*Journal of Developmental Origins of Health and Disease*

**www.cambridge.org/doh**

# Sex- and age-dependent differences in nicotine susceptibility evoked by developmental exposure to tobacco smoke and/or ethanol in mice

André Luiz Nunes-Freitas[1], Alex C. Manhães[1], Ana Carolina Dutra-Tavares[1], Pedro Henrique Leal-Rocha[1], Claudio C. Filgueiras[1], Anderson Ribeiro-Carvalho[2] and Yael Abreu-Villaça[1]

[1]Laboratório de Neurofisiologia, Departamento de Ciências Fisiológicas, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Av. Prof. Manuel de Abreu 444, 5 andar – Vila Isabel, Rio de Janeiro, RJ, 20550-170, Brazil and [2]Departamento de Ciências, Faculdade de Formação de Professores da Universidade do Estado do Rio de Janeiro, São Gonçalo, RJ, 24435-005, Brazil

**Original Article**

**Cite this article:** Nunes-Freitas AL, Manhães AC, Dutra-Tavares AC, Leal-Rocha PH, Filgueiras CC, Ribeiro-Carvalho A, and Abreu-Villaça Y. (2021) Sex- and age-dependent differences in nicotine susceptibility evoked by developmental exposure to tobacco smoke and/or ethanol in mice. *Journal of Developmental Origins of Health and Disease* **12**: 940–951. doi: 10.1017/S2040174420001191

Received: 15 June 2020
Revised: 17 October 2020
Accepted: 8 November 2020
First published online: 9 December 2020

**Keywords:**
Alcohol; addiction; polydrug; gestation; development

**Address for correspondence:**
Yael Abreu-Villaça, Laboratório de Neurofisiologia, Departamento de Ciências Fisiológicas, Instituto de Biologia Roberto Alcantara Gomes, Centro Biomédico, Universidade do Estado do Rio de Janeiro, Av. Prof. Manuel de Abreu 444, 5 andar, Vila Isabel, Rio de Janeiro, RJ, 20550-170, Brazil.
Emails: yael_a_v@yahoo.com.br; yael_a_v@pq.cnpq.br

## Abstract

Either tobacco smoking or alcohol consumption during pregnancy sex-selectively increases susceptibility to drugs of abuse later in life. Considering that pregnant smoking women are frequently intermittent consumers of alcoholic beverages, here, we investigated whether a short-term ethanol exposure restricted to the brain growth spurt period when combined with chronic developmental exposure to tobacco smoke aggravates susceptibility to nicotine in adolescent and adult mice. Swiss male and female mice were exposed to tobacco smoke (SMK; research cigarettes 3R4F, whole-body exposure, 8 h/daily) or ambient air during the gestational period and until the tenth postnatal day (PN). Ethanol (ETOH, 2 g/Kg, 25%, i.p.) or saline was injected in the pups every other day from PN2 to PN10. There were no significant differences in cotinine (nicotine metabolite) and ethanol serum levels among SMK, ETOH and SMK + ETOH groups. During adolescence (PN30) and adulthood (PN90), nicotine (NIC, 0.5 mg/Kg) susceptibility was evaluated in the conditioned place preference and open field tests. NIC impact was more evident in females: SMK, ETOH and SMK + ETOH adolescent females were equally more susceptible to nicotine-induced place preference than control animals. At adulthood, SMK and SMK + ETOH adult females exhibited a nicotine-evoked hyperlocomotor profile in the open field, with a stronger effect in the SMK + ETOH group. Our results indicate that ethanol exposure during the brain growth spurt, when combined to developmental exposure to tobacco smoke, increases nicotine susceptibility with stronger effects in adult females. This result represents a worsened outcome from the early developmental dual exposure and may predispose nicotine use/abuse later in life.

## Introduction

Ethanol and tobacco are widely used and abused by women of childbearing age[1–3]. Even though smoking rates are decreasing worldwide[4], this general trend has not always been evident among women[5]. Regarding ethanol, 32% of women are current drinkers and, among those, 20% are heavy episodic drinkers[6]. Pregnancy is perceived by many women as a window of opportunity to quit drugs of abuse[4,7–9]. Despite that, smoking rates and alcohol drinking prevalence during pregnancy have not changed substantially over the last 2 decades[3,10]. Recent prevalence values are 8.5%–14.5% of smokers during gestation[10–12] whereas 11%–20% of women drink alcoholic beverages while pregnant[3,13–15]. Both drugs disrupt the development of the nervous system, producing long-lasting neurological and behavioral outcomes in the offspring[16–18]. These include increased risk of attention deficit hyperactivity disorder[19–21], learning and memory deficits[22–24] and impaired sensory processing[25–27].

