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The effects of co-administration of opium and morphine with nicotine during pregnancy on spatial learning and memory of adult male offspring rats

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ABSTRACT

Objective(s): Smoking opium/cigarette is a global health concern. The aim of this study was to examine learning and memory of rat male offsprings whose mothers had been exposed to either opium or morphine with nicotine during pregnancy.

Materials and Methods: Wistar rats were used for the experiments. In the female rats, opium, morphine and nicotine dependencies were induced by daily injections of drug solution for 10 days before mating. Spatial memory was tested by Morris water maze test in male pups at the postnatal day 60. The duration that took until the rats found the platform in the maze and also their swimming speed were recorded.

Results: An increase in the platform finding duration was observed for the pups of dependent mothers in comparison with the control in the training trial (P<0.05). Prenatal exposure to opium/morphine and nicotine significantly decreased the time spent in the trigger zone to find the hidden platform (P<0.05) but had no significant effect on the swimming speed in the probe test. However, no significant difference was observed in the learning and memory behavior of offspring whose mothers received morphine, opium, nicotine or the co-administration of either morphine or opium with nicotine.

Conclusion: The present study showed that the opium, morphine and nicotine abuse and co-administration of opium/morphine with nicotine during pregnancy may cause deficits in spatial learning of male rat offspring. Based on our data, no synergistic effects of co-drug administration were observed on learning and memory in male rat offspring.

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Introduction

The results of many studies have confirmed that cigarette smoking is associated with increased risk of mortality due to causing cardiovascular diseases, cancer, and chronic obstructive pulmonary disease. The risk associated with cigarette smoking is cultural independent (1). Tobacco has been responsible for 24% of all male deaths and 7% of all female deaths in developed countries; however, no precise estimate can be made for the number of deaths attributable to smoking in developing and undeveloped countries. Cigarette smoking is responsible for at least 3 million deaths per year worldwide (2). Cigarette smoking increases dopamine release in ventral striatum which underlies the reinforcing properties of nicotine (3). Nicotine influences the brain regions involved in arousal and reward that may be linked to motivationally significant aspects of tobacco dependence (4). This is a source of addiction to tobacco and alters mood and performance (5). Maternal smoking during pregnancy increases the risk of having underweight newborns, prenatal morbidity, mortality and sudden infant death syndrome and having babies with impaired learning and behavior (6-8). Although nicotinic acetylcholine receptors are in the fetal brain, nicotine involved multiple transmitter pathways including cholinergic and catecholaminergic systems. It may be implicated

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in the developmental events of fetal brain and the eventual programming of synaptic competence (9-11). Prenatal nicotine exposure has been associated with neurochemical and behavioral consequences. Comparable alterations occur in peripheral autonomic pathways, leading to increased susceptibility to hypoxia-induced brain damage, prenatal mortality and sudden infant death (12).

Opium abuse and addiction is a serious health problem in many eastern countries, including Iran. Opium abuse can result in a set of barriers to development including increasing the rate of crime and community violence, low productivity and political instability (13). Addiction in pregnancy has become an important health problem owing to the tendency of drug-dependent women to neglect general health care and seeking prenatal care (14). Long-term behavioral and developmental consequences of prenatal opium consumption have been reported by other investigators. Joffe *et al*, (1990) reported that parental methadone injection prior to mating can produce physiological and behavioral changes in rat progeny which are not dependent on detectable effects on early viability or growth (15). Sarkaki *etal* (2008) suggest that the addiction of both parents to morphine may cause memory deficiency through reduction of long-term potentiation (LTP) in hippocampus (16). Prenatal opioid exposure regulates dendritic growth and spine formation in the development of the rat's brain (17). It also impairs the performance of adult male and female rats on tasks requiring learning and spatial memory (18) such as radial arm maze (19, 20) and causes selective regional effects on mu-receptor ontogeny in the rat's brain (21). Previously, we showed that opium dependency of both parents can weaken the learning and memory process in their offspring (22).

