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Social interaction of rats is related with baseline prepulse inhibition level

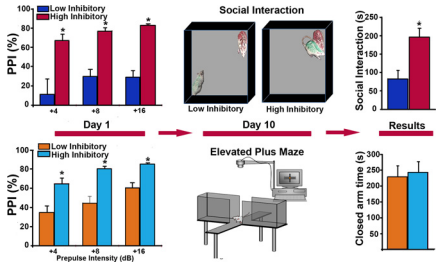
Gokhan Goktalay [a], Hakan Kayir [b,*], Gokhan K. Ulusoy [b], Tayfun Uzbay [c]

[a] Department of Medical Pharmacology, Faculty of Medicine, Uludag University, Bursa, Turkey
[b] Department of Medical Pharmacology, Psychopharmacology Research Unit, Gulhane Military Medical Academy, Ankara, Turkey
[c] Neuropsychopharmacology Application and Research Center (NPARC), Uskudar University, Istanbul, Turkey

HIGHLIGHTS

- Disrupted prepulse inhibition (PPI) is a model for positive and cognitive symptoms.
- Low PPI rats spent less time in social interaction (SI) than high PPI rats.
- Low and high PPI rats had similar anxiety levels in the elevated plus maze test.
- Sensorimotor gating (PPI) deficits may be responsible for the social withdrawal.

GRAPHICAL ABSTRACT



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ABSTRACT

The symptoms of schizophrenia are evaluated in three general categories: positive, negative and cognitive symptoms. Disruption of prepulse inhibition (PPI) of the acoustic startle reflex is commonly used to model positive and cognitive symptoms in experimental animals. On the other hand, deficient social interaction (SI) is a common model of negative symptoms. Here we tested whether PPI provides information about negative symptoms by using a SI test. Baseline PPI and its relation with anxiety-like behavior were also examined with elevated plus maze (EPM) test. In the first experiment, baseline PPI levels of 30 Wistar rats were measured and animals with the highest 1/3 and the lowest 1/3 of PPI scores were respectively assigned in high-inhibitory (HI) and low-inhibitory (LI) groups. Subsequently, rats in the HI and LI groups were paired with animals from the same group and tested for SI. In the second experiment, another batch of animals was assigned to HI and LI groups and they were investigated in the EPM test. The results demonstrate a significant difference between the PPI values of HI and LI groups. Both the SI time and the moving distance of LI rats were significantly lower, and the average distance between rat pairs was significantly longer than HI rats. In the EPM test LI and HI rats showed similar levels of anxiety-like behaviors, however our results imply that performance of the rats in the SI test is related to baseline PPI levels. Thus PPI test can provide predictive information about the outcome of animal models for negative symptoms in rats.

1. Introduction

Filtering external sensory stimuli, integration of sensory and cognitive information and the execution of appropriate motor responses are related to a healthy sensorimotor gating system [4]. Sensorimotor gating can be evaluated by prepulse inhibition (PPI) of the acoustic startle reflex. Prepulse inhibition refers to the attenuation of the startle response caused by a strong auditory stimulus

* Corresponding author at: Department of Medical Pharmacology, Psychopharmacology Research Unit, Gulhane Military Medical Academy, Etlik, 06018 Ankara, Turkey. Tel.: +90 312 304 4771; fax: +90 312 304 2010.
E-mail addresses: hakankayir@yahoo.com, hkayir@gata.edu.tr (H. Kayir).

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when it is abruptly preceded by a weaker, non-startling stimulus. Initially PPI was proposed as an automatic response related to pre-attentive processes [12]. However, today, a more expanded role has been attributed to PPI, especially for regulation of the input of sensory stimuli to the brain, which prevents sensory overload and cognitive fragmentation [4].

PPI test has a great importance in the schizophrenia research since deficient PPI has been extensively studied in patients with schizophrenia [6], and it is accepted as an endophenotype for schizophrenia spectrum disorders [11]. As an animal model, the disruption of PPI can be induced by several manipulations, such as drug treatments (i.e. dopaminergic agonists or NMDA receptor antagonists), brain lesions and genetic manipulations. Recently, baseline PPI was shown as an important variable and could influence the experimental results in rats [10,19,20]. Moreover, in humans, selecting low PPI subjects from a healthy population according to the baseline PPI levels and comparing them with high PPI subjects was shown to have a predictive value for evaluating the effects of drugs on PPI [16].

Social dysfunction/withdrawal is one of the core components of the negative symptoms of schizophrenia [1]. Social withdrawal in patients with schizophrenia may arise from general anhedonia, anxiety, and/or social cognitive deficits [13]. In rats, decrease in social interaction (SI) after several manipulations such as drug treatments (i.e. phencyclidine and MK801), neonatal brain lesions [29] and genetic manipulations [24,25] is a widely accepted model for the negative symptoms of schizophrenia [26,29]. SI measures in rodents are directly analogous to SI measures in humans. The SI test was initially developed to measure anxiety-like behavior [8], subsequently it was used to determine social behaviors in potential animal models of schizophrenia [30].