Of special interest to this work, both prenatal ethanol and tobacco smoke exposure may predispose offspring to subsequent drug use and abuse. Maternal smoking increases the risk of early tobacco experimentation[22], tobacco use[28] and accelerates the progress to regular daily smoking in the offspring[29] with evidence of a faster transition in women[30]. Besides, Electronic Nicotine Delivery Systems (ENDS) such as e-cigarettes are becoming progressively more popular at a fast rate[31–33] and maternal smoking during gestation may play a role in this burst in consumption[34]. Regarding ethanol, there is a positive association between prenatal exposure and problematic ethanol consumption in adolescents and young adults[35,36], as well as evidence for a cross talk, in that prenatal ethanol exposure also increases the risk for tobacco and illicit drug addiction[37].



CAMBRIDGE UNIVERSITY PRESS

Parallel evidence from animal models corroborates these findings and suggests that early exposure to nicotine or ethanol increases the reinforcing effects of the drug later during postnatal life[38–40].

There is a strong association between smoking and alcohol drinking. Accordingly, the prevalence of smoking combined with intermittent consumption of alcoholic beverages is high among reproductive-age women[41,42] and even during pregnancy[43–45]. This is particularly worrisome since shared detrimental effects of smoking and drinking could lead to worsened outcomes in the offspring. In this regard, our group has recently shown that the dual exposure to nicotine and ethanol during early development of mice leads to more consistent hyperlocomotor effects, memory/learning deficits and cAMP and cGMP signaling disruption than either drug on its own[46]. Other consequences of tobacco smoke and ethanol dual exposure, such as the possibility of increased susceptibility to drugs of abuse later in life, still need to be investigated.

Studies in experimental models of co-exposure have specifically assessed negative consequences of combined nicotine + ethanol[46–48]. However, tobacco smoke contains thousands of components and there has been a growing body of evidence that identifies important contributions of non-nicotine components to tobacco smoke effects in the central nervous system[49–60]. Accordingly, an alternative approach to more closely investigate the impact of smoking is to use animal models of tobacco smoke exposure. Despite that, there are scant experimental studies on the effects of tobacco smoke and ethanol co-exposure. This lack of information is particularly disconcerting when one considers that pregnant women who smoke cigarettes as well as drink alcoholic beverages expose themselves and their babies to a substantial number of substances that are present in the tobacco smoke and that may interact with nicotine and/or ethanol in affecting the developing central nervous system.

Considering that: 1) pregnant smoking women may also be intermittent consumers of alcoholic beverages, 2) both epidemiological and animal models suggest that early exposure to nicotine/tobacco or to ethanol increases the susceptibility to drugs of abuse later in life and, 3) exposure to both nicotine/tobacco and ethanol may lead to worsened outcomes when compared to exposure to either drug on its own; here, we investigated the possibility that even an early short-term intermittent exposure to ethanol when combined to chronic exposure to tobacco smoke aggravates susceptibility to nicotine re-exposure later in life. Ethanol exposure was limited to the brain growth spurt, a neonatal period which, in rodents, roughly corresponds to the third trimester of human gestation. As for tobacco smoke, the animals were exposed during the period equivalent to the entire human gestation. Regarding nicotine re-exposure, ENDS such as e-cigarettes are becoming progressively more popular at a fast rate, particularly among adolescents[31–33]. Currently, ENDS are three times more common among adolescents and young adults than among older adults[61]. ENDS contain nicotine as the addictive ingredient[62,63], which, despite some controversy[64], has been shown to differentially impact adolescent and adult brain[65]. Accordingly, here, nicotine susceptibility was evaluated both during adolescence and adulthood in the open field (OF) and conditioned place preference (CPP) tests. Considering that the male–female differences in drugs of abuse consumption and associated disorders have decreased over the last decades[66], plus the evidence of significant gender differences in drugs of abuse susceptibility[67–70], the examination of potentially sex-selective consequences of early tobacco and ethanol dual exposure is critical; therefore, both males and females were tested.

## Methods

### Animals and treatment

All Swiss mice were bred and maintained in our animal facility at 21°C–22°C on a 12:12 h light/dark cycle (lights on at 1:00 a.m.). Food and filtered water were available *ad libitum*. Male Swiss mice were paired with females (1:2) and daily exposed either to tobacco smoke or to ambient air. Once pregnancy was confirmed, female mice were housed singly and daily exposure continued until birth. After birth (postnatal day 1 = PN1), dams and pups were exposed until PN10. Tobacco smoke was generated from the burning of reference research cigarettes (University of Kentucky, Lexington, KY, USA) type 3R4F (nicotine = 0.73 mg/cigt; total particulate matter = 11.0 mg/cigt; tar = 9.4 mg/cigt; carbon monoxide = 12.0 mg/cigt). Whole body exposure was for 8 h/d, from 8:00 a.m. to 4:00 p.m., 7 d/week, in a chamber that received the smoke generated in an automatic tobacco smoking machine (Teague Enterprises, Davis, CA, USA). The smoking machine generates a single 35-ml, 2-s puff per min, containing 89% sidestream smoke (smoke released from the burning end of a cigarette) and 11% mainstream smoke (smoke from the puff stream), as a surrogate for active smoking[49–51,71,72]. The pattern of tobacco smoke exposure used in this study[72] generates cotinine (nicotine metabolite) serum levels that are within the range of those found in smokers[73–76]. Control mice were exposed to ambient air in a chamber identical to the one used for smoke exposure. As for ethanol exposure, from PN2 to PN10, pups received a single injection of ethanol (5 mg/Kg, i.p., 10% in saline solution) every other day[77–80]. The ethanol dose was chosen based on previous studies[72,78,81], which have shown that it generates blood ethanol concentrations within the range that a human fetus would be exposed to after maternal ingestion of a moderate to heavy dose of ethanol[82]. Control mice were exposed to equivalent volumes of saline. Accordingly, mice were distributed into four exposure groups: VEH (air + saline), ETOH (air + ethanol), SMK (tobacco smoke + saline) and those receiving the combined treatment: SMK + ETOH (tobacco smoke + ethanol).

