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Original investigation

Effect of Prenatal Exposure to Waterpipe Tobacco Smoke on Learning and Memory of Adult Offspring Rats

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Abstract

Introduction: Waterpipe tobacco smoking has increased in prevalence worldwide, including among pregnant women. In this study, we investigated the effect of prenatal maternal waterpipe tobacco smoke (WTS) exposure during different stages of pregnancy on learning and memory of adult offspring rats.

Methods: Pregnant rats received either fresh air or mainstream WTS (2 hours daily) during early, mid, late, or whole gestational period. Male offspring rats were followed through 20 weeks. Outcomes included (1) spatial learning and memory using the radial arm water maze (RAWM), (2) levels of brain derived neurotrophic factor (BDNF) in the hippocampus, and (3) oxidative stress biomarkers (superoxide dismutase, catalase, glutathione peroxidase and thiobarbituric acid reactive substances).

Results: Relative to offspring whose mothers were exposed to fresh air, prenatal exposure to WTS at any stage of pregnancy resulted in short- and long-term memory impairment in adult offspring rats ($p < .05$). This impairment was associated with reduced levels of BDNF in hippocampus ($p < .05$). However, prenatal WTS did not affect the level of oxidative stress biomarkers in hippocampus. Prenatal WTS during late gestation increased the activity of catalase as compared to control.

Conclusion: Prenatal maternal WTS exposure can impair the memory of adult male offspring. These results support development of interventions that target pregnant women who smoke waterpipe during pregnancy.

Implications: We examined for the first time the effect of prenatal waterpipe tobacco smoke exposure on learning and memory of offspring. The results showed that in utero exposure to waterpipe tobacco smoke was associated with impaired memory and decreased brain derived neurotrophic factor in hippocampus of adult male offspring rats.

Introduction

The tobacco use epidemic includes around one billion users worldwide and is responsible for about five million deaths each year.[1] The prevalence of tobacco smoking during pregnancy has been increasing globally.[2] The harmful consequences of prenatal tobacco smoke on offspring begin early and include low birth weight[3] and increased infant mortality.[4] They are also apparent later in life, including increased risk of developing hypertension,[5] asthma[6] and cognitive and behavioral disturbances[7] that are a result of compromised maturation of brain neurons.[8] All of these detrimental effects observed in the offspring of pregnant smokers likely are due to the many toxicants delivered by tobacco products, including carbon monoxide and nicotine.[9]

Smoking tobacco using a waterpipe, also known as hookah, shisha, goza, narghile, arghileh, and hubble-bubble, is one form of tobacco consumption that is increasing in prevalence worldwide[10] and it is now recognized as a public health problem.[10] With increased prevalence comes greater acceptability, particularly in some populations. For example, evidence suggests that, relative to cigarette smoking, waterpipe tobacco smoking is gaining acceptability among girls and women, especially in the Arab world.[11] In some Arab countries, around 4–9% of pregnant women smoke waterpipe.[10,12]

Waterpipe tobacco smoke (WTS) contains many toxicants such as carbon monoxide, nicotine, tar, volatile aldehydes, heavy metals, and a variety of carcinogens such as polycyclic aromatic hydrocarbons and nitrosamines.[13] Waterpipe tobacco smoking during pregnancy has been shown to be associated with low birth weight, newborn length, and head circumference.[14] In rodents, prenatal exposure to WTS produces low birth weight, low growth rate, and lower survival rates of offspring.[15]

The central nervous system develops rapidly *in utero* and hence is highly susceptible to environmental insults.[16] In animal models, prenatal exposure to cigarette smoke reduces the cortical thickness and neuronal density as compared to unexposed groups.[17] Human studies suggest that prenatal cigarette smoke exposure results in reduced head circumference and brain volume in neonates.[18,19] However, the effect of prenatal WTS on learning and memory is still unclear. Herein, we examined, for the first time, the effect of prenatal WTS exposure during different stages of pregnancy on learning and memory of adult offspring rats and also investigated the involvement of oxidative stress and brain derived neurotrophic factor.

Methods

Animals

Adult male and female Wistar rats, 200–250 g, were purchased from the Animal Care Unit at Jordan University of Science and Technology (JUST; Irbid, Jordan). All experimental procedures were in accordance with JUST's Animal Care and Use Committee (ACUC). Rats were kept at 12 hours light and 12 hours dark cycle at 25°C and free access to food and water. Female and male pairs were kept in the same cage overnight for mating and were checked the next morning for the appearance of a vaginal plug, a marker of pregnancy.[20] The day that pregnancy was confirmed, was considered pregnancy day 0. Female rats were assigned randomly to receive fresh air (control) or WTS during early gestation (pregnancy days 0–7), mid gestation (pregnancy days 7–14), late gestation (pregnancy days 14–21) or whole gestation (pregnancy days 0–21).

