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Original article

# Fear memory in a neurodevelopmental model of schizophrenia based on the postnatal blockade of NMDA receptors

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ABSTRACT

*Background:* Epidemiological data have indicated that memory impairment is observed during adolescence in groups at high risk for schizophrenia and might precede the appearance of schizophrenia symptoms in adulthood.
*Methods:* In the present study, we used a neurodevelopmental model of schizophrenia based on the postnatal blockade of N-methyl-D-aspartate (NMDA) receptors in rats to investigate fear memory in adolescence and adulthood. The rats were treated with increasing doses of CGP 37849 (CGP), a competitive antagonist of the NMDA receptor (1.25 mg/kg on days 1, 3, 6, 9; 2.5 mg/kg on days 12, 15, 18 and 5 mg/kg on day 21). Fear memory was analysed in delay and trace fear conditioning. Sensorimotor gating deficit, which is another cognitive symptom of schizophrenia, was also determined in adolescent and adult CGP-treated rats.
*Results:* Postnatal CGP administration disrupted cue- and context-dependent fear memory in adolescent rats in both delay and trace conditioning. In contrast, CGP administration evoked impairment only in cue-dependent fear memory in rats exposed to trace but not delay fear conditioning. The postnatal blockade of NMDA receptors induced sensorimotor gating deficits in adult rats but not in adolescent rats.
*Conclusions:* The postnatal blockade of NMDA receptors induced fear memory impairment in adolescent rats before the onset of neurobehavioral deficits associated with schizophrenia.


## Introduction

The concept of schizophrenia as a neurodevelopmental disorder posits that psychotic symptoms are the end state of abnormal brain development starting years before illness onset [1]. The first symptoms of schizophrenia are observed in late adolescence or early adulthood; however, epidemiological, genetic and retrospective studies have also shown some behavioural impairment during the adolescent period of life. Epidemiological analyses have shown that children at genetic risk for schizophrenia have more developmental problems than control children, and many of these studies have revealed characteristics that are predictive of future schizophrenia [2]. Children at high risk for schizophrenia demonstrated problems in motor and neurological development, deficits in attention and verbal short-term memory or poor social competence [2]. Thus, the above findings suggested that developmental abnormalities appear before the manifestation of psychosis and might be observed as behavioural impairments prior to the onset of schizophrenia.

Individuals with schizophrenia have well-known deficits in a range of cognitive domains [3], and several findings indicate the influence of emotion on cognitive processing in schizophrenia because schizophrenia patients showed more cognitive disabilities in response to negative than neutral stimuli [4,5]. These observations might suggest that a more vulnerable cognitive system in those with this illness might lead to a stronger influence of emotion on cognition [6]. Thus, people with schizophrenia have difficulties in cognitive control associated with emotion, but whether emotional cognitive deficits are present before the onset of illness remains unknown [7]. This seems to be an important question in light of evidence that people at high clinical or genetic risk of schizophrenia were shown to have reduced recognition of emotional expression, in particular impairments in fear processing [8], which might reflect pathophysiological processes involved in the risk of schizophrenia.

*N*-Methyl-D-aspartate (NMDA) receptors are crucial for synaptic plasticity, and they play an important role in the pre- and early postnatal stages of brain development. Proper expression and

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regulation of NMDA receptors in the brain are critical for learning and memory processes, as well as synapse plasticity and maturation [9]. Thus, the impairment of NMDA receptor function by environmental or genetic risk factors in the pre- or early postnatal stage might disturb normal synaptic development and cause subsequent impairments in learning and memory processes. In fact, the impairment of fear memory in adulthood was observed in a model of schizophrenia based on the pharmacological blockade of NMDA receptors in adolescent [10,11] and adult animals [12,13]. Despite the availability of several animal neurodevelopmental models of schizophrenia, relatively little is known about the behavioural impairments in adolescence that precede the typical deficits of schizophrenia that manifest in adulthood. There is limited data [14–16] indicating that the disruption of NMDA receptor function early in life might cause impairments in spatial learning and memory during adolescence, before the onset of schizophrenia; however, the function of other forms of memory during adolescence, i.e., emotional memory, is still under investigation.

Therefore, to extend the available data, in the present study, we used a neurodevelopmental model of schizophrenia based on the postnatal blockade of NMDA receptors [17–20] to determine whether emotional memory impairment might precede the onset of schizophrenia and continue during the illness manifestation. Thus, the efficacy of fear learning and memory formation processes were investigated in adolescent and adult animals. Available data obtained from adult animals examined in a model of schizophrenia showed incoherent results related to fear memory function, depending on the conditioning task and model of schizophrenia used [10,12,13,21–23]. Therefore in the present study, associative emotional learning and memory processes were investigated in two fear conditioning tasks. We used trace (TFC) and delay (DFC) fear conditioning protocols because they modulate diverse aspects of fear memory formation by recruiting different brain regions [24–26].

