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A-582941, cholinergic alpha 7 nicotinic receptor agonist, improved cognitive and negative symptoms of the sub-chronic MK-801 model of schizophrenia in rats

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ABSTRACT

OBJECTIVES: Nicotinic receptor systems are involved in a wide variety of behavioural functions, including cognitive function, and nicotinic medications, may provide beneficial treatment for cognitive dysfunctions such as schizophrenia. According to the results of postmortem studies of schizophrenic patients, alpha 7 nicotinic acethylcholine receptor (*α*7 NAChR) binding levels and protein expressions were found to be decreased in cognition-related areas such as hippocampus and prefrontal cortex. In addition to this the fact that the prevalence of tobacco consumption is greater than healthy individuals encouraged research of nicotinic receptors in schizophrenia pathogenesis and treatment. In this study, we examined the effect of A-582941, a partial agonist of *α*7 NAChR, in the sub-chronic MK-801 model of schizophrenia in rats.

METHODS: Wistar Hannover rats were divided into five groups as follows and and all drug administrations were done intraperitoneally (i.p.) excluding the last dose of MK-801, which was done subcutaneously (s.c.): control (saline), vehicle (DMSO), MK-801 (0.2 mg/kg), MK-801 + A-582941 (1 mg/kg) and a positive control group (MK-801 + Clozapine) (5 mg/kg) ($n = 8–10$ in each). MK-801 was injected twice a day for 7 days. Prepulse inhibition of the acustic startle response (PPI) test was conducted after the last dose of MK-801 and animals were allowed to wait for a week for a washout period. A-582941 and Clozapine treatments were given for 10 days. The novel object recognition test (NORT) and social interaction (SI) and Morris water maze (MWM) tests were conducted.

RESULTS: PPI, discrimination index in NORT, social following behaviour in SI and swimming time in platform area in the MWM test were decreased by MK-801 injection, while latencies to platform finding in MWM and social avoidance in SI tests were increased. Clozapine increased the prepulse inhibition, discrimination index swimming in platform area and decreased platform-finding latencies and social avoidance in comparison with the MK-801 group. Although the A-582941 treatment has no effect on PPI, it increased the discrimination index, swimming time in platform area, following behaviour and decreased avoidance and platform-finding latencies.

CONCLUSION: Clozapine treatment improved the disruptive effect of MK-801 on PPI, NORT, and SI tests as expected. A-582941 treatment improved social deficits and cognitive dysfunctions on both visual and spatial memory. Therefore, A-582941 had a stronger effect on the negative symptoms and cognitive dysfunctions compared to that of Clozapine. The results of this study clearly suggested that *α*7 NAChR ligands might be a better treatment option, especially on cognitive and negative symptoms of schizophrenia.

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A-582941; MK-801; Clozapine; alpha 7 nicotinic acethylcholine receptors; Wistar Hannover; schizophrenia

Introduction

Schizophrenia is a complex and devastating psychiatric disease whose prevalence is estimated to be at least 0.45% by certain studies [1]. Positive and negative symptoms such as hallucination, delusion, paranoia, social withdrawal, and anhedonia are more prominent symptoms of schizophrenia, while 80% of schizophrenic patients have cognitive impairments in different types of memory [2]. Even though positive symptoms can be improved by both typical and atypical antipsychotics, negative symptoms and cognitive deficits are still unmet needs for schizophrenia treatment.

It is well known that glutamatergic hypoactivity plays an important role in schizophrenia pathophysiology. Studies have reported decreased glutamate levels in the cerebrospinal fluid and decreased NMDA receptor expressions in the hippocampus and prefrontal cortex of postmortem brain [3–5]. It has also been shown that NMDA receptor antagonists such as phencyclidine (PCP) and ketamine induce psychosis-like behaviours in healthy people [6]. On this basis, NMDA receptor

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antagonists (PCP, MK-801 and ketamine) have been used to induce schizophrenia-like behaviours in rodents [7]. Acute injection of these antagonists is commonly used for modelling psychosis-like behaviours, while low-dose sub-chronic injections (commonly 7 days, twice a day) create long-lasting negative and cognitive symptoms in rodents [7].

Nicotinic acetylcholine receptors (NAChR) are ligand-gated ionotropic receptors, which are located in pre- and/or postsynaptic positions of nerve cells in the central nervous system. When an agonist stimulates these receptors, the sodium channel is opened and facilitates the stimulation of the nerve cell by positive ion entry into the cell [8]. These receptors are grouped as hetoremeric or homomeric subtypes due to ingredients of their subunits. Heteromeric $\alpha4\beta2$ and homomeric $\alpha7$ receptors are the most common subtypes of NAChR [9]. The main differences of $\alpha7$ NAChR from other subtypes are (i) rapid desensitization, (ii) high ion permeability, (iii) high voltage dependence, and (iv) distinct pharmacological profile. It has been reported that $\alpha7$ NAChR is located especially in the cortex, hippocampus, substantia nigra, and cerebellum in mammalian brains [10].