Waterpipe Tobacco Smoke Exposure

Female rats (n = 10) in experimental groups were exposed to WTS for 2 hours/day with one hour rest between the exposure sessions during the specified pregnancy days. Animals were exposed to waterpipe smoke in the first quarter of light onset. The exposure period/protocol were based on a previous study.[15] This exposure protocol has been shown to be associated with adverse effects on pregnancy outcomes including growth retardation.[15] The exposure to WTS was through whole-body exposure apparatus as described previously.[21] Briefly, the apparatus is composed of waterpipe machine and an exposure chamber. The waterpipe machine uses a diaphragm pump to draw the smoke and discharge it into the exposure chamber. The smoking apparatus is performed in accordance with Beirut Method that is designed to provide 171 puffs of 2.6 seconds duration and 17 seconds between the puffs. The puffs were monitored by a puff topography instrument to provide the mean puff volume of 530 ml, similar to human puff topography that was measured during actual waterpipe smoking sessions.[22] Nakhla Double Apple tobacco, one of the most popular and widely smoked Moassel, was used. The company did not give the exact ingredient of Moassel. However, it usually contains tobacco, molasses, glycerin and natural flavors. During the whole exposure period, the level of carbon monoxide (CO) in the exposure chamber was adjusted to maintain the level of CO exposure at similar levels for all pregnant rats (950 ± 134 ppm, mean ± SD).

At the end of the exposure session, WTS was stopped and pregnant female rats were kept at clean fresh air until the next session. After completing the assigned period of WTS-exposure, the pregnant rats were kept at clean fresh air until delivery.

Behavioral Test

Radial arm water maze (RAWM) was used to test spatial learning and memory of the adult male offspring rats as described previously.[23] RAWM was carried out on 10 male adult offspring rats (20 weeks old, one pup/litter) from each group (WTS exposed groups and control). Male offspring rats were used due to male advantage for different spatial learning and memory tasks.[24] Briefly, The RAWM is a black, circular, water-filled tub (water temperature: 24 ± 1 °C; dimensions: 167 cm diameter, height 55 cm, 43 cm deep) with six V-shaped stainless steel plates (49 cm height, 55 cm length) arranged to form a swimming field of an open central area and six arms (arm width 35 cm). This test was composed of a learning phase that consisted of twelve consecutive trials; the first six trials followed by 5 minutes rest then another six trials. Short-term memory testing was done 30 minutes, and long-term memory 5 and 24 hours after the end of the last trial of the learning phase. The above described sequence was done only one time in one day. In each trial, the animal was started in a different starting arm, except the goal arm, which was fixed for a particular day for a particular rat. To avoid odor trail, no consecutive animals were trained on the same goal arm. In each trial, the rat was allowed 1 minute to swim freely in the maze to find the hidden platform. Once the rat is on the platform, the rat was allowed 15 seconds to observe visual cues before the next trial. The cues were available for the rats in fixed positions on the walls throughout the days of the experiments. When a rat was unable to find the platform within the 1 minute period allowed, it was guided toward the platform and left there for a 15 second. During the 1 minute period, each time the rat entered an arm other than the goal arm, an error was counted. Entry occurred when the whole body of the rat (not including the tail) was inside the arm. Although rats

naturally swim, swimming is considered as aversive stimulus for rats that might help in reducing learning time.[25] Thus, all rats had the motive to swim in an effort to find the hidden platform.

Hippocampus Dissection

Animals were sacrificed by decapitation 48 hours after testing of long-term memory. Dissection of hippocampus was performed in accordance with previous report.[23] Briefly, hippocampus was isolated from the brain and was immersed in liquid nitrogen then stored at −80°C till tissue homogenization.