Opium addiction is usually accompanied by cigarette smoking. The interaction between nicotine and opioids has been reported by some investigators (23-25). Mello *et al* reported that opioid agonist administration is associated with increased cigarette smoking (26). Opioid receptors play an important role in behavioral sensitization to nicotine (27). Despite the fact that prenatal exposure to abused drugs is common in most parts of the world, in the clinical population, it is difficult to determine the independent effects of gestational exposure to a single drug on brain development; this is in part due to the confounding effects of additional risk factors that are encountered in the substance-abusing population (28). Most of the previous studies have focused on mothers' exposure to pure opioids (such as morphine, heroin and codeine) and cigarette smoking during pregnancy and the ability of these substances to alter many biological functions in offspring, including learning and memory process (4, 10, 16, 22, 29). However, there is no documented data on the effects of prenatal opium exposure on learning and memory process. On the other hands, there is no report regarding the effect of co-administration of opium and morphine with nicotine during pregnancy on the learning and memory process in offspring. The present study investigated to what extent adult male offspring, whose mothers had been exposed to opium or morphine with nicotine during pregnancy, showed learning and memory deficits. To the researchers' knowledge no study has been carried out so far on this issue.

Materials and Methods

Animals

Wistar rats (250–300g) were kept at a controlled temperature (23±1˚C) and 12/12 hr light/dark cycle. Food and water were available continuously. Experiments were performed at the same time on light cycle for all groups.

All of the procedures were in concordance with the guidelines for caring and using laboratory animals. The protocol was also approved by the research committee of Kerman Neurosciences Research Center and the Neuroscience Ethic Committee, Kerman, Iran (EC/KNRC/88-27).

Induction of dependency

Opium dependency was induced by daily injections of aqueous opium solution (10 mg/kg, SC, two times per day) for 10 days before mating. Morphine dependency was induced by daily injection of morphine (10 mg/kg, SC, two times per day) for 10 days before mating (22, 30-32). Opioid dependency of parents was confirmed following naloxone injection (2 mg/kg, IP) to 6 rats (one rat from each group). All tested animals showed opioid withdrawal signs such as wet dog shake, diarrhea, jumping and weight loss as the standard opioid withdrawal signs (16). The tested animals showed opioid withdrawal signs such as wet dog shake diarrhea, jumping and weight loss as the standard opioid withdrawal signs. However, all the naloxone treated rats were eliminated from the research group. Nicotine (1mg/kg) was injected subcutaneously with the same protocol as morphine (12).

In each breeding, two males were housed with five females. The presence of avaginal plug designated as gestation day (E0). At least 30 sperm positive females were removed from the breeding cages and housed individually in nesting boxes with *ad libitum* food and water. Sperm positive dams were randomly assigned to 7 groups as follow:

1. Nicotine group: offspring of female rats received nicotine (n=7).
2. Opium group: offspring of opium exposed female rats (n=7).
3. Morphine group: offspring of morphine exposed female rats (n=7).
4. Morphine+nicotine group: offspring of morphine+ nicotine exposed female rats (n=7).

5. Opium+ nicotine group: offspring of opium+ nicotine exposed female rats (n=7).
6. Saline group: offspring of female rats received saline (n=7).
7. Control group: offspring of untreated female rats (n=7).

Treatment with opium, morphine and nicotine was continued through breeding and gestation until parturition. Thereafter, opium and morphine doses were gradually decreased (1 mg every other day) to prevent the signs of opioid withdrawal. However, none of mothers/offspring showed any opioid withdrawal signs following opioid dose reduction. Following parturition, the rats were reared by their biological mothers, weaned at postnatal day 25. In this experiment, the focus was on the male offspring. Since in female rats, due to sex hormones and the stage of estrous, spatial learning is affected (33-35). Therefore, we limited our experiments to male rat, although the next experiments can perform on female rats in the next steps. The exposed offspring remained undisturbed until testing commenced and only one adult male rat was collected from each mother in order to investigate its behavior or to undergo behavioral testing.