In the final set of experiments, we verified the developmental aspects of the schizophrenia model. We postnatally administered the NMDA receptor antagonist and investigated the appearance of cognitive symptoms in adolescent and adult rats by measuring sensorimotor gating as the prepulse-induced inhibition of the startle response [27], and the impairment in sensorimotor gating might be considered to be the onset of schizophrenia [28].

## Materials and methods

### *Animals and treatment*

Pregnant dams (Wistar Han rats) were obtained from an animal provider (Charles River, Germany) at foetal embryonic day 15 (E15), were individually housed in polycarbonate cages (42 × 26.5 cm) with ad libitum access to food and water; they were kept under an artificial 12/12-h light/dark cycle (lights on at 7 a.m.). The day of parturition was designated as postnatal day 0. The rat pups were injected subcutaneously (*sc*) with increasing doses of CGP 37849 [(E)-(±)-2-amino-4-methyl-5-phosphono-3-pentenoic acid] (Tocris, UK), as previously described for CGP 40116 [20], an active isomer of racemate CGP 37849. The drug dosage was 1.25 mg/kg on days 1, 3, 6 and 9, followed by 2.5 mg/kg on days 12, 15, and 18 and a final dose of 5 mg/kg administered on day 21. On postnatal day 22, the rats were separated from their mothers. The drug administration protocol used in our study was not lethal to any animals used in the experiment. Male rats were randomly assigned to groups of five animals per cage with food and water available ad libitum, and a maximum of 2 rats per litter were used in the analysis conducted for each age. The experiments were performed on either adolescent animals (P35, P36, P37) or adults (P60, P61, P62), and distinct groups of rats were used in different experiments (n = 8 per group). The same groups of rats were used in adolescence and in adulthood for acoustic startle response test. In the case of cued fear conditioning, the separate groups of rats were trained in adolescence (P35) or in adulthood (P60). However, in conditioning experiments, each training group was later tested according to the schema on Figs. 1 and 2.

The study was carried out in strict accordance with the recommendations in the European Council Guide for the Care and Use of Laboratory Animals (86/609/EEC). The protocols were approved by the Committee for Laboratory Animal Welfare and the Ethics of the Institute of Pharmacology, Polish Academy of Sciences in Kraków. All efforts were made to minimise animal suffering and to reduce the number of animals used.

### *Cued fear conditioning*

The experimental procedure was similar to that described by Han et al. [25] and performed in the TSE Fear Conditioning System.

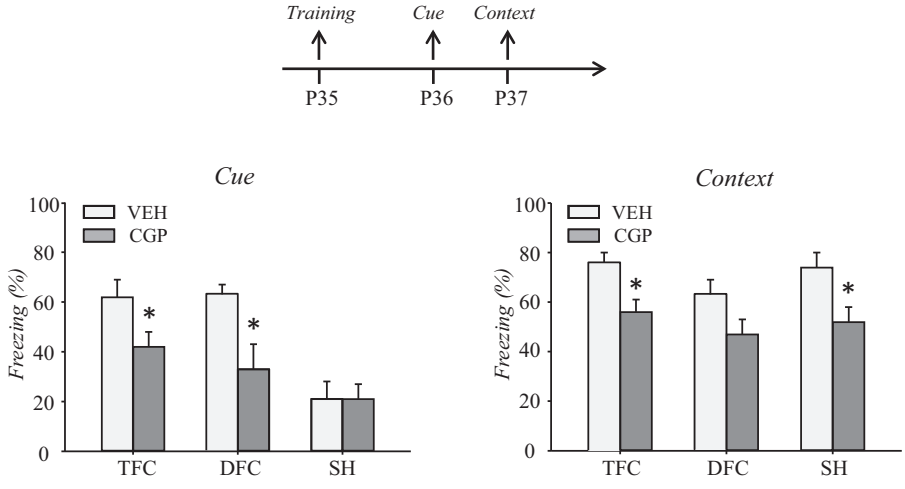


**Fig. 1.** The effects of postnatal CGP administration on cued fear memory in adolescent animals. Rats were trained at P35. Tests were conducted 1 (cue) and 2 (context) days later (P36 and P37, respectively). TFC—trace fear conditioning, DFC—delay fear conditioning, SH—shock only. Each data point represents the mean ± SEM; n = 8 per treatment and age. *$p < 0.05$ *vs.* appropriate VEH group (two-way ANOVA followed by Newman–Keuls test).