, while it elicited significant changes only in the new substrain in the total power and in alpha, beta and gamma2 bands.
Conclusions: The validation of the translational utility of this new rat substrain by electrophysiological investigations revealed that these rats show abnormalities that may model a part of the neurophysiological deficits observed in schizophrenia.

Abbreviations: EEG, electroencephalography; ERP, event related potential; NMDAR, *N*-methyl-D-aspartate receptor.

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1. Introduction

Schizophrenia is a common neurodevelopmental and highly heritable neuropsychiatric disorder [1,2]. Over the past few decades, researches using electroencephalography (EEG) have identified several neurophysiological alterations in this disease, indicating neural circuit disruptions. Unfortunately, the results are controversial, and they may depend on the subtype or phase of the disease; therefore, its modeling in preclinical research field is a big challenge [3–9]. It is argued that perfect simulation of inherently human diseases in animals might be impossible, but the recreation of endophenotypes related to the disorders is a possibility. Therefore, developing animal models with abnormal EEG activity may help in the clarification of the mechanisms in the background of this neuropsychiatric disease [10,11]. Previous studies using different rodent models of schizophrenia showed widely diversified alterations in the power of EEG oscillations and event related potentials (ERP) [12–23].

Preclinical and clinical studies focusing on pharmacological and genomical changes support the hypothesis that hypofunction of *N*-methyl-D-aspartate receptor (NMDAR) signaling contributes to the pathophysiology of schizophrenia; therefore, NMDAR antagonists, including ketamine, have been used extensively to probe questions related to its neurobiology [24–28]. Ketamine administered to healthy controls in subanesthetic dose mimics several symptoms of schizophrenia, and it worsens these signs in schizophrenia patients [24,29,30]. Furthermore, NMDAR antagonists or silencing of these receptors are used in animal models of neuropsychiatric disorders showing several alterations in EEG activity, too [13,18,25,31–34].

We developed a complex animal model by selective breeding based on behavioral alterations after combined subchronic ketamine treatment and postweaning social isolation [35,36]. It is thought that selective breeding for phenotypic extremes increases the homozygosity of genes that affect the selected trait, whereby the allelic frequency of trait-irrelevant genes remains unaffected [37]. Therefore, animals selectively bred for a behavioral given phenotype are increasingly used to study pathophysiological mechanisms underlying certain disorders. For example, rats have been successfully bred for anxiety [38], reduced sensorimotor gating [39] and for seizure susceptibility [40]. Several aspects of schizophrenia were found in the new substrain, i.e., disturbances in pain sensitivity, sensory gating, memory functions, motor activity and stereotypic behaviors [35,36]. Our recent data indicated that both heritable and environmental factors (i.e., juvenile social isolation and ketamine treatment) are important in the generation of the behavioral alterations, but the most significant changes were observed after the combination of treatments with selective breeding [35,36,41]. In order to keep the number of animals used in the experiments at minimal level, we decided to compare two groups of animals, i.e., naive rats without any intervention and the new substrain after juvenile isolation and subchronic ketamine treatment. In this report, the electrophysiological phenotype of this new rat substrain was characterized by the investigation of ERPs, their gating, and the basal frequency bands with and without acute ketamine treatment, to test the potential usefulness of the substrain in studying the neurophysiological deficits related to schizophrenia.

2. Methods

All experiments involving animal subjects were carried out with the approval of the Hungarian Ethics Committee for Animal Research (registration number: XIV/03285/2011). Animal suffering and the number of animals per group were kept to a minimum.

2.1. Selective breeding process

The paradigm for selective breeding was described previously [35,36]. Briefly, the parental generation consisted of male and female (10-10) outbred Wistar rats. Offsprings of the rats in the next generations were tested after weaning with the tail-flick test (48 °C hot water) to assess pain sensitivity, and then housed individually for 28 days. The animals were treated with ketamine (Calypsol, Richter Gedeon Plc., Budapest, Hungary; 30 mg/kg IP, 4 mL/kg, daily, 5 times/week, 15 injections in total) from 5 to 7 weeks of age. Then the animals were re-housed (4–5 rats/cage) and 1 week of recovery was provided to them with no treatment. Starting at the age of 9 weeks, the pain sensitivity, the sensory gating with prepulse inhibition, and the cognitive functions and stereotypic behavior on novel object cognition test were assessed (Table 1). Animals (5 male with 10 female) with the highest level of disturbances in these parameters were used for selective breeding throughout several generations.

2.2. Experimental paradigm for EEG experiments

Two experimental groups of 8–8 rats were compared: naive socialized male rats without any interventions; and the 17th generation of selectively bred male rats with social isolation and ketamine treatment as new substrain. After the above-mentioned behavioral tests, the animals were involved in the EEG experiments (Table 1).

Rats were anesthetized with a mixture of ketamine hydrochloride and xylazine (72 and 8 mg/kg intraperitoneally, respectively), and transferred into a stereotaxic frame. Afterwards, small burr holes were drilled in their skull for electrode placement according to coordinates found in the rat brain atlas [42]. The target area for the epidural stainless steel electrodes and coordinates relative to bregma were the following at both sides: recording electrodes: parietal cortex 6 mm posterior, 2 mm lateral to bregma; reference electrodes: 1.5 mm posterior to bregma, 2 mm lateral, and a ground electrode 2.5 mm posterior to bregma, 1 mm lateral. Finally, electrodes were placed in a miniature 6-pin connector, which was fixed with dental cement.

After the surgery, the animals were injected with gentamycin (10 mg/kg, subcutaneously) to prevent infection, and were housed individually. They were allowed to recover for one week with a 12:12-h light–dark cycle, an ambient temperature regulated at 23 °C, water and food with ad libitum access.

On the testing days (between 8:00 AM and 12:00 PM), animals were placed in the recording chamber (L: 34 cm, W: 14.5 cm, H: 33.5 cm), recording cables were attached to commutators allowing the free movement of the rats, and they were allowed to accommodate to the test environment for 10 min while auditory stimuli were not present.

Following the acclimatization, the 20 min test session was initiated. For generation of ERPs to the sensory gating paradigm, two consecutive clicks (70 dB clicks with broad spectrum for 5 ms: S1 and S2) were presented with interstimulus interval of 500 ms. The interval between the pairs of clicks was 5 s. Clicks were driven by a computer program and delivered via loudspeakers.

To habituate the animals to the task and to minimize the potential discomfort during the tests, three recording sessions were performed on three consecutive weeks without any intervention. Then EEG recordings were repeated after acute ketamine (20 mg/kg intraperitoneally) or vehicle (saline) injection on the subsequent two weeks. Each animal was given both injections with 7 days apart, and the order of vehicle and drug administration was counterbalanced. After the injections, the rats were placed in their cage for 20 min before putting them to the recording chamber for EEG registration.