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Research report

mGluR3 knockout mice show a working memory defect and an enhanced response to MK-801 in the T- and Y-maze cognitive tests

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HIGHLIGHTS

- Cognition of mGluR3-KO mice was analyzed in T- and Y-maze tests after MK-801.
- T-maze spontaneous alternation was significantly reduced in mGluR3-KO mice.
- MK-801 enhanced locomotion more in mGluR3-KO than in WT mice in the Y-maze.
- Both of these genotype effects depended on the side of forced or habituation arm.
- Findings suggest side bias or left-right asymmetry in responses of mGluR3-KO mice.

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ABSTRACT

Polymorphisms in the metabotropic glutamate receptor 3 (mGluR3) encoding gene *GRM3* have been linked to schizophrenia and cognitive performance in humans. Our aim was to analyze the role of mGluR3 in basal working memory and attentional processes, and also when these functions were distracted by the psychotomimetic N-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine (MK-801). mGluR3 knockout (KO) mice were used. Spontaneous alternation in a T-maze test was significantly reduced in mGluR3-KO mice compared to wildtype (WT) mice, particularly after a low dose of MK-801 (0.03 mg/kg, i.p., 30 min). In a Y-maze novelty discrimination test, the locomotor stimulatory effect of MK-801 (0.1 mg/kg) was enhanced in mGluR3-KO mice. Interestingly, mGluR3-KO mice showed the significantly reduced alternation in the spontaneous alternation T-maze test and the significantly enhanced sensitivity to MK-801 in the Y-maze test only when forced to enter the right arm first, not when the forced arm was on the left. A side-biased response was also found in a rewarded alternation T-maze test, where mGluR3-KO mice made significantly more incorrect visits to the left arm than the right arm after a 25-s delay. No genotype difference was found in the novelty discrimination in the Y-maze test, rewarded alternation with a 5-s delay, preference for left or right when free to enter either arm or in MK-801-induced circling. Our findings indicate cognitive disturbance and left-right asymmetry in certain behavioral responses of mGluR3-KO mice. This novel observation warrants further elucidation, and should also be considered in other studies of mGluR3 in brain functions.

1. Introduction

Group II metabotropic glutamate receptors 2 and 3 (mGluR2 and mGluR3) have been implicated in brain functions related to psychiatric disorders. Pharmacological activation of mGluR2/3s shows antipsychotic and anxiolytic properties in preclinical animal models [1,2] and also in clinical settings [3,4]. However, the antipsychotic efficacy of mGluR2/3 agonists has not yet been confirmed in other clinical studies [5]. Both mGluR2 and mGluR3 modulate neuronal excitability by regulating glutamate (and GABA) release from presynaptic terminals, and postsynaptically by regulating activity of adenylate cyclase and various ion channels [6,7]. Although mGluR3 is widely expressed in neurons and glia with high expression in the cerebral cortex, hippocampus, thalamus, striatum and substantia nigra [8–10] its role in neuronal and glial communication is poorly understood [11]. mGluR3s located in the astrocytes appear to be important in neuroprotection [12].

Abbreviations: ANOVA, analyses of variance; *GRM3*, mGluR3 gene; KO, knockout; mGluR, metabotropic glutamate receptor; MK-801, dizocilpine; NMDA, N-methyl-D-aspartate; WT, wildtype.

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Glutamate N-methyl-D-aspartate (NMDA) receptor antagonists [phencyclidine, ketamine and dizocilpine (MK-801)] have been used to model symptoms of schizophrenia in rodents because of their psychotomimetic effects [13–15]. Typical behavioral responses to MK-801 include disruption of working memory and attention, as well as induction of locomotor activity and stereotypic behaviors. Sensitivity to the effects of MK-801 or phencyclidine is often analyzed in transgenic mice to elucidate functional roles of targeted genes in schizophrenia related behaviors [16,17]. In preclinical models, antipsychotic drugs inhibit psychotomimetic effects of NMDA receptor antagonists. Also, activation of mGluR2/3s has been shown to reverse phencyclidine induced hyperlocomotion indicating the role of mGluR2/3s in reversing neuronal processes elicited by NMDA receptor blockade [2]. This effect of the mGluR2/3 agonists is mediated through activation of the mGluR2 as it is abolished in mGluR2-KO (knockout), but not in mGluR3-KO mice [18,19]. Although, these preclinical findings indicate the importance of mGluR2 in antipsychotic-like actions, several human genetic studies suggest association of the mGluR3 gene (*GRM3*) with schizophrenia [11]. Schizophrenia-associated polymorphisms of *GRM3* have been linked to changes in markers of glutamate transmission, to poorer performance in cognitive tests and changes in mismatch negativity [20–23]. In addition, involvement of mGluR3 in schizophrenia-related functions is suggested by studies where increased brain levels of N-acetylaspartylglutamate, an endogenous agonist for mGluR3 [24–27], have prevented psychotomimetic effects of phencyclidine and cognitive deficits induced by MK-801 [28–30]. It is also important to note that we use here the same mGluR3-KO mouse line with Hsd:ICR (CD-1®) genetic background as utilized in a study showing the abolished antipsychotic effect of the elevated N-acetylaspartylglutamate levels due to genetic disruption of mGluR3 [29]. Clearly, the role of mGluR3 in basal and disrupted cognitive performance requires further investigation.mGluR2-KO and mGluR3-KO mouse lines have been utilized to dissect which receptor mediates various behavioral effects of mGluR2/3 agonists [12,18,19,29,31–33] and antagonists [34]. However, very few basal phenotypic features have been reported for either knockout line. Recently, it has been shown that mGluR2/3-double KO mice display deficits in spatial working memory and reference memory tests when motivated by food reward, but not when motivated by avoidance of aversive situation [35]. Specific roles of mGluR2 or mGluR3 were not addressed in that study. Our aim here was to analyze the role of mGluR3 in spatial working memory and attentional processes in T- and Y-maze tests, respectively, in basal conditions and after cognitive disturbance with the NMDA receptor antagonist MK-801.

