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What is This?
Nicotine-induced impairments of spatial cognition and long-term potentiation in adolescent male rats

G Han¹,², L An¹, B Yang¹, L Si¹ and T Zhang¹

Abstract
The aim of the present study was to investigate whether cognitive behavioral impairment, induced by nicotine in offspring rats, was associated with the alteration of hippocampal short-term potentiation (STP) and long-term potentiation (LTP) and to discuss the potential underlying mechanism. Young adult offspring rats were randomly divided into three groups. The groups include: control group (CC), nicotine group 1 (NC), in which their mothers received nicotine from gestational day 3 (GD3) to GD18, and nicotine group 2 (CN), in which young adult offspring rats received nicotine from postnatal day 42 (PD42) to PD56. Morris water maze (MWM) test was performed and then field excitatory postsynaptic potentials elicited by the stimulation of perforant pathway were recorded in the hippocampal dentate gyrus region. The results of the MWM test showed that learning and memory were impaired by either prenatal or postnatal nicotine exposure. In addition, it was found that there was no statistical difference of the MWM data between both nicotine treatments. In the electrophysiological test, LTP and STP were significantly inhibited in both NC and CN groups in comparison with the CC group. Notably, STP in CN group was also lower than that in the NC group. These findings suggested that both prenatal and postnatal exposure to nicotine induced learning and memory deficits, while the potential mechanism might be different from each other due to their dissimilar impairments of synaptic plasticity.

Keywords
Nicotine, learning and memory, Morris water maze, long-term potentiation, short-term potentiation

Introduction
Nicotine is one kind of alkaloid that exists in solanaceous plants, which is also a psychoactive component of tobacco and appears to play an important role in tobacco dependence.¹,² Prenatal nicotine exposure caused reproductive teratogenicity and could affect the neural tissue development including differentiation, synaptogenesis, and synaptic function in the developing brain,³,⁴ therefore, contributing in a major way to the responsibilities associated with maternal smoking during pregnancy.⁵,⁶ Although cigarette smoking during pregnancy was associated with adverse fetal and developmental outcomes, 15–20% of all women smoke throughout the duration of pregnancy.⁷,⁸ Consequently, extensive studies related to malformation following prenatal and postnatal nicotine exposure were conducted.⁹,¹⁰

In pregnant women who smoke, nicotine crosses the placenta, concentrates in fetal blood and amniotic fluid, and is detectable in breast milk during lactation.¹¹,¹² When nicotine is absorbed into human body, it can be transmitted by blood and gets into brain by crossing the blood–brain barrier. For prenatal nicotine exposure, numerous long-term neurological effects have also been documented, such as attention-deficit hyperactivity disorder, learning disabilities, behavioral problems, and increased risk of nicotine addiction.¹³–¹⁵ Additionally, prenatal nicotine exposure disrupted the procedure of neurodevelopmental events and induced central nervous system (CNS) dysfunction, which include changing the normal processes

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on a cellular level, disturbing the programming that neurons replicate and differentiate into functional neuronal cells, and affecting initiation of synapses and localization of specific nerve cell populations. In addition, the adolescent brain was much more sensitive to nicotine than that of the adult brain, enhancing synaptic and behavioral impairments that contributed to dependence and addiction. The neurochemical and morphological results suggested that the abnormal effects of nicotine on the development of immature CNS could persist into young adulthood. Furthermore, due to its adverse effects on the development of CNS, including hippocampus, cerebellum, and sensory cortex, postnatal exposure to nicotine severely impaired cognition and higher sensory regions.