The period of exposure to tobacco smoke intended to parallel human exposure during gestation. In this regard, the prenatal development of rodents roughly corresponds to the first two trimesters of human pregnancy, while the third trimester comprises the first 10-day period of postnatal life in mice and rats, a period during which the brain undergoes a growth spurt[83,84]. Nicotine interferes with neurogenesis and early synaptogenesis during gestation, as well as with dendritic arborization, late synaptogenesis and migration of multiple neuronal populations, which occur in great intensity during the first 10 days following birth in mice[85–87]. Despite evidence that most women stop drinking when they verify that they are pregnant, it is frequent to resume consumption during the third trimester[88]. Given this epidemiological finding, together with evidence of cell loss, reduced neurogenesis[89,90], locomotor hyperactivity and memory/learning deficits in animal models of intermittent exposure to ethanol during the third trimester equivalent of human gestation[46,78,81,91], here, ethanol exposure was restricted to the early postnatal period. Exposure on alternate days was chosen to mimic episodic drinking.

At weaning (PN21), animals from each litter were separated by sex and housed in groups of 2–5 mice by cage. Body mass of the dams and pups was monitored every other day during ethanol exposure (PN2–PN10). Offspring body mass was also measured at PN30 and PN90. The timeline of the experiment is shown in Fig. 1.

https://doi.org/10.1017/S2040174420001191 Published online by Cambridge University Press

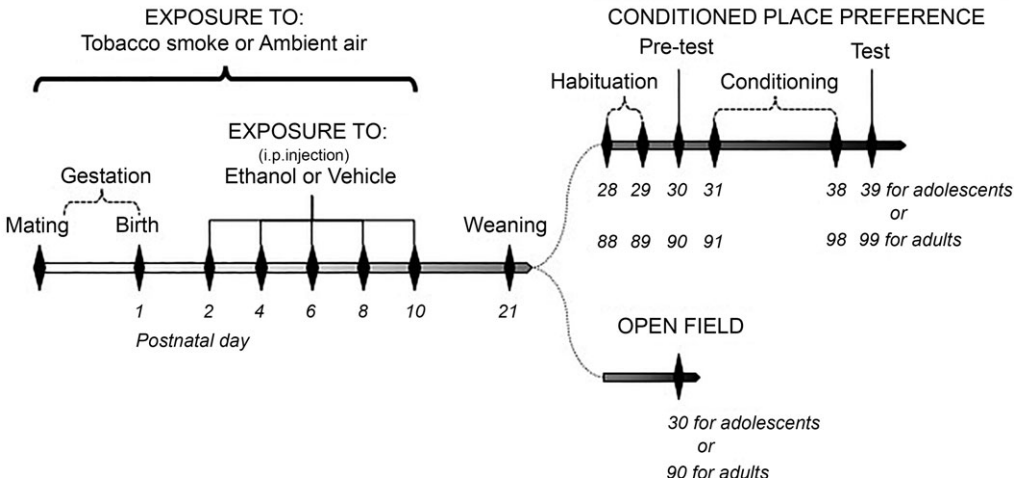


**Fig. 1.** Timeline of the experiment.

### *Behavioral tests*

Mice from each litter were semi-randomly assigned to the OF or CPP test either during adolescence (PN30) or adulthood (PN90). No more than one male and one female from each litter were assigned to each experimental group, age and behavioral tests. For each experimental group, age and sex, 7 to 12 animals were examined. All tests were performed in a sound attenuated room and the test apparatus was cleaned with paper towels soaked in 35% ethanol and dried before each test.

