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Original Article

Effects of Nicotine Administration in an Enriched Environment on the Behavior of Male MK-801-Exposed Rats

Neda Salmani[1#], Fatemeh Darvishzadeh Mahani[2#], Mahdieh Parvan[2], Masoumeh Nozari[3*]

[1]Department of Psychology, Zarand Branch, Islamic Azad University, Kerman, Iran
[2]Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
[3]Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran

Abstract

Background: Smoking is more common in patients with schizophrenia than in healthy populations. Some controversial hypotheses connect the disease with the high prevalence of smoking. Moreover, environmental factors affect the severity of the positive and negative symptoms of schizophrenia. The current study aimed to assess the effect of enriched environment (EE) and nicotine on the MK-801 animal model of schizophrenia.

Methods: Male Wistar rat pups randomly received saline or MK-801 (dose:1 mg/kg) for five days from the sixth postnatal day (P) until the tenth. The pups were placed in EE or standard cages (SCs) after weaning (P21). Morris water maze (MWM) was used to assess spatial learning and memory. The rats received 0.6 mg/kg nicotine twice for three days at the end of the second month and were examined in an open-field box and three-chamber social interaction test.

Findings: MK-801 rats' behaviors were the same as those of the saline rats when they were exposed to nicotine. No positive effects of EE were observed when the animals were exposed to nicotine.

Conclusion: The results suggested that nicotine decreased schizophrenia-like symptoms and covered the positive effects of EE.

Keywords: Behavior, Enriched environment; Nicotine, NMDA receptor blockade, MK-801, Schizophrenia

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Introduction

Nicotine consumption rates are high in patients with mental illness. Recent studies have shown that smoking is four to five times more prevalent in adults with mental disorders than in healthy adults. Based on previous studies, more than 60%-64% of patients with schizophrenia are smokers; however, its prevalence in healthy individuals is 19%.[1,2] About 1% of the world's population is affected by schizophrenia, a chronic brain disorder. The symptoms of schizophrenia include emotional disorders, behavioral or thought disorders, and isolation.[3] The relationship between schizophrenia and nicotine dependence is complex. Although the leading cause of nicotine dependence in patients with schizophrenia is unknown, previous studies have pointed out the role of dopamine systems and reported that central nicotine receptor activation leads to an increase in dopamine and serotonin levels.[4,5] Nicotine can also inhibit monoamine oxidase A and B and induce antidepressant effects. Improvement of the extrapyramidal effects of antipsychotic drugs and the symptoms of the disease is another positive effect of nicotine consumption on these patients. These outcomes may be the cause of the higher prevalence of smoking and nicotine self-medication in these patients. However, others believe that nicotine increases psychosis risk due to the increased sensitivity of D2 receptors.[6-8]

Our recent study showed nicotine-induced conditioned place preference in a schizophrenia animal model.[9] The current research project examines the effect of nicotine consumption on cognitive behaviors in an enriched environment (EE) to know if EE and nicotine have synergic effects in an MK-801 rat model of schizophrenia. In experimental studies, EE is a cage bigger than the standard cages (SCs) that include various objects with different tissues, shapes, and sizes to increase sensory, cognitive, and motor stimulation. Total brain weight, cortical thickness, synaptogenesis, and dendritic branching can be increased by EE.[10,11] Previous research has reported the beneficial roles of EE on animal models of schizophrenia; however, this is the first study to examine the combined effect of EE and nicotine on an MK-801 rodent model of schizophrenia. MK-801 (dizocilpine) is a non-competitive N-methyl-D-aspartic acid (NMDA) receptor antagonist, and behavioral alterations such as repetitive movements and cognitive deficits as well as many histochemical changes representing schizophrenia may occur if rodents are exposed to it during the neonatal period.[12,13]

*Corresponding Author: Masoumeh Nozari, Emails: Masoumeh.Nozari@gmail.com; m.nozari@kmu.ac.ir
#Both authors contributed equally to this work