As mentioned above, these neurodevelopmental effects of prenatal and postnatal exposure have been consistently demonstrated in animal models, and they have been found as early as the newborn periods in humans as well. Although the literature on both prenatal and postnatal exposure was remarkably consistent in showing associations with increased rates of behavioral problems, a more pivotal issue about the relative effects of prenatal and postnatal exposures on CNS and their potential different mechanisms still remain an issue to investigate. Accordingly, a hypothesis has been raised that the different mechanisms of synaptic plasticity may be involved in the cognitive deficits induced by either prenatal or postnatal nicotine exposure. Therefore, two different animal models were established, and the performance of learning and memory was estimated by the Morris water maze (MWM) test during the adolescent period. In addition, to investigate the potential cellular mechanism and distinguish the possible different mechanisms between prenatal and postnatal nicotine exposure, field excitatory postsynaptic potentials (fEPSPs) from the perforant pathway (PP) to the hippocampal dentate gyrus (DG) region were recorded, while the long-term potentiation (LTP) and short-term potentiation (STP) were measured accordingly. The potential contribution of nicotine to hippocampal synaptic plasticity, associated with nicotine exposure during fetal and postnatal development, was carefully examined and the underlying mechanisms were cautiously discussed.

**Materials and methods**

**Animals and drug treatments**

The experiments were carried out according to the guidelines of the Beijing Laboratory Animal Center, China, and were approved by the Ethical Commission at Nankai University, China.

A total of 16 Wistar rats (8 males and 8 females) were obtained from the Academy of Military Medical Sciences of People’s Liberation Army. Food and water were freely available during all phases of the experiment. After allowing the animals to acclimatize to the environmental conditions for 2 days, eight females were randomly paired with eight males (8:8). Then the four pairs were assigned to the control (CON) group and the other four pairs to nicotine group (NIC). Female rats were subcutaneously injected with nicotine solution (concentration 0.1 mg/mL and 0.5 mg/kg bodyweight) at about 09:00 h from the gestation day 3 (GD3) to GD18 daily. The next day morning, when the vaginal suppository or sperm was found in the vaginal smear, the day was denoted as GD1. Meanwhile, the control group was received with an equivalent volume of saline solution (0.9% sodium chloride (NaCl)) for the same period of time daily. The day of birth was identified as postnatal day 0 (PD0). On PD21, offspring rats were weaned and the litter size was randomly culled to assure uniformity of litter size between nicotine and control groups. A total of 16 offspring male rats were randomly selected from CON group, and were divided into CC group and CN group. Simultaneously, eight offspring male rats were randomly chosen from NIC group and marked as NC group. On PD42, which was in the adolescent period of development, rats in CC (n = 8) and NC groups (n = 8) were subcutaneously injected with saline solution (0.9% NaCl), while CN group (n = 8) received nicotine (0.5 mg/kg) once a day for 14 days. The offspring rats were treated the same way as mothers in other aspects of life. All efforts were made to minimize the number of animals used and their suffering.

The median lethal dose of nicotine for rats is 50 mg/kg body weight. A high dose of nicotine could lead to symptoms of nausea, vomiting, or even death. Nicotine at 0.07–1.1 mg/kg body weight could induce toxicity; however, it did not cause serious damage. In addition, although the oral route is safe, the rate of absorption of nicotine is slow. The rate of rise of nicotine in the brain and resultant psychological effects are dissimilar to those of intravenous nicotine. Consequently, nicotine was administered subcutaneously at moderate dose of 0.5 mg/kg body weight in this study, which was in line with that in a previous investigation.
MWM experiment

On PD56, rats in all the three groups were trained and tested in MWM to measure the spatial cognition. MWM is an established test of spatial learning and reference memory.29 As described previously with modifications,30,31 the maze was placed in a room with soft lighting. All light fixtures in the room were covered with white sheets to reduce the brightness of the lights and eliminate their reflection in the water. All experiments were performed at 08:00 and 18:00 h. The water maze tank had a diameter of 150 cm and a height of 60 cm and was filled with water (23 ± 1°C) to the depth of 45 cm. The water was made opaque using black nontoxic ink. It was divided into four quadrants by two imaginary perpendicular lines crossing the center of the tank. The four quadrants were named as northeast (NE), southeast, southwest, and northwest, respectively. A movable black circular platform (10 cm in diameter) was located in the center of NE quadrant. The top of the escape platform was submerged 2–3 cm below the water surface. The swimming path was recorded using a camera mounted 2.0 m above the center of the pool and analysis was performed using a computerized video tracking system (Ethovision 2.0, Noldus, Wagenigen, The Netherlands). Training and testing in MWM comprised of two consecutive stages: acquisition phase (AP) and retention phase (RP). In AP stage, rats received eight trials (two trials per day) from four start positions with a 5-min intertrial interval during which animals rested in their home cage. They were put in the pool facing the sidewall and allowed 60s to locate the platform. If animals failed to locate the platform within 60s, they were guided by hand to the platform. All rats were allowed to sit on the platform for 10s before being removed from the pool. The order of starting points was taken randomly but the same for all animals. During this stage, the hidden platform was consistently located in the NE quadrant. Two parameters were recorded, which were escape latency (the time required to find the platform) and swimming speed. In RP stage, animals were given a single 1-min probe trial test at 18:00 h on the fifth day. The platform was taken out. The trial started from a starting point facing north. Numbers passing platform area (Platform crossings) was recorded.