Apparatus

Spatial memory was tested by Morris' water maze (MWM) apparatus. The MWM was a black circular pool with a diameter of 150 cm and a height of 60 cm, filled with 22±1˚C water to a depth of 25 cm. The maze was divided geographically into four equal quadrants and held release points that were designed at each quadrant as N, E, S, and W. A hidden circular platform (10 cm in diameter), made of Plexiglass, was located in the center of the southwest quadrant, and submerged 1.5 cm beneath the surface of the water. Fixed, extra maze visual cues were present at various locations around the maze (i.e., computer, MWM hardwares, and posters). A camera was mounted above the center of the maze and animal motion was recorded and sent to the computer. A tracking system by a commercial software (Noldus, Netherlands; version:6 XT) was used to measure the escape latency and swimming speed (12).

Behavioral procedure

1- Habituation: Before orientation training, animals were habituated to the swimming pool environment. They were placed individually on the platform for 20 sec and in the pool for 180 sec and were allowed to swim.

2- Training trial: In orientation training, rats were trained to escape from drowning by climbing onto the hidden platform; they were allowed to swim for maximum trial duration of 90 sec, and if they failed to find it themselves, they were placed on the platform for 10 sec (reinforcement). The rats were given four trials per day for 4 consecutive days. In each trail, the rats were gently placed into the MWM at the middle of the circular edge in a randomly selected quadrant, and with their nose pointing toward the wall. Each training session comprised four trials, with an intertrial interval of 60 min, and was performed routinely between 10:00 AM and 17:00 PM. The escape latency (i.e. the time required for the rat to find and climb onto the platform) was recorded for each trial. The average of the four trials per training day was also recorded.

In case a rat could not find the platform during the testing time (that is 90 sec), or remained on the platform for less than 3 sec, the experimenter would assist the rat and so the rat was granted the opportunity to remain there for some time (10 sec). If a rat failed to find the platform within the testing time (90 sec) or if it stayed on the platform for less than 3 sec, it was assisted by the experimenter and allowed to stay there for the same period of time (10 sec).

3- In the fifth day (the probe test), the platform was removed from the pool and each rat was placed into the pool from the opposite quadrant. This day consisted of 60 sec free swim period without a platform. The time spent in the trigger zone to find the hidden platform and the swimming speed of the rats was also recorded.

Statistical analysis

Prism software was used for data analysis. Repeated measures ANOVA was used to analyze the quantitative differences in swimming escape latency in Morris water maze to find the platform. The time spent in the trigger zone to find the hidden platform and swimming speed was analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni *Post hoc* tests. Data were expressed as mean± SEM of 7 rats per group. *P*-value<0.05 was considered statistically significant.

Results

Data showed that there was no difference in the acquisition parameter between control and saline treated rats, so the results of drug exposed offspring (opium, morphine, nicotine and combination treatment) were compared with control (Figure 1).

The mean escape latency of the 4 days of training in MWM in prenatal morphine/opium exposed male rat offspring

The results of this study showed that the escape latency to find hidden platform in MWM was progressively and significantly reduced during the 4 trial days in all control and test groups ($P<0.0001$, $F_{3,373}=42.9$). Data analysis showed that the mean escape latency to find hidden platform in MWM was significantly increased in opium and/ morphine exposed offspring as compared to the control ($P<0.0001$, $F_{2,373}=11.8$). Bonferroni *Post hoc* test showed a significant increase in the escape latency to