### *Nicotine Conditioned Place Preference*

The CPP test is a widely accepted model to study the rewarding properties of addictive drugs in rodents[92,93]. In this test, the animals are conditioned to associate the effects of the drug with the environment where the drug is administered. The CPP apparatus (Insight, SP, Brazil) consists of a box with three distinct chambers. Briefly, the animals received, for 2 consecutive days (habituation period), one i.p. injection of saline. On the following day, the animals received the i.p. injection of saline and were allowed to freely explore the apparatus for 15 min (Pre-test). The time spent in each chamber except the central one during the Pre-test was used to determine the preferred and non-preferred chambers. For the next 8 days (conditioning period), mice from each group were randomly assigned into nicotine or saline groups. The nicotine groups ($VEH_{NIC}$, $ETOH_{NIC}$, $SMK_{NIC}$ and $SMK + ETOH_{NIC}$) were administered (challenged with) nicotine (0.5 mg/Kg) paired with the non-preferred side (biased design) in one session and, in the other session, received saline paired with the preferred side. The sequence was alternated along the conditioning period. The saline groups ($VEH_{SAL}$, $ETOH_{SAL}$, $SMK_{SAL}$ and $SMK + ETOH_{SAL}$) received saline in both sessions/chambers. Immediately after each injection, mice were confined to the appropriate chamber for 15 min. On the 9th day (Test day), no injections were administered. The animals were placed in the central chamber and freely explored the CPP apparatus for 15 min. The time spent in each chamber was quantified. The CPP extended from PN28 to PN39 (adolescents) or from PN88 to PN99 (adults).

The CPP effect is expressed as an increase in the time the animal spends in the non-preferred chamber after the conditioning sessions. Both for the Pre-test and the Test, the place preference values ware calculated as follows: %Time NPref = time in the non-preferred chamber in the session/total session time. Considering that the percentage of time spent in the non-preferred chamber in the Pre-test varies as a function of the experimental group, age, sex etc., in order to visualize more clearly differences between groups in the figures, the %Time NPref values of the Test session will be expressed as a function of the values in the Pre-test session (calculated as follows: %Time NPref Test/%Time NPref Pre-test). Therefore, Pre-test values for all groups will be normalized to 100% and Test values (and S.E.M.) will be corrected accordingly.

### *Open Field test*

Nicotine administration enhances locomotor activity and this effect is associated with dopamine release in striatum/nucleus accumbens[94,95]. In this sense, locomotor activity in the OF represents a useful model to evaluate the acute reinforcing effects of nicotine. The OF arena (Insight, SP, Brazil) consists of a transparent acrylic box that is equipped with infrared beams. At PN30 or PN90, each animal that was assigned to the OF received an i.p. injection (challenge) of nicotine (0.5 mg/Kg, $VEH_{NIC}$, $ETOH_{NIC}$, $SMK_{NIC}$ and $SMK + ETOH_{NIC}$ groups) or saline ($VEH_{SAL}$, $ETOH_{SAL}$, $SMK_{SAL}$ and $SMK + ETOH_{SAL}$ groups). Immediately after the injection, mice were individually placed in the center of the arena and were allowed to explore it for 5 min. Spontaneous locomotor activity was determined on the basis of the traversed distance.

### *Cotinine and ethanol serum levels*

Cotinine and ethanol levels were assessed in a separate group of mice. These animals were not used for the behavioral analyses.

To evaluate tobacco exposure, immediately after the last day of exposure (PN10), the animals were individually removed from the exposure chamber, decapitated and trunk blood was collected from SMK (10 males and 10 females) and SMK + ETOH (10 males and 10 females) mice for cotinine (the most important nicotine metabolite in mammalian species) quantification. Cotinine serum levels were determined using a cotinine assay kit from Orasure Technologies (Pennsylvania, USA) in accordance with the manufacturer's recommendations.

On PN10, 1 h (ETOH: 7 males and 3 females, and SMK + ETOH: 13 males and 14 females) and 18 h (ETOH: 6 males and 5 females, and SMK + ETOH: 6 males and 8 females) after the last ethanol injection, the animals were decapitated and trunk blood was collected. Ethanol serum levels were determined using an enzymatic kit from Alcohol Reagent Set from Pointe Scientific Inc. (Michigan, USA) in accordance with the manufacturer's recommendations.

https://doi.org/10.1017/S2040174420001191 Published online by Cambridge University Press

**Table 1.** Body mass (g)

| DAMS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | PN2 | PN4 | PN6 | PN8 | PN10 | | | | |
| VEH | 45.8 ± 0.8 | 46.1 ± 0.8 | 46.8 ± 0.8 | 47.6 ± 0.8 | 48.8 ± 0.8 | | | | |
| SMK | 42.3 ± 0.7** | 41.9 ± 0.6*** | 42.1 ± 0.6*** | 41.8 ± 0.6*** | 41.9 ± 0.9*** | | | | |
| OFFSPRING | | | | | | | | | |
| | | | | | | PN30 | | PN90 | |
| | PN2 | PN4 | PN6 | PN8 | PN10 | Female | Male | Female | Male |
| VEH | 2.0 ± 0.04 | 2.9 ± 0.13 | 3.7 ± 0.11 | 4.7 ± 0.15 | 6.1 ± 0.58 | 21.2 ± 1.2 | 24.1 ± 1.7 | 38.8 ± 1.1 | 43.7 ± 1.5 |
| ETOH | 2.0 ± 0.05 | 2.9 ± 0.07 | 3.7 ± 0.10 | 4.8 ± 0.17 | 5.7 ± 0.24 | 21.6 ± 1.2 | 21.5 ± 1.4 | 35.5 ± 0.7*# | 43.4 ± 1.2# |
| SMK | 1.8 ± 0.04** | 2.3 ± 0.07*** | 3.1 ± 0.11** | 3.9 ± 0.13** | 4.8 ± 0.16** | 20.7 ± 0.8 | 24.1 ± 1.1 | 38.3 ± 0.8 | 46.1 ± 1.2** |
| SMK + ETOH | 1.8 ± 0.04** | 2.4 ± 0.08** | 3.2 ± 0.14** | 4.2 ± 0.17* | 5.0 ± 0.22* | 21.3 ± 0.9 | 24.4 ± 1.4 | 36.8 ± 1.0 | 43.4 ± 0.8# |