Measuring Oxidative Stress Markers and Brain Derived Neurotrophic Factor

Hippocampus tissues were homogenized using a homogenizer (Tissue Master-125, Omni International, Kennesaw, GA, USA) with lysis buffer and protease inhibitor cocktail (Sigma–Aldrich Corp., MI, USA) as described previously.[23] Total protein concentration was measured using a commercially available kit (BioRAD, Hercules, CA, USA). The activity of anti-oxidative enzymes superoxide dismutase (SOD) (Sigma–Aldrich Corp., MI, USA), catalase (Cayman Chemical, MI, USA) and glutathione peroxidase (GPx) (Sigma–Aldrich Corp., MI, USA) were measured in hippocampus homogenates following manufacturer instructions. Levels of brain derived neurotrophic factor (Sigma–Aldrich Corp., MI, USA) and thiobarbituric acid reactive substances (TBARS) (Cayman Chemical, MI, USA) were measured in hippocampus homogenate. ELISA plates were read at the specified wave lengths determined by the kits using Epoch Biotek microplate reader (BioTek, Winooski, VT, USA). The enzyme activities, TBARS and BDNF levels were normalized to total protein in each sample.

Statistical Analysis

All statistics were carried out using the GraphPad Prism (4.0) computer program. Comparisons of the number of errors during the RAWM were made using two-way ANOVA; followed by Bonferroni posttest. The two factors were time (repeated measures factor, 3 levels corresponding to testing occurring 0.5, 5, and 24 hours after the learning phase) and treatment (between subjects' factor, 5 levels corresponding to control group and WTS exposure at early-, mid-, late-, or whole-gestation period) were the independent variables. Comparisons of number of errors during short- and long-term memory, oxidative stress biomarkers and BDNF levels were made using one-way ANOVA with between-group 5 levels. $p < .05$ was considered significant. All values were represented as mean ± standard error means (SEM).

Results

The Effects of Prenatal WTS on Learning and Memory

All rat groups, either control or WTS, learned the location of the hidden platform (Figure 1) and there was no significant difference between the groups ($p > .05$) in all learning trials (trials 1–12).

In short-term memory test, that was performed 30 minutes after the last learning trial, and long-term memory, that was performed after 5 and 24 hours of the last learning trial, all offspring rats who were exposed to WTS prenatally made significantly more errors as compared to control offspring rats ($p < .05$) (Table 1). However,

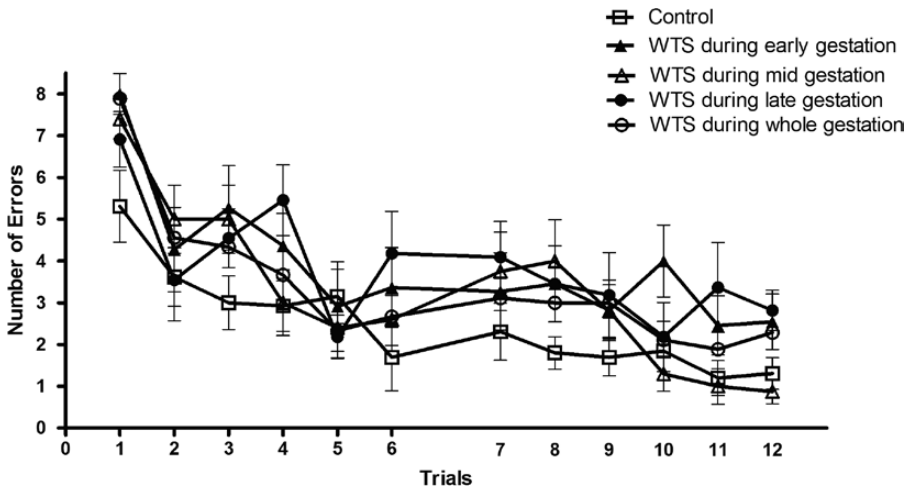

Figure 1. Performance of adult offspring rats during learning phase of radial arm water maze Offspring rats that were exposed prenatally to fresh air (control) or WTS for 2 hours per day during early gestation (0–7 days), mid gestation (7–14 days), late gestation (14–21 days) or whole gestation (0–21 days) ($n = 10$). Each offspring rat was trained for 12 trials, each 6 was separated by 5 minutes rest phase. Performance of animals was recorded as the average number of errors in each trial. Values were expressed as mean ± SEM. $p < .05$ was considered significant.

Table 1. Memory Performance in the Radial Arm Water Maze

WTS exposure					
Memory	Control	Early gestation	Mid gestation	Late gestation	Whole gestation
Short-term memory	0.7 ± 0.2	2.7 ± 0.5*	2.9 ± 0.5*	3.2 ± 0.7*	3.6 ± 0.9*
Long-term memory at 5 h	0.9 ± 0.3	3.9 ± 0.6*	3.9 ± 0.9*	4.0 ± 0.9*	4.1 ± 0.8*
Long-term memory at 24 h	0.5 ± 0.2	3.6 ± 0.8*	3.5 ± 0.8*	5.0 ± 0.9*	4.1 ± 1.0*

* Indicates significant difference from control group. Values were expressed as mean ± SEM. $P < 0.05$ was considered significant.

the time and duration of prenatal WTS exposure did not affect the number of made errors (Table 1).