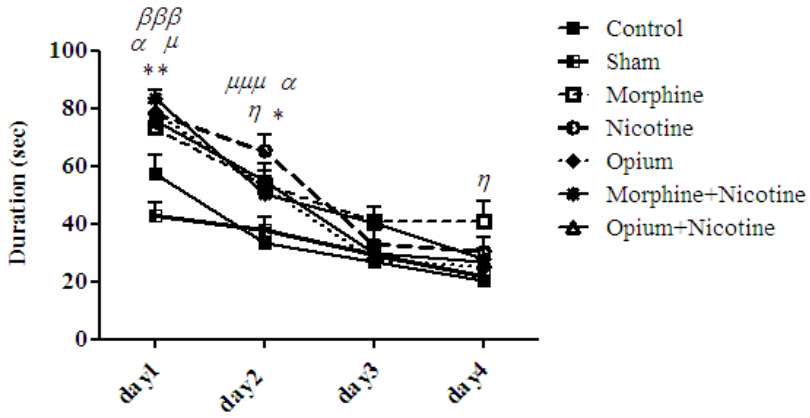

Figure 1. The effect of prenatal administration of opium and morphine, nicotine and co-administration of opium+nicotine and morphine+nicotine on average duration of the 4 days (16 trials) of training to find hidden platform in the Morris water maze of male offspring. Data are expressed as *mean± SEM*. *P*- values <0.05 was considered statistically significant (n=7).
*=*P*<0.05, **=*P*<0.01 Opium group *vs.* Control group
η=*P*<0.05 Morphine group *vs.* Control group
μ=*P*<0.05, μμμ=*P*<0.001 Nicotine group *vs.* Control group
α=*P*<0.05 (Opium+nicotine) group *vs.* Control group
βββ =*P*<0.001 (Morphine+nicotine) group *vs.* Control group

find hidden platform in MWM in the mother opium exposed offspring on the first day ($P<0.01$), 2nd day ($P<0.05$), and also for morphine exposed offspring on the 2nd and 4th day ($P<0.05$), as compared to the control. Data showed no significant correlation between the training trial days and spatial memory ($P=0.12$, $F_{6,373}=1.7$; Figure 1). There was no significant difference in the escape latency to find hidden platform in MWM between the opium exposed offspring as compared to the morphine exposed offspring.

The effect of prenatal administration of nicotine on average escape latency of the 4 days of training to find hidden platform in MWM of male rat offspring

Mean escape latency to find the hidden platform in MWM showed a progressive significant decrease during the 4 days of training in nicotine treated group of male offspring rats ($P<0.0001$, $F_{3, 223}=27.3$). Also mean swimming time to find the hidden platform in MWM test in nicotine treated rats was significantly increased as compared to the control ($P<0.0001$, $F_{1,223}=21$).

Bonferroni *Post hoc* test showed a significant increase in the escape latency to find the hidden platform in MWM in the mothers of nicotine exposed offspring on the first day ($P<0.05$), and also on the 2nd day ($P<0.001$) as compared to the control group. Data showed no significant correlation between the training trial days and spatial memory ($P=0.07$, $F_{3,223}=2.29$; Figure 1).

The effect of co-administration of opium and/morphine with nicotine on the mean escape latency of the 4 days of training in MWM

According to the results of this study, mean escape latency to find hidden platform in MWM test of male offspring whose mother received nicotine + morphine and/ nicotine+opium significantly increased as compared to the control group ($P<0.0001$, $F_{2,340}=11.3$). Bonferroni *Post hoc* test showed a significant increase in the escape latency to find hidden platform in MWM in the nicotine+ opium exposed offspring on the first and 2nd day ($P<0.05$), and also in nicotine +morphine exposed offspring on the first day ($P<0.001$), as compared to the control. Data showed no significant correlation between the training trial days and spatial memory ($P=0.32$, $F_{6,340}=1.16$; Figure 1). There was no significant difference in the escape latency to find hidden platform in MWM between the opium+ nicotine exposed offspring as compared to morphine+ nicotine exposed offspring.

The effect of prenatal exposure to opium, morphine and nicotine on the total average swimming duration to find hidden platform in MWM

Data showed that means of total time spent on the 4 trial days to find hidden platform in MWM in opium ($P<0.05$), morphine ($P<0.0001$) nicotine ($P<0.05$), morphine + nicotine ($P<0.01$) and opium + nicotine ($P<0.01$) were significantly increased as compared to the control group. There was no significant difference in the total time spent on the 4 trial days to find hidden platform in MWM between the opium+ nicotine exposed offspring and morphine+ nicotine exposed offspring (Figure 2).