Certain studies have shown that there is a link between $\alpha7$ NAChR dysfunction and schizophrenia. Freedman et al. [11] have suggested a link between impaired auditory sensory gating and $\alpha7$ NAChR gene single-nucleotide polymorphism on the promoter regions of chromosome 15q14 locus. Furthermore, postmortem studies demonstrated that $\alpha7$ NAChR binding levels and protein expressions decreased in dentate gyrus, hippocampal CA3 region, cingulate cortex, and prefrontal cortex, which are important regions for modulating cognitive function [12–14]. In addition to this, the fact that the prevalence of tobacco consumption of schizophrenia patients is higher compared with healthy individuals encourages researchers to examine nicotinic receptors in schizophrenia pathogenesis and treatment [15]. It has been considered that patients consume tobacco for their self-medications due to its nicotine content. It was also demonstrated that $\alpha7$ NAChR knock-out mice showed worse performance than wild-type animals in cognitive tasks [16]. These data have indicated that $\alpha7$ NAChR dysfunction can be responsible for schizophrenia pathophysiology and a new drug target for the treatment of schizophrenia.

Early synthesized members of full agonists of $\alpha7$ NAChR were not sufficiently selective to subtypes of receptors and they caused common nicotinic side effects. Later, newer agonists of NAChR such as A-582941 were synthesized, which had a much better profile in terms of side effects [17]. The effects of A-582941 were investigated on certain animal models including psychosis, and the promising effect of it was demonstrated in certain studies [18]. It has been shown that A-582941 has beneficial effects on attention, spatial and visual learning and memory in rodents and monkeys. Likewise, in behavioural studies, it has been proved that after NMDA receptor antagonist injection, there was reduction in parvalbumin and glutamic acid decarboxylase 67 content due to GABAergic dysfunction, which was ameliorated following $\alpha7$ NAChR agonists [19].

In this study, we aimed to examine the effects of A-58294, a partial agonist of $\alpha7$ NAChR, on sensorimotor gating, social, and cognitive functions in the sub-chronic MK-801 model of schizophrenia that is commonly used for investigating schizophrenia.

Materials and methods

Animals and housing

All experiments documented in this study were conducted in accordance with the Regulation of Animal Research Ethics Committee in Turkey (6 July 2006, Number 26220). The Marmara University Animal Research Ethics Committee granted ethical approval (017.2016.mar). Before starting the experiments, rats were allowed to habituate to the laboratory environment and the experimenters for 2 weeks. Male Wistar Hannover rats (8–12 weeks and 180–250 g) were housed in temperature-controlled (22 ± 1°C), 12/12 light and dark cycle room conditions. Animals were housed at standard animal cages (four animals per cage) and fed ad libitum bait and water. Experiments were conducted at the light phase of light/dark cycle. Animals were grouped as control (saline), vehicle (dimethyl sulfoxide; DMSO), MK-801, A-582941, and Clozapine (n = 8–10 per group).

Drugs

MK-801 ((+)-MK-801 hydrogen maleate, cat no:1000 9019, Cayman) was dissolved in trace amounts of DMSO (Merck) as a stock solution because of its high stability in DMSO and daily diluted to an appropriate volume with saline. A-582941 (Santa Cruz, cat no: Sc-362706A) and Clozapine (Leponex®, Novartis) also were dissolved in DMSO and diluted with saline. Saline, vehicle and drug treatments were administered at a volume of 0.1 ml/100 g.

Experimental design and treatments

After habituation and handling periods at the laboratory, MK-801 (0.2 mg/kg) was intraperitoneally (i.p.) injected twice a day (at 7:00 am and 7:00 pm) for 7 days. The last dose of MK-801 was administered subcutaneously (s.c.) for prepulse inhibition (PPI) of acoustic startle response test. Single doses of A-582941 (1 mg/kg, i.p.) and Clozapine (5 mg/kg, i.p.) were also treated in the PPI test. A seven-day washout period was conducted after the PPI test for avoiding acute effects of MK-801.

After the washout period, A-582941 and Clozapine were injected once a day for 10 days. The novel object recognition test (NORT) and social interaction (SI) were performed on the 6th day of treatment, while Morris's water maze (MWM) test was conducted at 6–10th days of treatments. The experimental design is summarized in Figure 1. For the PPI test, MK-801 was administered 15 min and A-582941 was injected 45 min before the test. Saline, DMSO, or Clozapine was administered to relevant groups 30 min and A-582941 45 min prior to the behavioural experiments. Only for the MWM test, drugs were injected before the first trial of the experiment for each day.

Prepulse inhibition of acustic startle response (PPI)

The SR-LAB system (San Diego Instruments, San Diego, CA) was used for evaluating the startle of rats. Two identical startle response chambers ($39 \times 38 \times 58$ cm) were used during the experiment. A chamber consists of a plexiglass cylinder cage (8.8 cm diameter, 25 cm length) with piezoelectric sensory under the cylinder cage for detecting the rat's startle, loudspeakers for creating acoustic stimulus, a control unit, and a computer with SR-LAB software.