Electrophysiological recordings

The day after the MWM experiment, electrophysiological tests were performed on the hippocampus of the brain. The animals were intraperitoneally anesthetized with urethane injection (1.2 g/kg). Then, they were placed in a stereotaxic frame (Narishige, Japan) for surgery and recording as described previously.32,33 Small holes were drilled in the skull to allow insertion of electrodes into the brain. The stereotaxic coordinates were derived from an atlas of the rat brain.34 A concentric bipolar stainless steel stimulating electrode with a tip separation of 0.5 mm was placed into the PP (coordinates: 8.0 mm posterior to bregma, 4.4 mm lateral to the midline, and 2.8–3.8 mm ventral from the cortical surface). A monopolar extracellular stainless steel recording electrode, 0.5 mm in diameter, was lowered into the DG region (coordinates: 4.2 mm posterior to bregma, 2.5 mm lateral to the midline, and 3.0–3.7 mm ventral from the cortical surface). After positioning the electrodes, the animals were left for a minimum period of 30 min for stability of baseline recordings before starting the experiment. Evoked field responses were recorded (Neurolog NL 104, NL 125, PowerLab/8 S; AD Instruments, Sydney, Australia). For induction of STP and LTP in the DG by stimulation of the PP, a probe stimulus was chosen at an intensity sufficient to produce a field response approximately 50% of spike threshold. Baseline responses (1 per 30 s) were recorded for 20 min followed by induced theta burst stimulation (TBS), consisting of one theta epochs which was consisted of 30 trains of 12 pulses (200 Hz) delivered at 5 Hz at the same stimulus intensity as the baseline pulse.35 In total, TBS lasted for 6s. Following TBS, single-pulse recording (0.05 Hz) resumed for 60 min. fEPSPs were amplified (100×), filtered at 5 Hz–5 kHz, digitized, and collected at 20 kHz sample frequency (Scope software, PowerLab, AD Instruments, Australia). A graphical presentation was produced by a running average of two fEPSPs. The postsynaptic effects were expressed as a percentage change in initial slope, relative to the mean initial slope in the baseline period. For example, the evoked fEPSP slope was measured as an average slope from 20 to 80% of the first positive deflection of the potential, which was from 10 to 11.5 ms after the stimulus pulse.36,37 The magnitudes of both STP and LTP were measured as the average of responses between 0 and 10 min and between 46 and 60 min after TBS, respectively.

Statistical analysis

All data were analyzed using version 16 of the Statistical Package for Social Sciences (SPSS 16.0; IBM,
Somers, New York, USA). For the MWM experiment, a two-way repeated measures analysis of variance (ANOVA) was used with “group” as the between subject factor and “day” as repeated measure. Bonferroni’s post hoc one-way ANOVAs were performed on the data from individual day. Electrophysiological recording data were analyzed offline with ES500 Scope and Chart Software (eDaq, Sydney, Australia). Again, a two-way repeated measures ANOVA was used with “group” as the between subject factor and “day” as repeated measure. Bonferroni’s post hoc one-way ANOVAs were performed on the data from LTP and STP results. Statistical significance was estimated using ANOVA with repeated measures. All the results were expressed as mean ± SEM. The significance level was set at 0.05.