The Effects of Prenatal WTS on BDNF Levels

The level of BDNF in hippocampus of offspring rats was reduced by prenatal WTS exposure during early (325.1 ± 28.4 ng/mg protein), late (478.5 ± 98.2 ng/mg protein) and whole pregnancy period (386.2 ± 88.9 ng/mg protein), but not mid (547.7 ± 45.5 ng/mg protein) gestation, as compared to control offspring (772.8 ± 81.9 ng/mg protein, $p < .05$) (Figure 2).

The Effects of Prenatal WTS on Oxidative Stress Markers

The activity of SOD, GPx and level of TBARS in hippocampus were not affected by prenatal exposure to WTS in early, mid, late and whole gestation relative to unexposed offspring ($p > .05$) (Table 2). Additionally, the catalase level did not differ significantly in hippocampus of rats whose mothers were exposed to WTS during early, mid and whole compared to unexposed offspring ($p > .05$). However, offspring that were exposed to WTS in late gestation had higher catalase level (28.32 ± 2.86, μM/mg protein) compared to unexposed offspring (19.5 ± 1.4 μM/mg protein, $p < .05$) (Table 2).

Discussion

The current study found that prenatal exposure to WTS did not alter learning but induced short- and long-term memory impairment in adult offspring rats regardless of level of WTS exposure duration during pregnancy. The impairment was associated with reduction of BDNF level in hippocampus. However, there was no evidence that prenatal WTS exposure affected the level of oxidative stress biomarkers in hippocampus, except catalase at prenatal WTS exposure at late gestation, reliably under the conditions reported here.

Prenatal WTS exposure induced cognitive impairment in adult offspring rats regardless of the exposure duration or the stage of pregnancy. The observed impairment of cognitive function in WTS-exposed offspring groups is consistent with previous results of prenatal cigarette smoke exposure. Passive tobacco smoking during pregnancy has been shown to induce learning and memory impairment of offspring mice as evaluated by the water maze test and long-term potentiation.[26] Amos-Kroohs and colleagues found that prenatal exposure to cigarette smoke and during lactation period resulted in long-term adverse effects on brain function that includes subnormal anxiety in a novel environment and impairs spatial and reference memory of offspring mice.[27] Though different brain regions play different roles in learning and memory, hippocampus is a critical player.[28] It has been shown that tobacco smoking reduces total hippocampus volume with age to a greater extent as compared to nonsmokers.[29] Injected nicotine (2.5 mg/kg/day) from gestational day 6–21 reduced the neuronal area of dentate gyrus, CA1 and CA3 regions of the hippocampus in offspring rats on postnatal day 40.[30] In addition, exposure to nicotine from gestational day 3 to day 18 induced learning and memory deficits in adult offspring mice.[31] Prenatal exposure to nicotine from day 7–21 of gestation affected the function and stoichiometry of glutamate receptors, the main modulator of learning and memory, in adult rat hippocampus.[32]

Different molecules are involved in hippocampus-dependent learning and memory and BDNF is an essential player[33] that has a critical role in synaptic plasticity and synaptogenesis.[34] Mice lacking BDNF in hippocampus showed impaired learning and spatial memory.[35] Human studies revealed that reduced level of BDNF was associated with low cortical thickness.[36] We found here that prenatal WTS reduced the level of BDNF in hippocampus in adult stage regardless of the WTS initiation. However, there was no difference between BDNF levels in offspring rats that were exposed to WTS during early, mid, late or whole gestation. Our result is consistent with a previous study where prenatal exposure to cigarette smoke