The test procedure of PPI has been described in previous literature [20]. Briefly, before the testing day, rats were put in the chambers and exposed to a background noise for 5 min and five startle stimuli for adaptation to apparatus and for testing of hearing and startle function. On the test day, rats were placed in the chambers and exposed 70 dB of background noises for 5 min. After the acclimation period, rats were exposed to three trial blocks. Block 1 consisted of five presentations of 40 ms 120 dB pulse-alone trials. Block 2 had 50 pseudo-random trials (average intertrial intervals of 15s) which consisted of 10 presentations of each prepulse + pulse trials (74, 78, and 86 dB of 20 ms duration and 100 ms before 40 ms 120 dB pulse), 10 presentations of 120 dB pulse-alone and 10 presentations of no stimulus. Block 3 had five presentations of pulse-alone trials. Startle response was defined as the average of 100 readings collected every 1 ms beginning at the onset of the acoustic startle stimulus. Only Block 2 was taken into account for per cent prepulse inhibition. Per cent prepulse inhibition of startle response was calculated for each rat as per the following formula: $\%PPI = 100 - (PP + P)/(P) \times 100$. "PP + P" and "P" mean the startle response after the presentation of prepulse and pulse stimulus and startle response after the pulse-alone stimulus, respectively.

Novel object recognition test

NORT was performed in a black plexiglass open field arena ($50 \times 50 \times 30$ cm) at dimly lit conditions. The test apparatus was cleaned with alcohol (70% ethanol) after each animal. The experiment consisted of two days as habituation and test days. This process is well described in previous studies [21] and summarized as follows:

Habituation (Day 1): All animals in the same group were put into the plexiglass chamber and were allowed to acclimate to the test environment for 60 min. No object was used in this period. The process was performed for each group.

Test (Day 2): The test consisted of two 3 -min trials with 1-h intertrial intervals. In trial 1 (Familiarization, T1), rats spent 3 min with two identical objects (A for each). The objects were placed in the opposite corners of the chamber which were 10 cm from the walls. At the end of 3 min, rats were returned to their home cage for 1 h. In trial 2 (Retention, T2), one of the identical objects was changed with a novel object (B) and rats were allowed to explore the object for 3 min. The sizes of familiar and novel objects were comparable to each other (about 10 cm height). The locations of A and B were changed with each other in half of the animals of each group. Exploratory behaviour was defined as sniffing, licking, and touching. All trials were recorded using a camera placed above the apparatus and connected with Noldus Ethovision XT® software. Exploration time (E) was scored by a treatments blind researcher. Discrimination index (DI) was calculated as per the following formula: $DI = (E_B - E_A)/(E_B + E_A)$.

Morris water maze (MWM) test

MWM was performed according to previous literature [22]. Briefly, the MWM test apparatus consisted of a cylinder water maze tank (160 cm diameter and 50 cm wall height), a platform (12 cm diameter and 30 cm height), and four cues (different shapes and colour cardboards). MWM was filled with water at approximately 25°C until 1 cm above the platform height. The pool was divided into four quarters theoretically and the quarters were assumed to represent North (N), South (S), West (W), and East (E) directions. Cues were attached to the opposite of each direction. A platform was located in the middle of SE quarter. Noldus Ethovision XT® was used to track and record the rats and the water was black coloured with Mixol® to ease the software's tracking of animals.

Rats were trained for four trials per day for 4 days (Acquisition period). In a trial, rats were released to water from one of the directions and allowed 75 s for finding the platform. If a rat did not find the platform, the researcher guided it for finding the platform. Rats were rested on the platform for 20 s. The trial was repeated for all directions for each animal in a day. The direction order was changed every day. Trials were recorded using a camera which was connected to Noldus

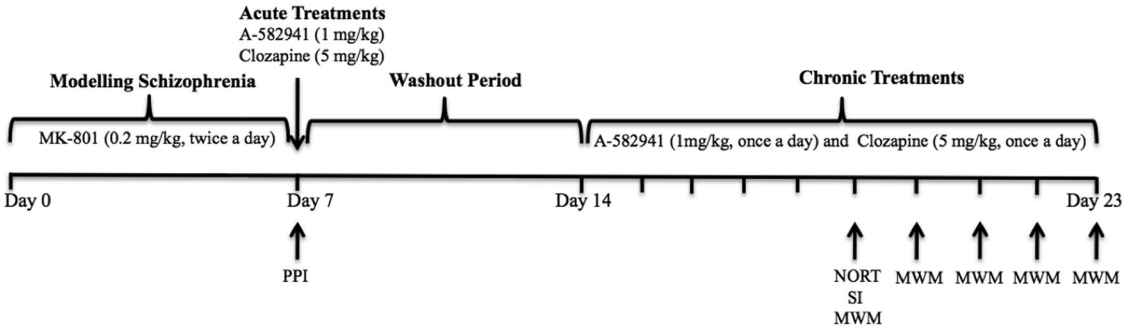


Figure 1. Experimental design of the study (PPI: Prepulse Inhibition of Acustic Startle Response, NORT: Novel Object Recognition Test, SI: Social Interaction, MWM: Morris's Water Maze).

Ethovision XT® and the platform-finding latency of each animal was calculated in this 4-day period. On the 5th day (Probe test), the platform was removed and the rats were released from the N direction. The swimming time in the SE quarter was scored for 60 s.