**Results**

*MWM experiment results*

The learning curves for MWM are shown in Figure 1(a). Two-way repeated measures ANOVA indicated that there were the statistical difference in day ($F_{(3,354)} = 98.790, p < 0.001$) and group ($F_{(2,118)} = 9.094, p < 0.001$), but no significant difference in day × group interaction ($F_{(6,354)} = 0.532, p > 0.05$). All three groups showed a progressive improvement in the escape latency over time in a day-dependent manner. One-way ANOVA for individual day showed that
there were significant differences among groups on day 1 ($F_{(2,181)} = 4.092, p < 0.05$), day 2 ($F_{(2,157)} = 9.576, p < 0.001$), day 3 ($F_{(2,157)} = 10.900, p < 0.001$), and day 4 ($F_{(2,159)} = 8.043, p < 0.001$). During those 4 days of training, Bonferroni’s post hoc test for multiple comparisons showed that escape latency of either CN or NC group was significantly prolonged compared with that of CC group (CN vs. CC: days 1, 2, 3, and 4): $p < 0.01$; NC vs. CC: day 1: $p < 0.05$, (days 2, 3, and 4): $p < 0.01$). Meanwhile, Bonferroni’s post hoc test showed that there was no significant difference between CN and NC groups on each day during this stage ($p > 0.05$, Figure 1(a)).

Similar findings were obtained in the percentage of time spent in the NE quadrant (Figure 1(b)). Two-way repeated measures ANOVA showed that there were statistical differences of percentage escape latency in day ($F_{(3,471)} = 7.991, p < 0.001$) and group ($F_{(2,157)} = 22.413, p < 0.001$), but no significant difference in day × group interaction ($F_{(6,471)} = 1.440, p > 0.05$). One-way ANOVA for individual day showed that there were significant differences of percentage escape latency among groups on day 2 ($F_{(2,180)} = 12.303, p < 0.001$), day 3 ($F_{(2,176)} = 10.826, p < 0.001$), and day 4 ($F_{(2,177)} = 18.564, p < 0.001$) except day 1 ($F_{(2,179)} = 2.455, p > 0.05$). Bonferroni’s post hoc test for multiple comparisons showed that percentage of time spent in the NE quadrant was shorter in either CN group or NC group than that in CC group in the four days (CN vs. CC: (days 2, 3, and 4): $p < 0.01$; NC vs. CC: (days 2, 3, and 4): $p < 0.01$). Meanwhile, Bonferroni’s post hoc test for multiple comparisons showed that there is no significant difference of groups ($F_{(2,12)} = 0.895, p > 0.05$) or time × group interaction ($F_{(2,144)} = 2.149, p > 0.05$) among three groups. Bonferroni’s post hoc test for multiple comparisons showed that the average fEPSPs slope of the last 15 min recording, which was usually used as an LTP index in our previous studies, was significantly lower in either CN ($p < 0.01$) or NC groups ($p < 0.01$) compared with that in CC group (Figure 2(c)). However, there was no significant difference of the fEPSPs slope between CN and NC groups ($p > 0.05$). In addition, the fEPSPs slope of the first 10 min recorded after TBS, which was generally called STP, was also measured. It can be seen that the values of STP are significantly lower in both CN ($p < 0.01$) and NC groups ($p < 0.01$) compared with that in CC group. Interestingly, there was a statistical difference of STP between CN and NC groups ($p < 0.05$, Figure 2(d)).