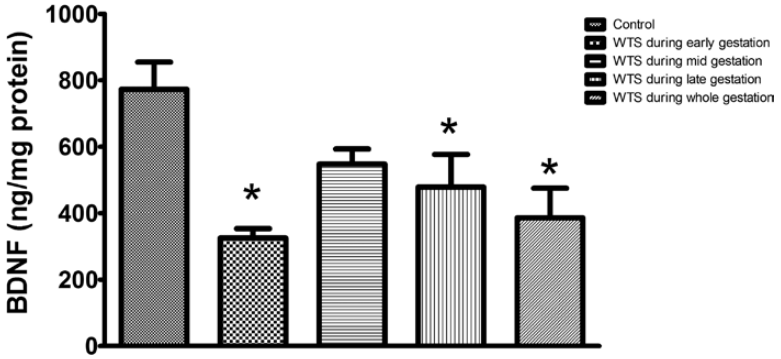

Figure 2. Hippocampal BDNF levels. BDNF level was measured in hippocampus of offspring rats that were exposed prenatally to fresh air (control) or WTS for 2 hours per day during early gestation (0–7 days), mid gestation (7–14 days), late gestation (14–21 days) or whole gestation (0–21 days) ($n = 10$). * indicates significant difference from control group. Values were expressed as mean ± SEM. $p < .05$ was considered significant.

Table 2. Hippocampal Oxidative Stress Levels

WTS exposure					
Marker	Control	Early gestation	Mid gestation	Late gestation	Whole gestation
SOD (unit/mg protein)	3.3 ± 0.2	3.0 ± 0.2	3.4 ± 0.2	3.2 ± 0.3	2.9 ± 0.3
Catalase (μM/mg protein)	19.5 ± 1.4	20.8 ± 1.7	23.3 ± 1.7	28.3 ± 2.9*	22.9 ± 1.7
GPx (unit/mg protein)	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.04	0.2 ± 0.03
TBARS (μM/mg protein)	8.8 ± 0.6	7.9 ± 0.6	8.5 ± 0.7	9.7 ± 0.7	8.5 ± 0.9

* Indicates significant difference from control group. Values were expressed as mean ± SEM. $P < 0.05$ was considered significant.

resulted in a significant reduction of BDNF mRNA expression and protein levels in striatal and cortical brain regions.[37] Additionally, adult smokers had lower levels of plasma BDNF as compared to nonsmokers.[38] Reduced BDNF level was also linked to the development of several behavioral abnormalities such as aggressive behavior, hyperactivity and depressive like behavior,[37] and neurological conditions such as Alzheimer's disease and Parkinson's disease.[39] Therefore, the reduced BDNF levels by prenatal WTS exposure points toward the alarming preventable consequences that could develop beside memory impairment. A study by Hawley and colleagues showed that exposure of animals to RAWM altered expression of BDNF in the hippocampus sub regions.[40] Thus, RAWM, in addition to waterpipe smoke exposure, might play a role in modulating BDNF expression in the hippocampus. However, in the current study both groups (control and waterpipe) were equally exposed to RAWM. Thus, the observed decrease in BDNF level in waterpipe group was most likely due to exposure to waterpipe smoke.

Alzoubi and colleagues reported that exposure of adult rats to WTS caused short- and long-term memory impairment that was associated with oxidative stress disturbances in hippocampus.[23] Exposure to WTS leads to the production of large amount of free radicals and the subsequent tissue inflammation and damage.[41] The level of SOD, catalase and GPx were reduced while oxidized glutathione was increased in hippocampus of rats that were exposed to WTS for 4 weeks.[23] Oxidative stress has been implicated in several cognitive impairments in different conditions such as aging and neurodegenerative diseases.[42] In this study, the activity of SOD and GPx in hippocampus of offspring rats were not affected by prenatal WTS exposure. However, there was a trend of increased catalase activity by prenatal WTS but the difference was not significant. The exposure to WTS at late gestation increased the catalase activity in hippocampus of offspring rats. A study by Khanna and colleagues reported reduced catalase activity in cerebrum and cerebellum of offspring rats that were exposed to nicotine, the major constituent of tobacco smoke, from gestational day 7–14, but not from gestational day 2–9.[43] Therefore, the effects of prenatal nicotine exposure varied during the different developmental stages of brain as well as the time of tissue collection and analysis. Prenatal WTS did not affect the level of TBARS, a byproduct of lipid peroxidation, in the hippocampus of offspring rats. The lack of an observed effect in the current study on oxidative stress biomarkers could be related to the adaptive response to exposure to WTS during prenatal/developmental period as opposed to adulthood exposure in our previous study.[23] To further confirm these findings, a larger experiment with more animals is needed. Current results are also inconsistent with a previous report of the increased oxidative stress level in the brain of offspring animals that were exposed to *in utero* cigarette smoke.[44] This effect could be due to differences in the exposure duration. Chan and colleagues exposed female mice to the smoke from two cigarettes twice daily for six weeks prior to mating and throughout gestation and lactation.[44] However, current results did not cover all oxidative stress biomarkers and more studies are needed to evaluate the effect of WTS on other oxidative stress markers such as glutathione species, hydrogen peroxide, malondialdehyde, and 8-isoprostanes among others with more rats. Therefore, more work is required to evaluate the oxidative stress level in pups and young adult offspring rats.