Social interaction (SI) test

SI was conducted immediately after NORT. SI was performed in a black plexiglass open field arena (50 × 50 × 30 cm) at dimly lit conditions. The test apparatus was cleaned with alcohol (70% ethanol) after each animal.

In this test, two unfamiliar rats in the same treatment group were placed in the test arena for 10 min. One of the rats was painted with temporary and non-odour ink for discriminating from the other before the test. Social behaviours of each rat were evaluated separately. The spending time for sniffing, climbing, and following behaviours was scored as an indicator of socialization and the spending time for avoiding was scored as an indicator of social withdrawal [23].

Statistical analysis

In this study, GraphPad Prism 6.0 for Mac was used in statistical analyses for all behavioural experiments. The most proper statistical test and its *post hoc* tests were chosen for each experiment. Briefly, one-way analysis of variance (ANOVA) was used for NORT, SI, and MWM probe tests, while two-way ANOVA was used for PPI and MWM acquisition tests. Dunnett's *post hoc* test was used to compare differences of groups in all experiments. Data were presented as mean ± standard error of mean (S.E.M.) for all experiments excluding the MWM acquisition test (presented as mean in this test). $p < .05$ was considered as a value of significance.

Results

The effects of A-582941 and Clozapine on MK-801-induced PPI disruption

In the PPI test, MK-801 injection significantly decreased PPI at +4 dB ($p < .05$), +8 dB ($p < .001$), and +16 dB ($p < .001$) prepulse intensities compared with the control group. Clozapine-treated rats had a higher PPI than the rats in the MK-801 group ($p < .001$) at +16 dB prepulse intensity, while the A-582941 treatment did not change the PPI in any of prepulse intensities. There was no significant difference between control and vehicle groups at any prepulse intensity (Figure 2).

The effects of A-582941 and Clozapine on MK-801-induced cognitive deficit in NORT

In NORT, the MK-801 group had a lower discrimination index compared to the control group ($p < .05$). Both of A-582941 and Clozapine groups had a higher discrimination index than the MK-801 group ($p < .05$ and $p < .01$, respectively). There was no difference between discrimination indexes of control and vehicle groups (Figure 3).

The effect of A-582941 and Clozapine on MK-801-induced cognitive deficit in MWM test

In the acquisition period of the MWM test, the rats in the MK-801 group found the platform at a later time than the control group on 1st ($p < .01$), 2nd ($p < .001$), 3rd ($p < .05$) and 4th ($p < .05$) days. A-582841-treated rats had significantly shorter latency on 2nd ($p < .05$) and 4th ($p < .05$) days compared to the MK-801 group, while latency of Clozapine-treated rats was markedly shorter than the MK-801 group on the 3rd ($p < .01$) and 4th ($p < .01$) days. There was no significant difference between the latencies of control and vehicle groups at any of these days (Figure 4).

In the probe trials of the MWM test, the MK-801 group spent less time in the platform area compared with the control group ($p < .05$). Clozapine-treated rats significantly increased ($p < .05$) the time in the platform area compared to the MK-801 group, whereas A-582941 did not have a clear effect on the decreased time by MK-801. The spent times in the platform area in control and vehicle groups were not statistically different in the probe trials of the MWM test (Figure 5).

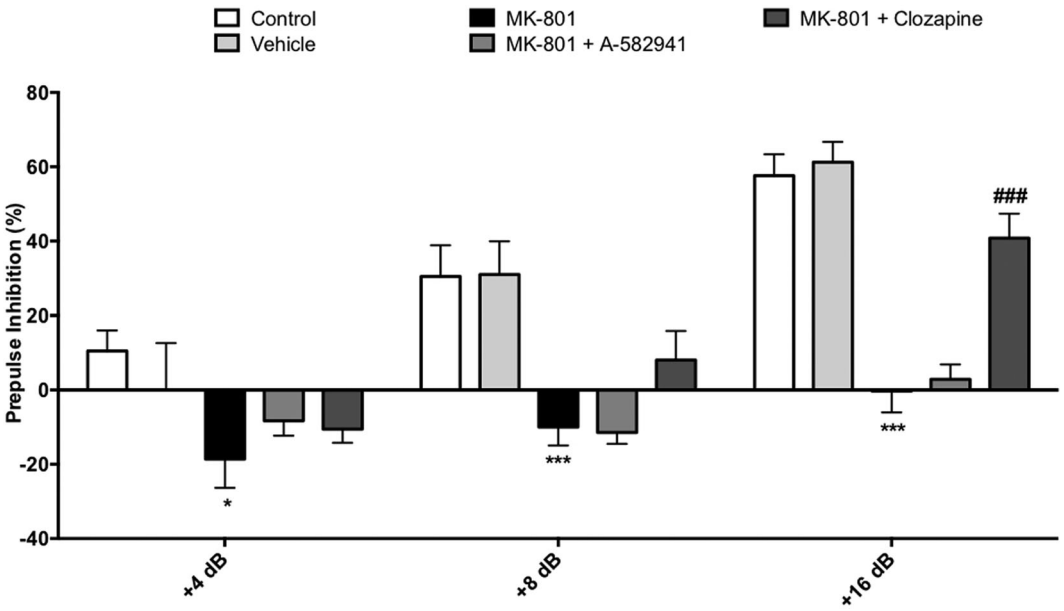


Figure 2. The effect of A-582941 and Clozapine on MK-801-induced PPI disruption. Data are expressed as mean ± S.E.M. and analysed by using two-way ANOVA followed by Dunnett's *post hoc* test. ***$p < .01$ compared with the control group and ###$p < .001$ compared with the MK-801 group.