**Discussion**

It is well known that prenatal nicotine acts as a neuro-teratogen that affects the crucial development phase of offspring’s CNS, especially hippocampus, which is closely related to learning and memory. Evidence from animal studies suggested that prenatal or postnatal nicotine exposure was associated with numerous deleterious neurological effects, such as
learning deficits, behavioral problems, and neurologic dysfunction.\textsuperscript{13–15,44} In the present study, the similar consequences of prenatal and postnatal nicotine exposure were identified on spatial cognitive impairments in the MWM tests; however, there was a different impact on hippocampal synaptic plasticity. The

\textbf{Figure 2.} Comparison of fEPSPs measurements under baseline and stimulation conditions among the three groups. LTP was induced using TBS after recording a stable baseline for 20 min. (a) Examples of the evoked LTP from the three groups. (b) Comparison of fEPSPs slopes among CC, CN, and NC groups. (c) Mean fEPSPs slopes obtained from the last 15 min recordings. (d) Mean fEPSPs slopes obtained from the first 10 min recordings. $n = 8$ for each group. Data are expressed as mean ± SEM. **$p < 0.01$: comparison between CC group and CN group; ###$p < 0.01$: comparison between CC group and NC group. *$p < 0.01$: comparison between NC group and CN group. fEPSPs: field excitatory postsynaptic potentials; LTP: long-term potentiation; TBS: theta burst stimulation.
effect of prenatal and postnatal nicotine treatments was evaluated, and the potential different mechanisms for synaptic plasticity were carefully discussed.

In the MWM test, it showed that escape latencies were decreased, while training days were increased in the first stage for acquiring information in all the three groups. The animals spent less time to find the platform, suggesting that substantial learning occurred in this period. Compared with the intergroup factors, it was found that rats in CC group spent least time to reach the target platform and expended the most time in the target quadrant during the trials among the three groups (Figure 1(a) and (b)). Meanwhile, the prolonged escape latencies of nicotine-treated rats in AP implied the unskillful and inefficient learning ability, which could contribute to the reduced number of platform crossings in the retention stage (Figure 1(d)). Multiple studies suggested that the action of nicotine was associated with the performance of spatial learning and memory in the MWM test, but results were not consistent across studies. A previous study reported that nicotine impaired performance of the water maze in a dose-independent manner.\(^{45}\) In addition, it was reported that nicotine improved acquisition in aged but not young rats.\(^{24}\) Nicotine treatment was able to reduce the learning ability and poor performance might be related to decreased neurogenesis\(^{46}\) and markers of synaptic plasticity\(^{47}\) in the hippocampus. It is possible that age, dosage, and treatment methods could contribute to the observed effects. In the present study, the spatial cognitions were damaged in either prenatal or postnatal nicotine-exposed rats, which were consistent with the previous study findings.\(^{48,49}\) However, except for spatial learning and reference memory deficits, prenatal nicotine exposure in rodents induced hyperactivity, increased anxiety, and somatosensory deficits.\(^{13,14,44}\) In addition, chronic postnatal nicotine exposure improved hippocampus-dependent working memory in radial arm maze\(^{50}\) but disrupted contextual learning in fear conditioning test.\(^{51}\) It is well known that the performance of different behavior tests provide dissimilar assessment of cognitive process and functions induced by nicotine prenatally. Considering the limitation of MWM test, reliability behavioral tools to predict other behavioral and cognitive alternations are needed to discriminate the potential difference between NC and CN groups in our further investigation.

Hippocampal LTP is a widely accepted model of synaptic plasticity that is thought to underlie learning and memory processes.\(^{52,53}\) Our results showed that nicotine induced impairment of LTP by seriously decreasing fEPSPs slopes in both CN and NC groups. Several previous studies reported that nicotine exposure early in fetal development adversely affected the synaptic development as well as suppress both presynaptic and postsynaptic elements required for neurotransmission.\(^{54,55}\) Recently, it was shown that nicotine caused reduction in cell genesis in the DG, so that it impaired learning in the water maze task test.\(^{46}\) The exquisite sensitivity of the developing CNS to nicotine exposure can lead to lasting neurobehavioral damage, including impairment of synaptic plasticity, which is related to underlying neural substrates damage and involved in the current poor behavioral performance.\(^{56}\) Moreover, in the MWM tasks, both prenatal and postnatal nicotine exposures were not limited to impairment of learning and memory, but might include deficits in attention, which was an ability to process spatial information and to develop efficient spatial search strategies.\(^{57,58}\) It is now widely accepted that processes such as sustained attention is regulated by central cholinergic systems.\(^{59}\) In addition, it has partly contributed to the effect of nicotine on nicotinic acetylcholine (Ach) receptor, which regulates both hippocampal glutamatergic and \(\gamma\)-aminobutyric acidergic neurotransmissions.\(^{60,61}\) It was further found that the abnormal cholinergic system induced by nicotine was probably altered in calcium-mediated cell signaling,\(^{62}\) which was importantly contributed to impair hippocampus-dependent synaptic plasticity and cognition. Therefore, nicotine exposure led to impairment of cholinergic system in the offspring, which may partly contribute to the MWM deficits observed.