Means ± SEM.
PN, postnatal day; VEH, control group; ETOH, ethanol exposure group; SMK, tobacco smoke exposure group; SMK + ETOH, tobacco smoke and ethanol exposure group.
*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ *vs.* VEH group; #$p \leq 0.05$ *vs.* SMK group.

### *Data analysis*

The Kolmogorov–Smirnov one sample test (K–S) was used to assess the normality of the distributions of each of the variables. Results on each variable were evaluated first by a global analysis of variance (ANOVA) or a repeated-measures analysis of variance (rANOVA). ANOVAs were performed for adolescent and adult body mass and locomotor activity in the OF. rANOVAs were performed for the analyses of dams' and pups' postnatal body mass, as well as %Time NPref data (within-subject factor: Day). Exposure (VEH, SMK, ETOH and SMK + ETOH), Challenge (SAL and NIC), Age and Sex were between-subject factors. Whenever Age or Sex interactions were identified, lower-order ANOVAs on each age and sex were performed. With this one-dimensional design (1-d), in which just one factor accounts for all groups of Exposure, lower-order ANOVAs were followed by planned Fisher's Protected Least Significant Difference (FPLSD) *post hoc* tests to investigate which groups were affected by the early drugs of abuse exposure and paired *t* tests whenever applicable. In order to assess the possibility that tobacco smoke and ethanol interacted, resulting in effects that were either more-than-additive (synergistic) or less-than-additive, a two-dimensional (2-d) ANOVA design was used[46,47,52,96,97] (for details, see supplementary materials). In this design, Smoke (exposed: SMK and SMK + ETOH; non-exposed: VEH and ETOH) and Ethanol (exposed: ETOH and SMK + ETOH; non-exposed: VEH and SMK) are used as two independent between-factors in the analyses.

Data are compiled as means and standard errors of the means. For the body mass analysis, data from males and females of the same litter were averaged separately within each exposure/challenge group to minimize litter effects and avoid over-sampling[98]. Effects were considered significant when $p < 0.05$ (two-tailed).

## Results

### *Body mass*

Body mass data are shown in Table 1. Dams exposed to cigarette smoke were lighter than VEH ones (e.g. PN2: −8%; PN10: −14%) at all time points evaluated here (Exposure: $F_{(1,112)} = 24.1$; $p < 0.001$; Exposure × Day: $F_{(3.2,366.9)} = 19.1$; $p < 0.001$).

Regarding the offspring, body mass increased during the first 10 postnatal days (Day: $F_{(4,448)} = 370.7$, $p < 0.001$). However, this increase was not similar among groups (Exposure: $F_{(3,112)} = 6.8$, $p < 0.001$). Body mass gain was reduced in the SMK group when compared to VEH (e.g. PN2: −10%; PN10: −21%) and ETOH (e.g. PN2: −10%; PN10: −16%) ones. Similar reductions were identified in the SMK + ETOH group when compared to VEH (e.g. PN2: −10%; PN10: −18%) and ETOH (e.g. PN2: −10%; PN10: −12%) ones (Table 1).

At PN30, no significant differences were observed among groups, indicating a post-exposure recovery in body mass. However, at PN90, a late-emergent sex-dependent effect was identified (Exposure × Sex: $F_{(3,362)} = 3.2$; $p < 0.05$). While in females (Exposure: $F_{(3,180)} = 2.8$; $p < 0.05$), ETOH mice were lighter than VEH (−8%) and SMK ones (−7%), in males (Exposure: $F_{(3,182)} = 4.1$; $p < 0.01$), SMK mice showed increased body mass when compared to VEH (+5%), ETOH (+6%) and SMK + ETOH (+6%) ones (Table 1).

### *Cotinine and ethanol serum levels*

Cotinine levels did not differ between SMK (females: 91.8 ± 8.0 ng/ml; males: 93.8 ± 8.5 ng/ml) and SMK + ETOH (females: 100.7 ± 9.8 ng/ml; males: 100.1 ± 10.1 ng/ml) offspring.