The effect of A-582941 and Clozapine on MK-801-induced social deficits in the SI test

In the SI test, the MK-801 group spent less time for following behaviour and more time for avoidance behaviour than the control group ($p < .05$ and $p < .001$, respectively). Clozapine treatment significantly decreased the time spent for avoidance ($p < .001$), while the A-582941 treatment not only decreased the avoidance time ($p < .01$) but also increased following time ($p < .01$) compared to the MK-801 group. There was no significant difference in the time spent for following and avoiding behaviours between control and vehicle groups (Figures 6 and 7). Besides, other parameters of the SI test did not change in any group (data not shown).

Discussion

Our results suggested that sub-chronic MK-801 administration impaired sensorimotor gating, novel object recognition, spatial learning and memory, and socialization in rats. A-582941 treatment could improve cognitive and social impairments, while it had no effect on sensorimotor gating deficit of rats in this model. Clozapine, the gold standard for treatment of schizophrenia, ameliorated the impairments of sensorimotor gating, cognitive, and social functions. It has also been shown that Clozapine only improved the defect of following behaviour that represents tendency to socialization, while A-582941 improved both of the impairments on following and avoiding behaviour that were thought to represent social withdrawal of schizophrenia patient.

It has been well demonstrated that information processing and sensorimotor gating which are thought to play a role in attention, cognitive function, and daily living are impaired in schizophrenia. PPI is a well-validated translational paradigm showing sensorimotor gating which includes both sensory stimulus and motor response [24]. It has been thought that PPI is associated with the cortico-thalamo-pallido-striatal neuronal pathway and it is demonstrated that the PPI paradigm was impaired in certain neuropsychiatric diseases such as schizophrenia and autism. In rodent studies, it has been shown that psychotomimetic drugs such as dopaminergic agonists (e.g. apomorphine) and glutamatergic NMDA receptor antagonists (e.g. PCP and MK-801) induce disruption of PPI paradigm via its acute effects and the effects are commonly transient [20,25]. Studies indicated that the disruptive effect of NMDA receptor antagonists on PPI was reversed by atypical antipsychotics but not typical in most times [26,27]. In our study, we showed that MK-801 administration induced deficits at all of the prepulse intensities and Clozapine reversed the disruptive effect of MK-801 at +16 dB prepulse intensity in the PPI test. Our results are consistent with previous studies. There are contradictory results of $\alpha 7$ NAChR agonists for PPI in certain models of psychosis. It has been shown that continuous administration of GTS-21, a partial agonist of $\alpha 7$ NAChR, treated MK-801 induced PPI deficit in rats and P40 sensory abnormality in DBA/2 mice [24,28]. In another study, it was demonstrated that $\alpha 7$ NAChR agonists GTS-21 and AR-R-17779 revealed a disruption on the PPI paradigm in both rats and DBA/2 mice [29]. In one study that investigated the effect of A-582941

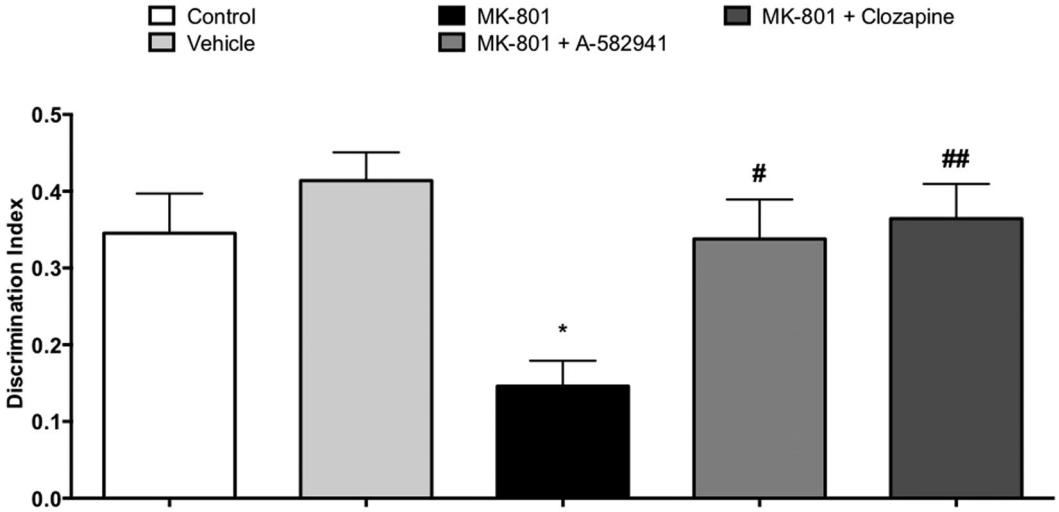


Figure 3. The effect of A-582941 and Clozapine on MK-801-induced cognitive dysfunction in NORT. Data are expressed as mean ± S.E.M. and analysed by using one-way ANOVA followed by Dunnett's *post hoc* test. *$p < .05$ compared with the control group and #$p < .05$ and ##$p < .01$ compared with the MK-801 group.

on the PPI paradigm, it has been suggested that $\alpha 7$ NAChR agonists A-582941, ABT-107 and PNU282987 did not improve sensory deficits in DBA/2 mice. In our study, we demonstrated that A-582941 did not improve MK-801-induced sensorimotor deficit in any prepulse intensity.