The effect of prenatal exposure to opium, morphine, and nicotine on the time spent in trigger zone and the swimming speed of male offspring

A significant decrease of the time spent in trigger zone was observed in the opium, morphine, nicotine

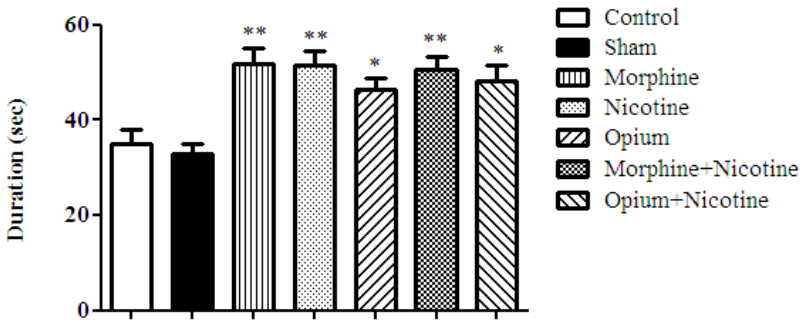

Figure 2. The effect of prenatal administration of opium, nicotine, opium+nicotine and morphine+nicotine on total average duration to find the hidden platform during the four days of training in the Morris water maze of male offspring. Data are expressed as mean± SEM. *P*-values <0.05 was considered statistically significant (n=7).
*=*P*<0.05, **=*P*<0.01 prenatal drug exposure group *vs.* Control group

and combination treatment groups compared to control (*P*<0.05; Figure 3A) in the fifth testing day. No significant differences were recorded in the swimming speed of offspring whose mothers received drug during their pregnancy (opium, morphine, nicotine, morphine + nicotine and opium + nicotine) in comparison to the control (Figure 3B).

Discussion

The results of this study indicate that prenatal exposure to opium, morphine; nicotine and co-administration of either morphine + nicotine or opium + nicotine alter spatial learning and memory in MWM test in male rat offspring. The spatial learning deficit was characterized by an increased escape latency to find the hidden platform in the prenatal opium, morphine/nicotine-exposed rats. Data showed that mean escape latency to find hidden platform in MWM was significantly longer in male rat offspring whose mothers were exposed to opium, morphine, nicotine or the co-administration of nicotine with either morphine or opium, as compared to the control. Based on our data, morphine/opium can reduce nicotine-induced spatial learning and memory damage on the second day. An analysis on nicotine; nicotine/morphine and nicotine/opium groups didn't show any significant difference at this day. In addition, prenatal exposure to opium, morphine/nicotine significantly decreased the time spent in trigger zone of pups in the fifth day, but no significant effect on the swimming speed of offspring.