Methods

Conditions of animals and housing

The researchers went to the Neuroscience Research Center in Kerman, Iran and purchased pregnant Wistar rats. The delivery day was designated as postnatal day (P0). Male pups were injected randomly with saline or MK-801 (obtained from Tocris) for 5 days from P6 to P10. After weaning (P21), the pups were placed in EE cages (four animals per cage) (100 × 100 × 100 cm) or SCs (four animals per cage) (40 × 20 × 15 cm). The EE cages had nesting material, colored plastic tunnels, running wheels, and shelters, which were changed every week.[9,14] The characteristics of the groups are summarized as follows:

1. EE: The rats were injected with saline from P6 up to P10 and were placed in EE cages.
2. Sham: The rats were injected with saline from P6 up to P10 and were placed in SCs.
3. MK-801: The rats were injected with MK-801 and were placed in SCs.
4. MK-801 + EE: The rats were injected with MK-801 and were placed in EE cages.

The animals received 0.6 mg/kg nicotine twice a day for three days at the end of the second month,[9] and until the end of the test, they grew up in their cages. Behavioral tests started one day after the last nicotine injection and were conducted from 07:00 AM to 2:00 PM. Eight rats were in each group. Figure 1 shows the experiment schedule.

The animal care protocols and test procedures were approved by the Animal Experiments Ethics Committee of Kerman University of Medical Sciences, Kerman, Iran (Ethics Code: IR.KMU.REC.1398.138). The study tests were consistent with the "NIH Guide for the Care and Use of Laboratory Animals". A standard room with laboratory food and water pellets was used to keep the rats. The temperature of the animal house was 22 ± 2 °C with 12 hours of light per day from 07:00 AM.

Open-field tests

The open-field tests determined the exploratory efforts and locomotor activity of rats. The rats were placed in the middle of a plexiglass frame (90 × 90 × 30 cm), the floor of which was separated into 16 equal squares using imaginary lines. For 5 minutes, a video tracking software (Borj Sanat Azma Co., Iran) automatically recorded the animal's activity, including the total movement distance (cm), the traveled distance, as well as the elapsed time in the peripheral (the squares around the center) and central (four squares in the center) areas of the box.

Moreover, a person blind to the status of the rats evaluated some parameters, including rearing (as a vertical movement) and grooming (head and body rubbing with paws or mouth) numbers. After each test, the box was cleaned to remove any olfactory cue that could change the behavior of the next animal.[15,16]

Social interaction test

The social interaction test was developed to quantitatively evaluate the social behavior of rodents. This test was conducted in a rectangular chamber with two side boxes and one box in the center (three-chambered box). The social interaction test involves familiarization, sociability, and social preference stages. In the first stage, each animal moved freely in the empty chamber for 10 minutes to acclimatize to the test environment. In the second stage, a wire cage was installed in each side chamber for sociability. A rodent of the same age and sex without any prior interaction with the sample (novel rat 1) was randomly sited in one wire cage. In each chamber, the elapsed time of the test was recorded for 10 minutes, and a video tracking system analyzed the data. In the third stage, a new rodent with the same age and sex (novel rat 2) was placed in another wire cage, and the video tracking system recorded the action of the rat. The following formulae were used to calculate the social preference index (SPI) and sociability index (SI):

$$SI = (\text{Time exploring novel rat}_1 - \text{Time exploring empty cage}) / (\text{Time exploring novel rat}_1 + \text{Time exploring empty cage})$$

$$SPI = (\text{Time exploring novel rat}_2 - \text{Time exploring known rat}) / (\text{Time exploring novel rat}_2 + \text{Time exploring known rat})$$

The SI and SPI scores were between -1 and 1; the values closer to 1 indicate that the animal is more social, while the negative scores show that the animal has no sociability or social preference.[17]

Spatial learning and memory in the Morris water maze

The MWM was utilized to assess the spatial learning and memory of the rodents. It was a circular pool with a diameter of 140 cm and a depth of 45 cm, and there were different black and white objects with various geometric shapes as visual cues around the pool.