At 1 h post-injection, ethanol serum levels did not differ between ETOH (females: 151.7 ± 10.4 mg/dL; males: 150.6 ± 10.2 mg/dL) and SMK + ETOH mice (females: 164.2 ± 12.4 mg/dL; males: 181.2 ± 11.0 mg/dL). There were only trace levels of ethanol 18 h post-injection (ETOH – females: 5.8 ± 2.2 mg/dL; males: 6.9 ± 4.3 mg/dL; SMK + ETOH – females: 6.6 ± 2.3 mg/dL; males: 4.7 ± 2.3 mg/dL).

For both cotinine and ethanol serum levels, there were no sex-dependent Exposure effects (no Exposure × Sex interactions).

### *Conditioned place preference test*

The higher-order 1-d rANOVA for the %Time NPref in the Pre-test and Test sessions identified significant Age and Sex interactions (Challenge × Age × Sex: $F_{(1,274)} = 4.4$; $p = 0.036$; Exposure × Day × Sex: $F_{(3,274)} = 5.7$; $p = 0.05$; Challenge × Day × Age × Sex: $F_{(1,274)} = 5.7$; $p = 0.017$). Accordingly, each age was analyzed separately.

https://doi.org/10.1017/S2040174420001191 Published online by Cambridge University Press

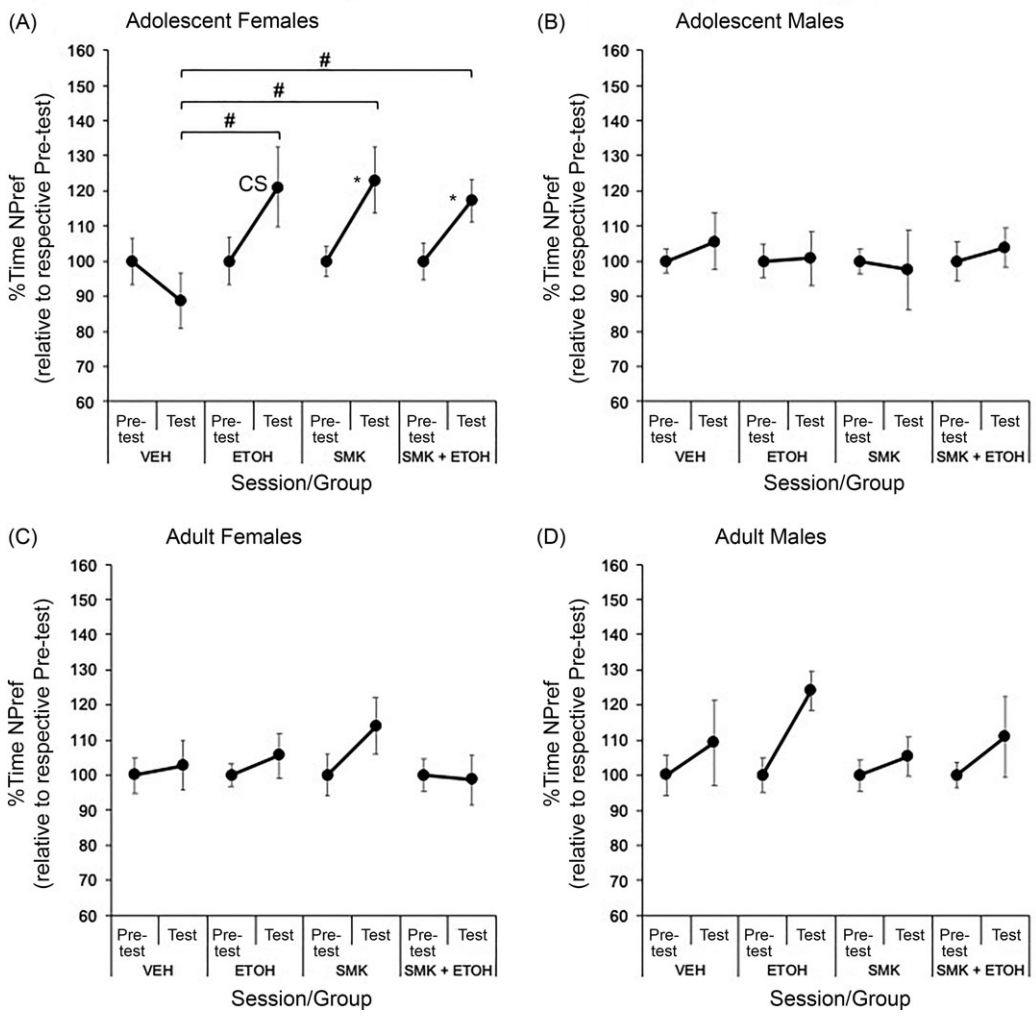


**Fig. 2.** %Time NPref data in the Conditioning place preference (CPP) test. Mice were exposed to tobacco smoke throughout the gestational period until PN10 and/or to ethanol (i.p. injection) every other day from PN2 to PN10. Animals were tested in the CPP either during adolescence (A: females; B: males) or at adulthood (C: females; D: males). Only data pertaining to animals that were nicotine-challenged during the CPP are shown. VEH, control group; ETOH, ethanol exposure group; SMK, tobacco smoke exposure group; SMK + ETOH, tobacco smoke and ethanol exposure group. %Time NPref data in the Test session is shown as a function of respective Pre-test data, which was normalized to 100%. Values are means ± SEM. *$p < 0.05$, significant difference between Test and Pre-test data within each experimental group. #$p < 0.05$, *vs*. VEH Test data. Differences revealed by FPLSD and paired *t*-tests.