Figure 2(b) showed that TBS led to a form of STP within 10 min, while it gradually degraded and nearly approached to the baseline in NC group. Also, TBS only induced a form of relatively weak STP in CN group. The different patterns of STP suggest that there are diverse underlying mechanisms of synaptic plasticity impairment in both NC and CN groups. Based on the requirement for macromolecular synthesis, LTP can be divided into at least two components, which are an early component (STP) and a late component (LTP).\(^{53}\) STP induced by brief stimuli has the associative properties vital for robust information storage.\(^{64}\) Alterations in STP could reflect a learning-specific shift in the processing state of hippocampal network and was essential for memory formation and the development of synaptic potentiation. As shown in Figure...
2(d), we obtained the higher value of STP in NC group compared with that in CN group. In addition, since the effect of prenatal nicotine exposure was to restrict fEPSPs maintenance and further impair long-term synaptic plasticity, it was different from sustaining lower TBS-induced fEPSPs in CN group. Therefore, it suggests that the STP impairment in CN group has an extremely deleterious effect on short-term memory compared with that in NC group, which could potentially serve as a crucial mechanism for memory formation, especially those forms dependent on the hippocampus.65,66 Previously, it was found that 10 pulses at 50 Hz produced an STP of the fEPSPs in hippocampal CA1, while this same stimulus induced an LTP of the response.67 One possible mechanism was that the STP tetanus stimulation evoked a sufficient degree of ACh release to suppress plasticity under controlled conditions, while nicotine possibly facilitated the threshold for LTP induced by alterations in calcium-mediated cell signaling.62 Strikingly, unlike acute nicotine exposure, the LTP of chronic nicotinetreated animals could be induced with normally subthreshold stimulation protocols.68 The activation of nicotinic receptor on interneurons inhibited nearby pyramidal cells and thereby prevented or diminished the induction of synaptic potentiation.69 Moreover, it was reported that nicotine enhanced synaptic transmission and converts STP to LTP in a cytoplasmic calcium-dependent pathway.70 To our knowledge, till date, the underlying STP mechanisms have been inadequately determined. The changes in synaptic plasticity may contribute to the complex neural adaptations and behaviors caused by nicotine. Therefore, it was hard to draw a distinct conclusion on why the effect of postnatal nicotine was more deleterious than that of prenatal for STP. Accordingly, further study in exploring potential mechanisms in STP between NC and CN groups is still needed.

In summary, this study investigated the effects of nicotine on rat spatial cognition and synaptic plasticity through both prenatal and postnatal nicotine exposures. Our findings suggest that (1) both prenatal and postnatal nicotine exposures impaired learning and memory abilities and inhibited LTP of offspring rats. (2) The different patterns of STP suggest that there are diverse underlying mechanisms of synaptic plasticity impairment between prenatal and postnatal nicotine exposure. However, these results only provide the preliminary data and future experiments need to be performed to explore the underlying cellular mechanisms.

Authors’ Note
The authors HG and AL contributed equally to this work.

Conflict of interest
The authors declared no conflicts of interest.

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References
9. Santiago SE and Huffman KJ. Postnatal effects of prenatal nicotine exposure on body weight, brain size and...
36. Quan MN, Tian YT, Xu KH, Zhang T and Yang Z. Post weaning social isolation influences spatial cognition, prefrontal cortical synaptic plasticity and hippocampal potassium ion channels in Wistar rats. *Neuroscience* 2010; 169: 214–222.