A hidden platform (15 cm wide, 35 cm high) was

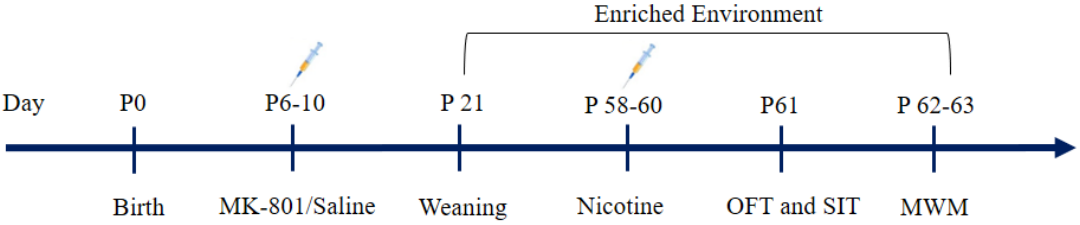

Figure 1. The study schedule. P: Postnatal day, OFT: Open-field test, SIT: Social interaction test, MWM: Morris water maze

installed 1.5 cm under the water level and the rats were trained for three days in the MWM to find the platform. Each block per day was composed of four successive trials with 60 seconds duration and about 60 seconds intervals. Different trials had specific semi-random entry points. Twenty-four hours after the trials, memory retention was tested by removing the platform as a probe trial. Then, the studied rat explored the pool. A camera above the maze recorded the swimming tracks of the rats in the trials and video-tracking software was also used to analyze the data (Borj Sanat Azma Co., Iran) [18,19].

Statistical analysis

Kruskal–Wallis test was performed to compare the data obtained from the social interaction and open-field tests. One-way analysis of variance (ANOVA) and Tukey's post hoc test were used to analyze the percentage of time and distance in the target quadrant during the MWM probe trials (parametric data). Moreover, two-way repeated-measures ANOVA was utilized to analyze the learning performance in the MWM (between-subjects factor: group and within-subjects factor: block). GraphPad Prism (version 6.0, GraphPad Software, Inc.) was also used to analyze the study data. The insignificant interaction term was removed from all analyses, and then the main effects p-values were calculated. The significance level was considered at $P < 0.05$.

Results

Effects of nicotine administration on the behavior of rats in the open-field box

Figure 2a shows the total distance moved (TDM). Rats that were raised in the EE moved less distance in the open field test (EE and MK-801 + EE compared with sham $P < 0.05$) when they received three days of nicotine, while MK-801 rats moved the same distance as sham rats (MK-801 compared to sham $P > 0.05$).

MK-801 rats in the EE cages had more rearing compared to EE rats ($P < 0.05$). Grooming was less in MK-801 in SCs and EE cages compared to the saline group ($P < 0.01$).

Effects of nicotine administration on the social behavior of the studied groups

The social behaviors of the animals exposed to MK-801 were the same as those of the sham (SI and SPI: $P > 0.05$ in the MK-801 and MK-801 + EE groups compared with the sham group). EE did not change the animals' behaviors in the EE and MK-801 + EE groups ($P > 0.05$; Figure 3).

Effects of nicotine administration on learning and memory

Figure 4 shows the MWM test results. After nicotine administration, the treated rats traveled shorter time and distance on the 2nd and 3rd days compared to the 1st day to find the hidden platform, revealing that spatial learning occurred in all the studied groups (repeated measures ANOVA; at least $P < 0.05$, Figure 4a, b). In the acquisition phase, no significant differences were detected among the groups in the traveled distance and elapsed time to find the platform ($p > 0.05$; Figure 4a, b).