In rats, differences in baseline sensorimotor gating (i.e. PPI) levels may play a role in differential responses in SI test, a hypothesis that was explored in the present study. The possible relationship between two animal models of positive and negative symptoms (PPI and SI) was investigated by assigning the rats as high-inhibitory (HI) and low-inhibitory (LI) according to their baseline PPI levels; the SI test was then performed with pairs of LI or HI rats. Additionally, an elevated plus maze (EPM) test was performed with a separate batch of rats to determine whether observed differences between HI and LI groups in SI test were attributable to the differences in general anxiety levels.

2. Methods

2.1. Animals and laboratory

Adult male Wistar rats (3–4 months old) were used for this study. They were housed in groups of three or four in a quiet, temperature- and humidity-controlled room ($20 \pm 2\,^{\circ}C$ and $55 \pm 5\%$, respectively) in which a 12 h:12 h light–dark cycle was maintained (lights on between 0700 and 1900 h). Food and water were available ad libitum. All experiments were performed at the same time of the day during the light period (0900–1130 h). All procedures in this study were in accordance with the Guide for the Care and Use of Laboratory Animals as adapted by the National Institutes of Health (Washington, DC, USA, 1996) and the Declaration of Helsinki. Local ethical committee approval was also obtained. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Prepulse inhibition of the acoustic startle reflex test

Prepulse inhibition of the acoustic startle reflex test was performed using Animal Acoustic Startle Response System (AASR, Habitest Model E10-21, Coulbourn, PA, USA) as previously described [19,20]. The background noise level was 65 dB [A] sound pressure level (SPL) during the study. Prepulse stimulus levels were determined according to the background noise. The software recorded the startle response of rats for 200 ms with a 1 ms resolution after the administration of pulse stimuli with or without a prepulse stimulus, and it expressed the peak startle response as grams.

All the rats were handled daily for three days before starting the experiments. Twenty-four hours before the test sessions, rats were habituated to the animal holders of the startle test system for 15 min. On the test day, rats were placed in the cages and the test protocol was applied. The standard test sessions began with a 5-min acclimatization period in which only 65 dB background noise was present. Then, five consecutive pulse alone stimuli (110 dB) were administered, and 8 blocks of five trials were administered subsequently. One block was consisted of these trails in random order:

i. Pulse alone stimulus.
ii. Prepulse (4 dB [A] SPL above background noise) + pulse stimulus.
iii. Prepulse (8 dB [A] SPL above background noise) + pulse stimulus.
iv. Prepulse (16 dB [A] SPL above background noise) + pulse stimulus.
v. No stimulus (only background noise was applied).

The session was ended with five consecutive pulse alone stimuli. A pulse stimulus was a broadband noise stimulus at 110 dB [A] SPL for 40 ms, whereas all three prepulse stimuli were tone stimuli at 3 kHz frequency for 20 ms. Prepulse stimulus levels were selected at intensities which did not elicit significant startle reflex when applied alone. Prepulse stimulus was applied 120 ms prior to pulse stimulus (onset to onset). The inter-trial interval varied randomly in a range of 10–30 s. This protocol approximately lasted for 20 min. PPI was defined as a decrease in the amplitude of startle reflex in the presence of the prepulse stimulus and was calculated for each of the three different prepulse intensities by using the following formula:

$$\%\mathrm{PPI} = 100 - \left(\frac{\text{average startle reflex in the presence of prepulse stimuli} \times 100}{\text{average startle reflex without any prepulse}}\right)$$

2.3. Social interaction test

A batch of 30 rats was assigned into three groups according to their baseline PPI values at 81 dB (background + 16 dB) prepulse level. The rats with the highest 1/3 and the lowest 1/3 of PPI scores were assigned to high-inhibitory (HI) and low-inhibitory (LI) groups ($n = 10$), respectively.

For the following week, rats remained in their home cages (i.e., no testing was performed and no handling by the experimenter). Then, the rats were familiarized with the SI test arena for 15 min. The SI test arena was a black Plexiglas cage (80 cm × 80 cm × 40 cm) equipped with a CCD camera mounted 260 cm above from the floor of the arena. The behavior of the animals was analyzed by a video tracking software (Ethovision v3.1.16, Noldus, NL). During familiarization, the recording system was active and the distance moved (m) was calculated for each rat to show exploratory activity. The arena was cleaned thoroughly before each test. After two days, each rat was paired with a rat from the same baseline (HI or LI) group. Such pairings were used to detect differences in the mean distance apart. Also, proximity measures were calculated for the pair instead of the individual. The maximum difference between body weights of the rat pairs was ±15 g. The pairs were from different cages and were not housed together. Rats in each pair were dyed using non-toxic paint. The dorsal surface of one rat was dyed with red paint