In this study, we used whole-body exposure system to study the effect of prenatal WTS on learning and memory of offspring rats. There are two methods in inhalational experiments; nose-only exposure where the animals are restrained and exposure is localized to nose, and whole-body exposure where the animals are unstrained and exposed to the atmosphere.[45] In the nose-only exposure system, the pregnant animals are under stress from the restraint that could influence the neuronal volume in the hippocampus of adult offspring animals.[46] Although whole-body exposure system excludes the effect of stressful restraint, oral ingestion could affect the extent of absorption.[45] However, whole-body exposure system is used frequently to assess the effect of cigarette tobacco smoke in small animals.[47]

Waterpipe smoke contains large amounts of CO, nicotine and several other toxic compounds.[13] Li and colleagues showed that prenatal exposure to nicotine resulted in learning and memory impairment in offspring.[48] Mactutus and Fechter reported in 1984 that prenatal exposure to CO resulted in learning and memory deficit in offspring.[49] Therefore, more studies are needed to investigate whether the impaired memory induced by prenatal WTS is due to nicotine, CO or other toxic compounds in WTS. Concentrations of nicotine and cotinine were not measured in the current study and hence future studies should focus on measuring their levels to correlate them with human levels. In addition, studies have shown that exposure of rodents to cigarette tobacco smoke during the early postnatal period, a very important period for the neurodevelopment of rodents, is associated with adverse effects on adult offspring.[27,50] Therefore, future studies directed toward examining the effect of waterpipe smoke exposure during this period is of interest and granted by future studies.

In conclusion, prenatal exposure to waterpipe tobacco smoke, at any stage of pregnancy, impaired short- and long-term memory in offspring animals, and this impairment was associated with reduced levels of brain derived neurotrophic factor in hippocampus. However, there is no evidence on the involvement of oxidative stress in the hippocampus in this impairment. Many countries' regulations focus on the appearance of health warnings on cigarette packs but few countries warn the public about the health effects of waterpipe tobacco smoke. The identified relationship of prenatal WTS exposure and memory impairment suggests a need for polices to increase public awareness about the harmful effects of waterpipe tobacco smoking during pregnancy and the importance of waterpipe tobacco smoking cessation.

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Declaration of Interests

Dr. Eissenberg is a paid consultant in litigation against the tobacco industry and is named on a patent application for a device that measures the puffing behavior of electronic cigarette users. The other authors declare that they have no conflict of interest.