As shown in Figures 4c and 4d, no significant differences were observed among different groups ($P > 0.05$) in the percentage of time and distance traveled in the target quadrant, indicating that the effects of

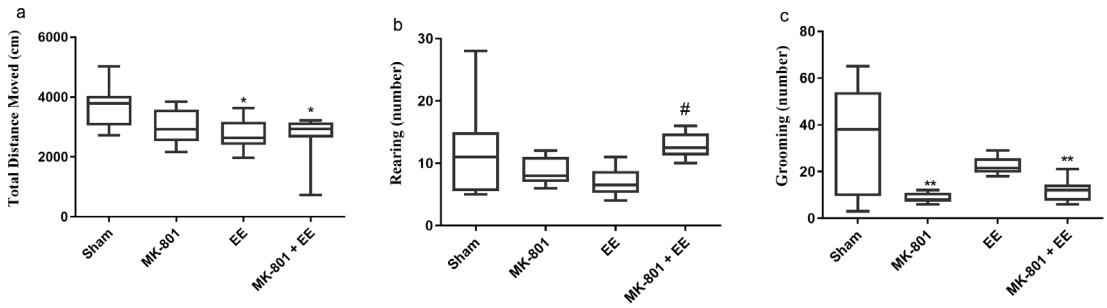

Figure 2. The behavior of animals after nicotine injection in the open-field test. The data are presented as min to max (n = 8). * $P < 0.05$, and ** $P < 0.01$, vs. sham # $P < 0.05$, vs. EE

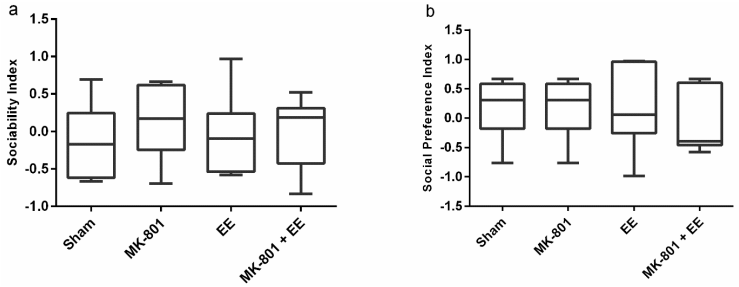

Figure 3. The behavior of animals after nicotine injection in the social interaction test. Data are presented as min to max (n = 8)