Visual learning and memory deficits are one of the main cognitive dysfunctions in schizophrenic patients. To research the cognitive dysfunction of schizophrenia, Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) and Treatment Unit for Research on Neurocognition and Schizophrenia (TURNS) initiatives recommend NORT as a translational preclinical paradigm to investigate procognitive effect of different therapeutics [24]. NORT is a cognitive test which evaluates non-spatial visual episodic memory and is based on the spontaneous exploratory behaviour of rodents. It has been thought that perirhinal cortex and hippocampus are involved in visual recognition memory in both rodents and human [24]. It has been well confirmed that acute and chronic administration of the glutamatergic NMDA receptor antagonists impaired the recognition memory in rodents. This cognitive deficit induced by NMDA receptor antagonists reversed by second-generation antipsychotics but not first-generation antipsychotics [30]. In many studies, it was reported that NAChR agonists have a cognitive enhancer effect in not only the schizophrenia model but also in the Alzheimer model or without any model of rodents. Potasiewicz and colleagues indicated that A-582941 had procognitive activity at recognition memory on natural

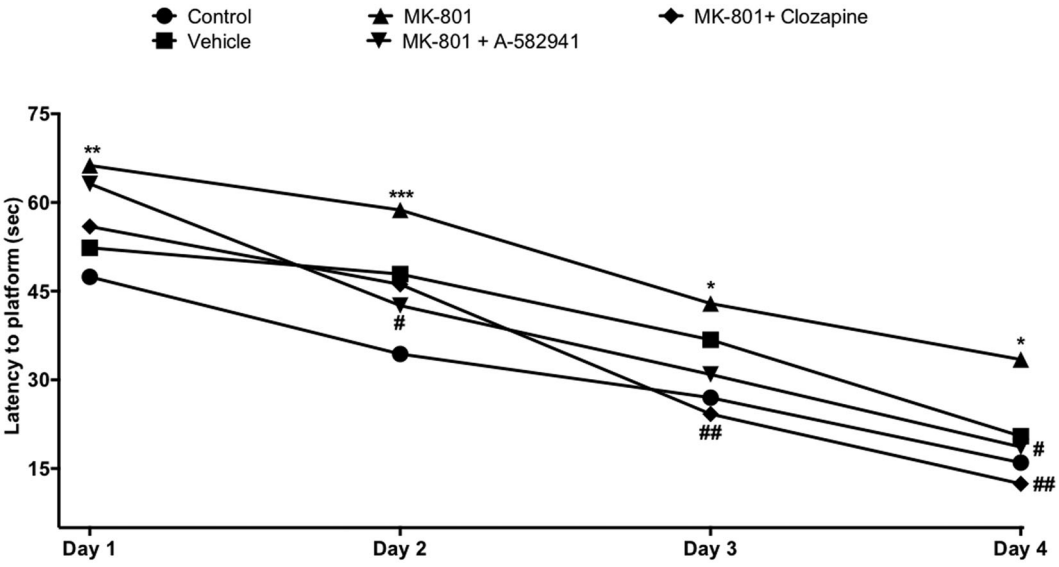


Figure 4. Latency to platform finding of Control, Vehicle, MK-801, A-582941 and Clozapine groups in the acquisition period of the MWM test. Data are expressed as mean and analysed by using two-way ANOVA followed by Dunnett's *post hoc* test. *$p < .05$, **$p < .01$, ***$p < .001$ compared with the control group, #$p < .05$ and ##$p < .01$ compared with the MK-801 group.