2. Materials and methods

2.1. mGluR3-KO mouse line

mGluR3-KO mouse line was generated by homologous recombination as described earlier [33]. For this study heterozygous mGluR3-KO mice obtained from Taconic Farms Inc. (Germantown, NY, USA; kindly provided by Lilly Neuroscience, Eli Lilly and Company, Indianapolis, IN, USA) were further backcrossed with Hsd:ICR (CD-1®; Harlan) mice at Helsinki University. Heterozygous breeding provided mGluR3-KO and littermate WT mice after at least five backcrosses for the first behavioral test. Homozygous breeding was used to produce a larger amount of age-matched mice for behavioral tests. Homozygous WT and KO breeding pairs were littermates of heterozygous parents after at least eight backcrosses to Hsd:ICR (CD-1®) strain. Age-matched WT and mGluR3-KO mice were 10–14 weeks of age unless otherwise stated.

The mice were maintained in same-sex groups of 3–8 (cage, 40 cm × 30 cm × 15 cm; Tecniplast, Buguggiate, Italy) with food pellets (Harlan BV, Horst, Netherlands) and tap water available ad libitum at standard housing conditions (12 h light–dark cycle, lights on at 6:00 A.M.; temperature, 20–23 °C; relative humidity, 50–60%; aspen chip beddings). Males were housed alone (cage, 20 cm × 27 cm × 13 cm) if overt aggressive behavior was observed, and when on food restriction (only in the rewarded T-maze alternation test). A wooden toy and a shelter were kept in each cage during housing. All behavioral tests and drug injections were performed between 8 A.M. and noon at the light intensity of approximately 175 lux, unless otherwise stated. The mice were let to adapt to testing laboratory for at least 1 h before all experiments. The mice were tested blindly for genotype. All animal procedures were approved by the Southern Finland Provincial Government. All efforts were made to minimize the number of animals used.

2.2. Drugs

(+)-MK-801 maleate (Asc-027, Ascent Scientific) was dissolved in saline and injected (i.p.) at the volume of 10 ml/kg. Mice were injected with MK-801 or saline, returned to their home cages for 30 min until transferred to the testing room and placed in the maze.

2.3. Spontaneous alternation T-maze test

A spontaneous alternation T-maze test for analyzing spatial working memory was performed as previously described [36,37]. A T-maze was made of gray plastic and consisted of three arms (50 cm × 10 cm, walls 15 cm height). A door separated a 12-cm start compartment, and two other doors separated 24-cm compartments of the other arms. Vehicle or MK-801 (0.03–0.1 mg/kg, i.p.) was injected 30 min before the test and the mouse returned to its home cage until transferred to the testing room and placed in the start compartment for 5 s. Thereafter, the door was opened and the latency for the mouse to visit the forced arm (another arm was closed) and to return to the start arm was recorded (forced trial). A side of the forced arm was balanced by the treatment and genotype. Immediately after the forced trial, the test trial started and the mouse was allowed to visit either arm. When it entered one arm, the opposite arm was closed, and when the mouse returned to the start arm, both doors were again opened for the next trial. This was continued for 15 min. A video camera was located above the T-maze and the experimenter observed the mouse through a video monitor and remotely operated the doors from an adjacent room. The maze was cleaned after each mouse with water-wet paper towel and dried (as all other mazes and arenas described below). A percentage of spontaneous alternation was calculated by dividing the number of alternated arm visits (visits to the other arm than the previously visited) by the total number of arm visits × 100. A percentage of forced arm preference was calculated by dividing the number of visits to the forced arm by the total number of arm visits × 100 (Table 1).

Experimentally naïve, age-matched WT and mGluR3-KO males and females were obtained from heterozygous breeding (Fig. 1) and for subsequent experiments from homozygous breeding (Figs. 2 and 3). First two T-maze experiments were performed all in the same room (Room 1, Figs. 1 and 2) so that the position of the T-maze was to the right from the door. As we realized that the side of the forced arm may affect the results, the next T-maze experiment was performed in another room (Room 2, Fig. 3) so that the position of the T-maze was left from the door mirroring the position of the T-maze in Room 1. The number of animals used in each test is indicated in Table 1.