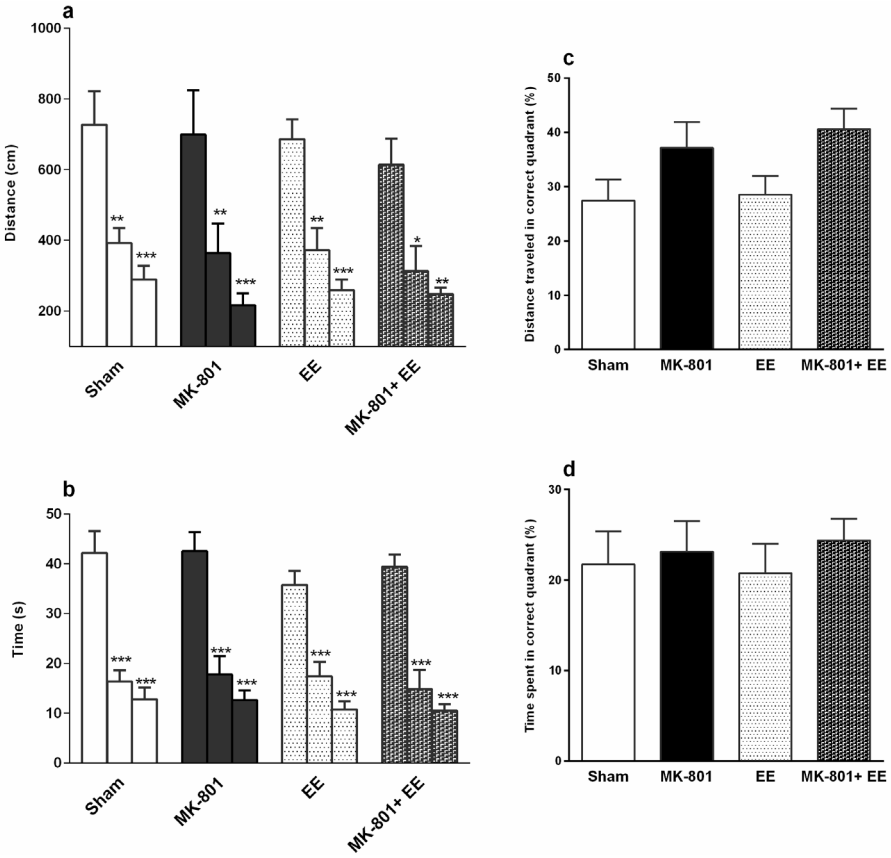

Figure 4. Effect of nicotine administration on the spatial memory and learning of the animals in MWM: (a) There was a significant decrease in the TDM on days 2 and 3 compared to the first day in all groups (each column represents a training day). However, there was no significant difference between different groups on each day. (b) The time spent on days 2 and 3 decreased significantly compared to the first day, while there was no significant difference between different groups on each day. Total traveled distance (c) and time (d) percentage in the correct quadrant. *p<0.05; **P<0.01; ***P<0.001 compared to first day within each study group

nicotine consumption were the same in MK-801 and saline animals in both environments.

Discussion

The results showed that the behaviors of MK-801 rats were the same as those of the sham group when they consumed nicotine. Even some stereotypic behaviors, such as grooming, were less in the MK-801 animals than in animals with saline treatment. Being raised in EE did not have any effect on the behavior of animals. Previous studies have confirmed that MK-801 administration can increase anxiety-like behaviors, locomotor activity, and stereotypic behaviors. The present study showed that nicotine decreased symptoms in the MK-801 psychosis rat model. Although the therapeutic effects of EE on the animal model of schizophrenia have been reported repeatedly, no positive effect of EE was observed when the animals were exposed to nicotine.[13,20,21]

Wang et al reported that chronic nicotine pretreatment (0.2 mg/kg for 2 weeks) diminished MK-801-induced memory impairments.[22] Shu et al showed acute nicotine injection alleviated memory deficits in lipopolysaccharide (LPS)-induced fear memory reconsolidation impairment.[23] Furthermore, Hambsch et al indicated that in a G72 mouse model of schizophrenia-like symptoms, chronic nicotine administration improved short-term memory impairments.[24] Enhanced social interaction in smokers has been previously reported, but less focus has been placed on this variable in patients with schizophrenia. Studies have shown that nicotine affects multiple targets, changing neurotransmitter systems such as dopamine and serotonin and inhibiting the monoamine oxidase A and B (MAO-A).[5,25]

Although previous studies have reported the beneficial roles of EE on MK-801-induced cognitive deficit, enhanced cognitive performance was observed after nicotine injection in MK-801-exposed rats, and EE did not have any positive effects when MK-801 animals received nicotine before behavioral tests. It seems that nicotine and EE do not reinforce each other and the capacity of EE to modulate rat brain circuits involved in schizophrenia-like behaviors decreases after nicotine exposure.

Conclusion

Despite the positive effects of nicotine, it is considered a dangerous health behavior due to the addictive nature of smoking. Thus, understanding why patients with schizophrenia smoke more than others is vital for developing addiction prevention and treatment strategies. The current study suggests that decreased

social interactions and stereotypic behaviors by nicotine may increase nicotine cravings.

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Authors' Contribution

Conceptualization: Masoumeh Nozari.
Data curation: Neda Salmani, Fatemeh Darvishzadeh Mahani.
Formal analysis: Neda Salmani, Fatemeh Darvishzadeh Mahani.
Funding acquisition: Masoumeh Nozari.
Investigation: Neda Salmani, Fatemeh Darvishzadeh Mahani.
Methodology: Neda Salmani, Fatemeh Darvishzadeh Mahani, Mahdieh Parvan.
Project administration: Masoumeh Nozari.
Supervision: Masoumeh Nozari.
Writing–original draft: Neda Salmani, Fatemeh Darvishzadeh Mahani.
Writing–review & editing: Neda Salmani, Fatemeh Darvishzadeh Mahani, Masoumeh Nozari.

Competing Interests

The authors declare that there is no conflict of interest.

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