Lower-order analyses (1-d rANOVAs) on each age confirmed that in adolescent mice, there were sex-selective effects (PN30 – Challenge × Sex: $F_{(1,134)} = 4.6$; $p = 0.033$; Challenge × Day × Sex: $F_{(3,143)} = 5.8$; $p = 0.017$), therefore, male and female data were analyzed separately. The analysis of adult mice failed to show significant effects and interactions. However, due to a trend toward a significant Exposure × Day × Sex interaction (PN90 – $F_{(3,131)} = 2.6$; $p = 0.051$), lower-order analyses were performed.

Subsequent analyses on each age and sex indicated that interactions between early exposure to tobacco smoke and/or ethanol and nicotine challenge later in life were only present in adolescent female mice (Exposure × Challenge × Day: $F_{(3,73)} = 3.5$; $p = 0.019$; Challenge × Day: $F_{(1,73)} = 8.7$; $p = 0.004$). Separate analysis for each challenge group indicated that, as expected, there were no significant differences in saline-challenged animals (data not shown). Distinctively, the analysis of nicotine-challenged adolescent females (Exposure × Day: $F_{(3,34)} = 2.9$; $p = 0.05$) indicated that $SMK_{NIC}$ and SMK + $ETOH_{NIC}$ ones significantly increased the time spent in the nicotine-paired chamber after the conditioning sessions (paired *t* tests, Fig. 2A). For $ETOH_{NIC}$ females, the increase in time spent in the nicotine-paired chamber was close to significant ($p = 0.07$, paired *t* test). In addition, $ETOH_{NIC}$, $SMK_{NIC}$ and SMK + $ETOH_{NIC}$ adolescent females spent more time in the non-preferred chamber in the Test session than $VEH_{NIC}$ ones (Fig. 2A). Consistent with the similar nicotine conditioning profile in all three groups early-exposed to the drugs of abuse, the effect of the combined exposure reflected a less-than-additive outcome (Smoke × Ethanol: $F_{(1,34)} = 6.5$; $p = 0.016$ in the 2-d design). This result indicates that the effect of SMK + ETOH on nicotine-induced CPP was equivalent to the effect caused by either drug.

There were no significant main effects or interactions in adolescent males (Fig. 2B) and in adult males and females (Figs. 2C and 2D).

### *Open field test*

The higher-order 1-d ANOVA for locomotor activity identified significant Age (Challenge × Age: $F_{(1,242)} = 18.3$; $p < 0.0001$;

https://doi.org/10.1017/S2040174420001191 Published online by Cambridge University Press