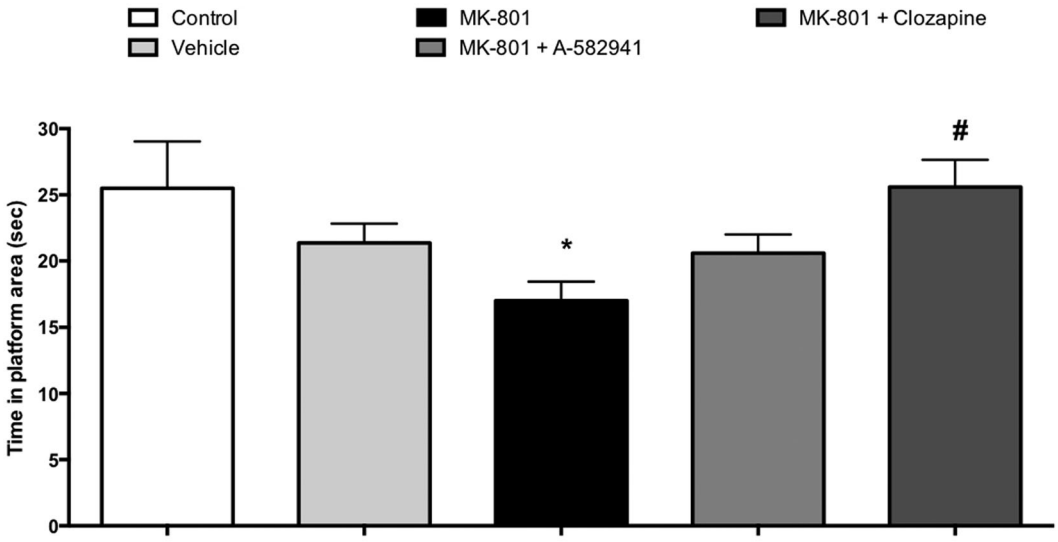


Figure 5. The time spent in the platform area of Control, Vehicle, MK-801, A-582941 and Clozapine groups in the probe trials of the MWM test. Data are expressed as mean ± S.E.M. and analysed by using one-way ANOVA followed by Dunnett's *post hoc* test. *$p < .05$, compared with the control group and #$p < .05$ compared with the MK-801 group.

forgetting of rats [31], while another study demonstrated that A-582941 ameliorated ketamine-induced cognitive dysfunction in NORT in rats [32]. In our study, it was shown that A-582941 and Clozapine enhanced MK-801-induced recognition memory deficit in rats.

MWM is one of the prevalent tests which evaluates the cognitive function of rodents in behavioural studies. It has been demonstrated that MWM is well validated for cognitive dysfunction of schizophrenia and visuospatial learning and memory were involved in the assessed cognitive function. It has been thought that MWM represents a revised version of the Brief Visual Memory Test which investigates the hippocampal-dependent spatial recognition memory in human and impaired in schizophrenia patients. Certain brain regions and neurotransmitter systems including hippocampus, striatum, basal forebrain, cerebral cortex, and cerebellum play an important role in the MWM performance of rodents [33]. Different studies showed that glutamatergic NMDA receptor activity is crucial for spatial learning and memory, and antagonists of these receptors revealed cognitive impairments on the MWM test in rodents [34–36]. Our results indicated that sub-chronic MK-801 administration induced persistent learning deficits in the acquisition period at all of the 4 days and memory defect in the probe test. Studies showing the effects of $\alpha 7$ NAChR agonists are very limited for spatial learning and memory. It has been reported that PHA 543613, selective agonist of $\alpha 7$ NAChR, improved learning and memory deficits of MWM in the beta-amyloid model of Alzheimer disease in mice [37]. It has been also demonstrated that nicotine, non-selective NAChR agonist revealed

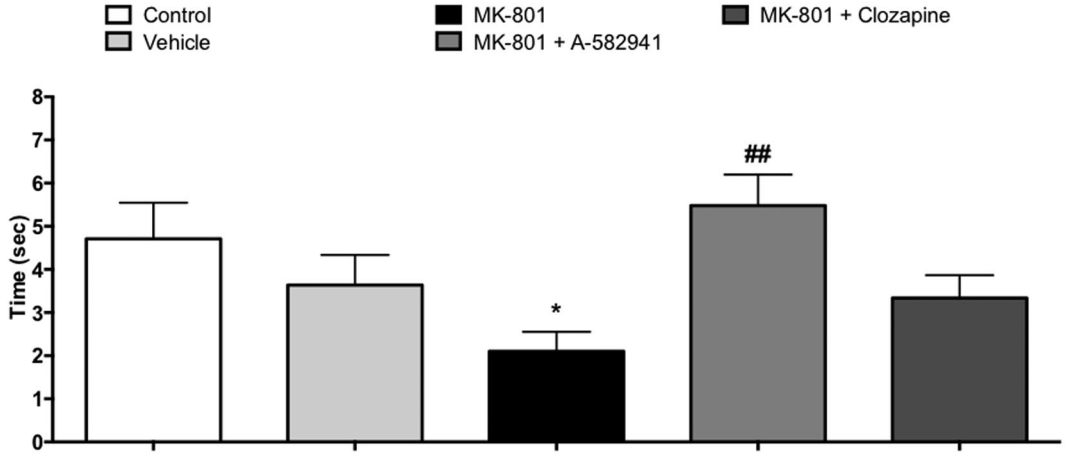


Figure 6. Time spent for following behaviour of Control, Vehicle, MK-801, A-582941 and Clozapine groups in SI test. Data are expressed as mean ± S.E.M. and analysed by using one-way ANOVA followed by Dunnett's *post hoc* test. *$p < .05$ compared with the control group and ##$p < .01$ compared with the MK-801 group.