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References

1. WHO. Tobacco, Fact sheet. 2016; http://www.who.int/mediacentre/factsheets/fs339/en/. Accessed January 3, 2017.
2. Krstev S, Marinković J, Simić S, Kocev N, Bondy SJ. Prevalence and predictors of smoking and quitting during pregnancy in Serbia: results of a nationally representative survey. *Int J Public Health*. 2012;57(6):875–883.
3. Wang X, Zuckerman B, Pearson C, et al. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. *JAMA*. 2002;287(2):195–202.
4. Wilcox AJ. Birth weight and perinatal mortality: the effect of maternal smoking. *Am J Epidemiol*. 1993;137(10):1098–1104.
5. Ryckman KK, Borowski KS, Parikh NI, Saftlas AF. Pregnancy Complications and the Risk of Metabolic Syndrome for the Offspring. *Curr Cardiovasc Risk Rep*. 2013;7(3):217–223.
6. Zacharasiewicz A. Maternal smoking in pregnancy and its influence on childhood asthma. *ERJ Open Res*. 2016;2(3):00042–2016.
7. Walker A, Rosenberg M, Balaban-Gil K. Neurodevelopmental and neurobehavioral sequelae of selected substances of abuse and psychiatric medications in utero. *Child Adolesc Psychiatr Clin N Am*. 1999;8(4):845–867.
8. Roy TS, Seidler FJ, Slotkin TA. Prenatal nicotine exposure evokes alterations of cell structure in hippocampus and somatosensory cortex. *J Pharmacol Exp Ther*. 2002;300(1):124–133.
9. England LJ, Aagaard K, Bloch M, et al. Developmental toxicity of nicotine: A transdisciplinary synthesis and implications for emerging tobacco products. *Neurosci Biobehav Rev*. 2017;72:176–189.
10. Akl EA, Gunukula SK, Aleem S, et al. The prevalence of waterpipe tobacco smoking among the general and specific populations: a systematic review. *BMC Public Health*. 2011;11:244.
11. Dar-Odeh NS, Abu-Hammad OA. The changing trends in tobacco smoking for young Arab women; narghile, an old habit with a liberal attitude. *Harm Reduct J*. 2011;8:24.
12. Tamim H, Yunis KA, Chemaitelly H, et al. Effect of narghile and cigarette smoking on newborn birthweight. *BJOG*. 2008;115(1):91–97.
13. Shihadeh A, Schubert J, Klaiany J, El Sabban M, Luch A, Saliba NA. Toxicant content, physical properties and biological activity of waterpipe tobacco smoke and its tobacco-free alternatives. *Tob Control*. 2015;24(suppl 1):i22–i30.
14. Al-Sheyab NA, Al-Fuqha RA, Kheirallah KA, Khabour OF, Alzoubi KH. Anthropometric measurements of newborns of women who smoke waterpipe during pregnancy: a comparative retrospective design. *Inhal Toxicol*. 2016;28(13):629–635.
15. Khabour OF, Alzoubi KH, Al-Sheyab N, Shihadeh A, Eissenberg T. Investigating the Effects of Exposure to Waterpipe Smoke on Pregnancy Outcomes Using an Animal Model. *Nicotine Tob Res*. 2016;18(5):585–589.
16. Balsevich G, Poon A, Goldowitz D, Wilking JA. The effects of pre- and post-natal nicotine exposure and genetic background on the striatum and behavioral phenotypes in the mouse. *Behav Brain Res*. 2014;266:7–18.
17. Roy TS, Sabherwal U. Effects of prenatal nicotine exposure on the morphogenesis of somatosensory cortex. *Neurotoxicol Teratol*. 1994;16(4):411–421.
18. Rivkin MJ, Davis PE, Lemaster JL, et al. Volumetric MRI study of brain in children with intrauterine exposure to cocaine, alcohol, tobacco, and marijuana. *Pediatrics*. 2008;121(4):741–750.
19. Ekblad M, Korkeila J, Parkkola R, et al. Maternal smoking during pregnancy and regional brain volumes in preterm infants. *J Pediatr*. 2010;156(2):185–190.e181.
20. Fung YK, Lau YS. Effects of prenatal nicotine exposure on rat striatal dopaminergic and nicotinic systems. *Pharmacol Biochem Behav*. 1989;33(1):1–6.
21. Al-Sawalha NA, Migdadi AM, Alzoubi KH, Khabour OF, Qinna NA. Effect of waterpipe tobacco smoking on airway inflammation in murine model of asthma. *Inhal Toxicol*. 2017;29(2):46–52.
22. Shihadeh A, Azar S, Antonios C, Haddad A. Towards a topographical model of narghile water-pipe café smoking: a pilot study in a high socioeconomic status neighborhood of Beirut, Lebanon. *Pharmacol Biochem Behav*. 2004;79(1):75–82.
23. Alzoubi KH, Khabour OF, Alharahshah EA, Alhashimi FH, Shihadeh A, Eissenberg T. The Effect of Waterpipe Tobacco Smoke Exposure on Learning and Memory Functions in the Rat Model. *J Mol Neurosci*. 2015;57(2):249–256.
24. Jonasson Z. Meta-analysis of sex differences in rodent models of learning and memory: a review of behavioral and biological data. *Neurosci Biobehav Rev*. 2005;28(8):811–825.
25. Hodges H. Maze procedures: the radial-arm and water maze compared. *Brain Res Cogn Brain Res*. 1996;3(3-4):167–181.
26. Yang J, Jiang LN, Yuan ZL, et al. Impacts of passive smoking on learning and memory ability of mouse offsprings and intervention by antioxidants. *Biomed Environ Sci*. 2008;21(2):144–149.
27. Amos-Kroohs RM, Williams MT, Braun AA, et al. Neurobehavioral phenotype of C57BL/6J mice prenatally and neonatally exposed to cigarette smoke. *Neurotoxicol Teratol*. 2013;35:34–45.
28. Broadbent NJ, Squire LR, Clark RE. Spatial memory, recognition memory, and the hippocampus. *Proc Natl Acad Sci USA*. 2004;101(40):14515–14520.
29. Durazzo TC, Meyerhoff DJ, Nixon SJ. Interactive effects of chronic cigarette smoking and age on hippocampal volumes. *Drug Alcohol Depend*. 2013;133(2):704–711.
30. Roy TS, Sabherwal U. Effects of gestational nicotine exposure on hippocampal morphology. *Neurotoxicol Teratol*. 1998;20(4):465–473.
31. Han G, An L, Yang B, Si L, Zhang T. Nicotine-induced impairments of spatial cognition and long-term potentiation in adolescent male rats. *Hum Exp Toxicol*. 2014;33(2):203–213.
32. Wang H, Dávila-García MI, Yarl W, Gondré-Lewis MC. Gestational nicotine exposure regulates expression of AMPA and NMDA receptors and their signaling apparatus in developing and adult rat hippocampus. *Neuroscience*. 2011;188:168–181.
33. Yamada K, Nabeshima T. Brain-derived neurotrophic factor/TrkB signaling in memory processes. *J Pharmacol Sci*. 2003;91(4):267–270.
34. Leal G, Afonso PM, Salazar IL, Duarte CB. Regulation of hippocampal synaptic plasticity by BDNF. *Brain Res*. 2015;1621:82–101.
35. Heldt SA, Stanek L, Chhatwal JP, Ressler KJ. Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. *Mol Psychiatry*. 2007;12(7):656–670.
36. Wang C, Zhang Y, Liu B, Long H, Yu C, Jiang T. Dosage effects of BDNF Val66Met polymorphism on cortical surface area and functional connectivity. *J Neurosci*. 2014;34(7):2645–2651.
37. Yochum C, Doherty-Lyon S, Hoffman C, Hossain MM, Zelikoff JT, Richardson JR. Prenatal cigarette smoke exposure causes hyperactivity and aggressive behavior: role of altered catecholamines and BDNF. *Exp Neurol*. 2014;254:145–152.
38. Bhang SY, Choi SW, Ahn JH. Changes in plasma brain-derived neurotrophic factor levels in smokers after smoking cessation. *Neurosci Lett*. 2010;468(1):7–11.
39. Murer MG, Yan Q, Raisman-Vozari R. Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. *Prog Neurobiol*. 2001;63(1):71–124.
40. Hawley DF, Morch K, Christie BR, Leasure JL. Differential response of hippocampal subregions to stress and learning. *PLoS One*. 2012;7(12):e53126.
41. Fahn HJ, Wang LS, Kao SH, Chang SC, Huang MH, Wei YH. Smoking-associated mitochondrial DNA mutations and lipid peroxidation in human lung tissues. *Am J Respir Cell Mol Biol*. 1998;19(6):901–909.
42. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuropharmacol*. 2009;7(1):65–74.
43. Khanna Sood P, Sharma S, Nehru B. Consequences of nicotine exposure during different phases of rat brain development. *Brain Dev*. 2012;34(7):591–600.
44. Chan YL, Saad S, Pollock C, et al. Impact of maternal cigarette smoke exposure on brain inflammation and oxidative stress in male mice offspring. *Sci Rep*. 2016;6:25881.