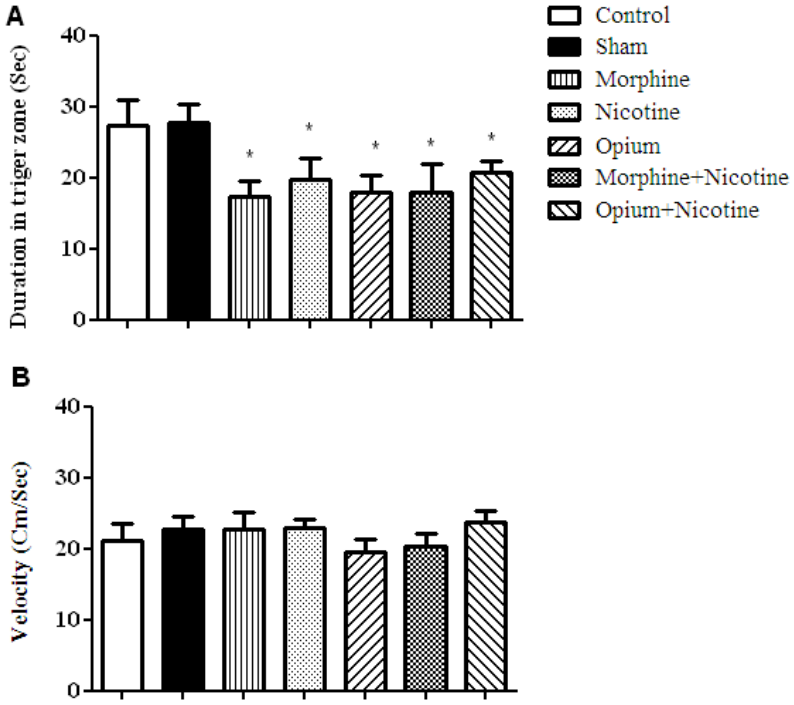

Figure 3. The effect of prenatal administration of opium, nicotine, opium+nicotine and morphine+nicotine on the time spent in the trigger zone (A) and the swimming velocity (B) in the fifth day of training in the Morris water maze of male offspring. Data are expressed as mean± SEM (n=7).
*=*P*<0.05, prenatal drug exposure group *vs.* Control group

Because morphine and nicotine can easily move through blood barriers and placenta, they could have direct effects in the fetal brain (36, 37). Many studies have focused on the exposure of mothers to morphine, heroin and codeine or nicotine/cigarette smoking during their pregnancy and the probable effects that these drugs have on prenatal exposed offspring (6, 8, 38-41); hence, this is an innovative study which demonstrates that prenatal exposure of mothers to opium, morphine/nicotine and the combination of opium + nicotine/morphine + nicotine causes deficits in spatial learning and memory in MWM test of male rat offspring. Our results are in concordance with the previous studies on the adverse effects of prenatal opioid or nicotine exposure on learning and memory of offspring (8, 22, 38, 40-42). Morphine exposure during pregnancy has been associated with an increase in congenital malformations, spontaneous abortions, fetal resorptions, childhood cancers, developmental, neurobehavioral, neuroendocrine, neurochemical abnormalities, and also low birth weight (6, 15, 20, 28, 43-45). Morphine alters the function of brain regions underlying drug-induced reward and reinforcement, and neural tube development in Wistar rats (28, 46, 47). Niu *et al* (2009) reported that prenatal morphine exposure caused impairment of synaptic plasticity in dentate gyrus and spatial memory of juvenile rats (48). Sarkaki *et al* (2008) suggest that both parental addiction to morphine may cause memory deficiency through the reduction of LTP in hippocampus (16). Ghafari *et al* (2014) reported that chronic morphine exposure caused pyramidal neurons of hippocampus loss in 18 and 32 days old infant mice (49). Add to that, cigarette smoking during pregnancy or prenatal nicotine exposure can result in increased fetal mortality, sudden infant death syndrome, and behavioral and attention disorders during childhood (6, 7, 12). Park *et al* (2000) reported that nicotine damaged spatial working memory (50). In addition, nicotine interaction with opioids has been reported by many investigators (51). There is a cross-tolerance between morphine- and nicotine-induced conditioned place preference in mice (24). It is additionally reported that central cholinergic and opioid receptor mechanisms might be involved in nicotine-induced antinociception (52). Prenatal nicotine exposure of rats can cause persistent changes of nicotinic cholinergic receptors (10). The present study confirms the results of both animal and human previous studies showing adverse effects of both opioids and nicotine on reproductive outcomes (12, 14, 19, 20, 46, 53-55). However there was no significant difference in learning and memory behavior of male offspring whose mother (s) had received morphine, opium, nicotine or the co-administration of either morphine or opium with nicotine. These data showed no synergistic effect of co-drug administration on learning and memory in male rat offspring. The underlying mechanism (s) is still unknown and needs further investigation.

Conclusion

The collected data showed that prenatal exposure to opium, morphine, nicotine and co-administration of either morphine + nicotine or opium + nicotine caused deficit in spatial learning and memory in MWM test of male rat offspring in comparison with the control; while, it had no significant effect on the swimming speed of the offspring. The mechanisms in the etiology of parentally-mediated adverse outcomes of opioid and nicotine exposure are still unknown. However, exploring the opium/morphine dose correlation in rats with human drug abuse dosage is of great clinical importance. Since there is no documented data on opium dose correlation with human drag abuse dosage, this pilot study regarding the effect of opium/morphine prenatal exposure on learning and memory process and further investigations are required to elucidate the effects of different doses of opium/morphine prenatal exposure on learning and memory process in offspring rats.

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