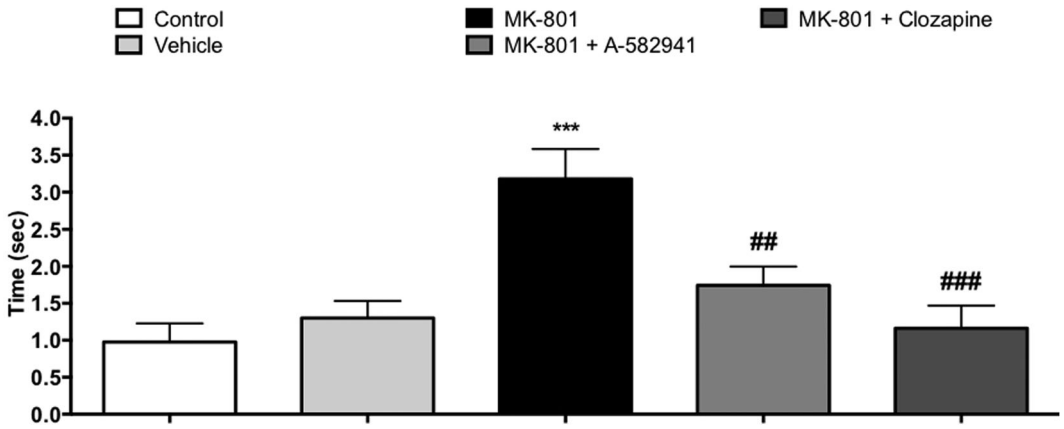


Figure 7. Time spent for avoiding behaviour of Control, Vehicle, MK-801, A-582941 and Clozapine groups in SI test. Data are expressed as mean ± S.E.M. and analysed by using one-way ANOVA followed by Dunnett's *post hoc* test. ***$p < .001$ compared with the control group, ##$p < .01$ and ###$p < .001$ compared with the MK-801 group.

an attenuation of beta-amyloid-induced learning and memory deficit in radial arm water maze in rats [38]. In our study, we showed that A-582941 reversed the disruptive effect of MK-801 on acquisition learning but not the probe memory of rats in the MWM test.

Negative symptoms such as avolition, anhedonia, and social withdrawal are some of the core symptom clusters of schizophrenia [39]. However, the exact neuronal mechanisms of negative symptoms are not known clearly; it has been thought that the frontal cortex plays an important role for this symptom in schizophrenia [40]. Although animal models of schizophrenia have some challenges for modelling the all negative symptoms, it has been demonstrated that glutamatergic NMDA receptor antagonists cause social deficits in rodents. It has also been shown that atypical but not typical antipsychotics can reverse the deficits induced by NMDA receptor antagonists [30]. It was reported that following, sniffing and climbing behaviours were decreased while avoiding bahaviour was increased in SI test [30,41]. Our study has shown that MK-801 cessation after sub-chronic administration caused social deficits in following and avoiding behaviours in rats. We did not observe clear impairments in sniffing and climbing behaviours, while certain studies showed deficits in all behavioural patterns. To our knowledge, a limited number of studies have investigated the effect of α7 NAChR on social symptoms of schizophrenia in rodents. In one study evaluating the effect of A-582941 on acute ketamine-induced schizophrenia rat model, it was indicated that A-582941 attenuated social deficits induced by ketamine in rats [32]. To investigate other agonists of α7 NAChR, it was shown that EVP-6124 and TC-5619 reversed social deficits in PCP and genetic (only TC-5619) model of schizophrenia in mice [42]. In our study, we have suggested that A-582941 not only reversed social withdrawal but also encouraged socialization, while Clozapine reversed only social withdrawal in the MK-801 model of schizophrenia in rats.

It has been well known that treatments of α7 NAChR agonists cause rapid desensitisation of these receptors and decrease the ratio of active receptors. On the contrary, it has been also shown that repeated administration of α7 NAChR agonists but not antagonists or modulators causes receptor up-regulation by increasing the numbers of receptors. It has been thought that the conformational changes after desensitisation, decreased cell surface turnover, increased receptor traffic, raised subunit production and reduction in subunit degradation are potential mechanisms of this up-regulation [43]. As the behaviuoral results of these conflicting conditions, it has been demonstrated that the up-regulation of these receptors contributes sustained procognitive activity of α7 NAChR agonists [44].

As a limitation of the present study, we could not exclude the role of 5HT3 receptors in the beneficial effects of A-582941. However, previous studies have shown that procognitive effects of A-582941 were abolished with pretreatment of selective α7 NAChR antagonists [31]. So, these data have shown that α7 NAChR is responsible for the beneficial effects of A-582941 more than 5HT3 receptors. Therefore, in our study, we think that α7 NAChR agonism plays a major role in the improving effect of A-582941 on the cognitive and social deficits in rats.

In literature, there are certain similarly designed studies that evaluate the effects of A-582941 on a schizophrenia model [32]. Our study is differentiated from those by using subchronic model of schizophrenia, subchronic treatment regimen of A-582941, evaluating sensorimotor gating function and spatial memory on MK-801 model of rats.

In conclusion, our study supported that A-582941 could be a candidate drug for treatment of especially cognitive deficits and negative symptoms of schizophrenia. Our results clearly showed that A-582941 had a better profile than Clozapine for social deficits in rats. Although A-582941 had promising results, our study had focused on behavioural effects of

A-582941 on a well-validated model of schizophrenia. Therefore, further studies on α7 NAChR agonists are necessary for fully understanding their role in schizophrenia pathogenesis and treatments, especially on cognitive and negative symptoms.

Disclosure statement

No potential conflict of interest was reported by the authors.

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