45. Cheng YS, Bowen L, Rando RJ, Postlethwait EM, Squadrito GL, Matalon S. Exposing animals to oxidant gases: nose only vs. whole body. *Proc Am Thorac Soc*. 2010;7(4):264–268.
46. Hosseini-Sharifabad M, Esfandiari E, Hosseini-Sharifabad A. The effect of prenatal exposure to restraint stress on hippocampal granule neurons of adult rat offspring. *Iran J Basic Med Sci*. 2012;15(5):1060–1067.
47. Small E, Shah HP, Davenport JJ, et al. Tobacco smoke exposure induces nicotine dependence in rats. *Psychopharmacology (Berl)*. 2010;208(1):143–158.
48. Li J, Bo L, Zhang P, et al. Exposure to nicotine during pregnancy and altered learning and memory in the rat offspring. *Nicotine Tob Res*. 2015;17(6):661–666.
49. Mactutus CF, Fechter LD. Prenatal exposure to carbon monoxide: learning and memory deficits. *Science*. 1984;223(4634):409–411.
50. Xiao L, Kish VL, Benders KM, Wu ZX. Prenatal and Early Postnatal Exposure to Cigarette Smoke Decreases BDNF/TrkB Signaling and Increases Abnormal Behaviors Later in Life. *Int J Neuropsychopharmacol*. 2016;19(5):pyv117.