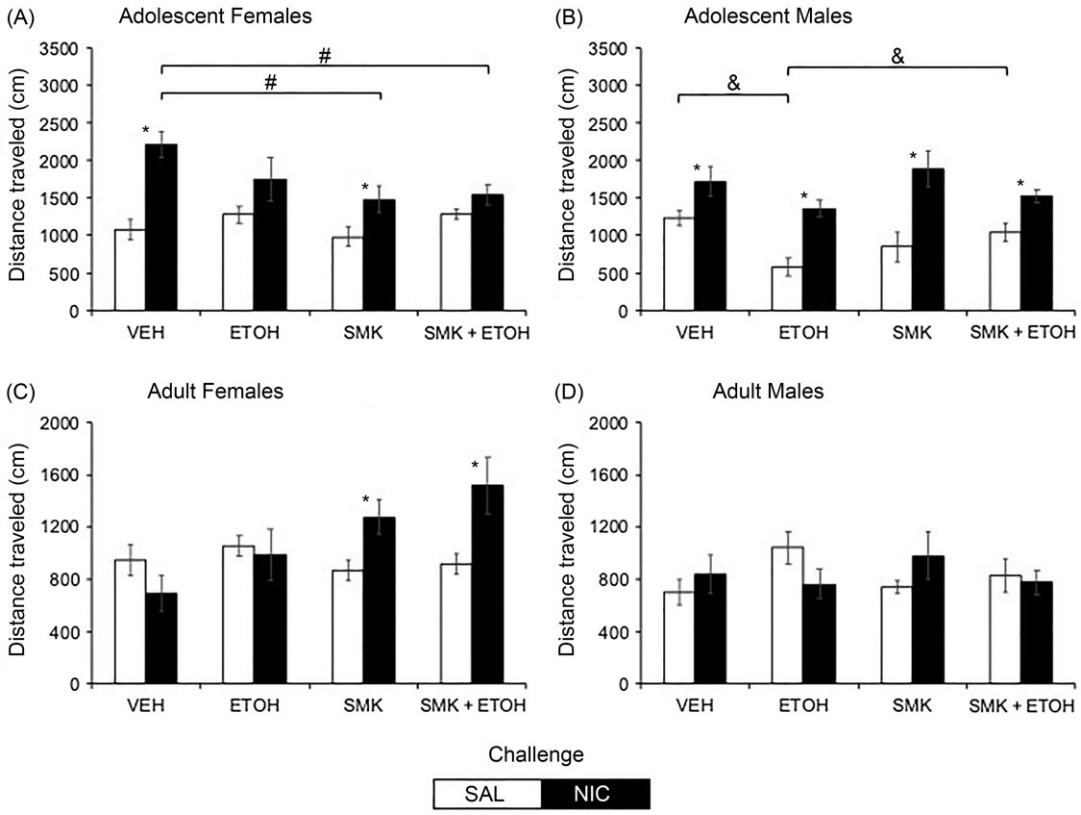


**Fig. 3.** Locomotor activity assessed as distance travelled in the Open Field test. Mice were exposed to tobacco smoke throughout the gestational period until PN10 and/or to ethanol (i.p. injection) every other day from PN2 to PN10. Animals were challenged with nicotine or saline and tested in the OF either during adolescence (A: females; B: males) or at adulthood (C: females; D: males). VEH, control group; ETOH, ethanol exposure group; SMK, tobacco smoke exposure group; SMK + ETOH, tobacco smoke and ethanol exposure group. SAL, saline challenge; NIC, Nicotine challenge. *$p < 0.05$, significant difference between SAL and NIC mice within each experimental group. #$p < 0.05$, comparisons among NIC-challenged animals. &$p < 0.05$, comparisons among SAL-challenged animals. Differences revealed by FPLSD.

Exposure × Challenge × Age: $F_{(3,242)} = 2.9$; $p = 0.034$) and Sex (Exposure × Challenge × Age × Sex: $F_{(3,242)} = 3.5$; $p = 0.015$) interactions. Accordingly, each age was analyzed separately.

Lower-order analyses (1-d ANOVAs) on each age further confirmed that in adolescent (PN30 – Exposure: $F_{(3.115)} = 3$; $p = 0.034$; Challenge: $F_{(1,115)} = 61.5$; $p < 0.0001$; Exposure × Sex: $F_{(3,115)} = 3.0$; $p = 0.037$) and adult (PN90 – Exposure × Challenge: $F_{(3,127)} = 3.4$; $p = 0.019$; Exposure × Challenge × Sex: $F_{(3,127)} = 2.8$; $p = 0.045$) mice, the impact of early exposure to the drugs of abuse and nicotine re-exposure on locomotor activity was sex-selective, therefore male and female data were analyzed separately.

In adolescent females, early exposure to the drugs of abuse failed to affect basal locomotion. However, as expected, the nicotine challenge resulted in a hyperlocomotor effect ($VEH_{SAL} < VEH_{NIC}$, Fig. 3A). Early exposure to the drugs of abuse interfered with this effect of nicotine: both tobacco smoke and the dual SMK + ETOH early exposures reduced the hyperlocomotor effect of the nicotine challenge ($VEH_{NIC} > SMK_{NIC}$, $VEH_{NIC} > SMK + ETOH_{NIC}$, respectively, Fig. 3A). The increase in locomotion was still significant in the SMK group ($SMK_{SAL} < SMK_{NIC}$) but not in the ETOH and SMK + ETOH ones ($ETOH_{SAL} = ETOH_{NIC}$; $SMK + ETOH_{SAL} = SMK + ETOH_{NIC}$) (Fig. 3A). This is consistent with the 2-d analysis, which showed that the effect of the dual exposure reflected the summation of the effects of tobacco smoke and ethanol (lack of Smoke × Ethanol interaction).

In adolescent males, early postnatal exposure to ethanol elicited a reduction in ambulation ($ETOH_{SAL} < VEH_{SAL}$, $ETOH_{SAL} < SMK + ETOH_{SAL}$, Fig. 3B). There were no differences in locomotor activity between VEH and SMK + ETOH groups ($VEH_{SAL} = SMK + ETOH_{SAL}$, Fig. 3B), which, together with the less-than-additive outcome of the 2-d analysis (Smoke × Ethanol: $F_{(1,28)} = 9.2$, $p = 0.005$) indicate that the dual exposure mitigated the effect of early ethanol exposure. Early exposure to the drugs of abuse failed to interfere with the hyperlocomotor effect of nicotine. Indeed, irrespective of the early exposure status, locomotor activity of adolescent male mice challenged with nicotine was higher than that of mice that were challenged with the saline injection (NIC vs. SAL groups, Fig. 3B), In addition, there were no significant differences between groups challenged with nicotine ($VEH_{NIC} = SMK_{NIC}$, $ETOH_{NIC}$ and $SMK + ETOH_{NIC}$, Fig. 3B.

In adult females, there were no lasting effects of early exposure to the drugs of abuse on basal locomotor activity ($VEH_{SAL} = SMK_{SAL}$, $ETOH_{SAL}$ and $SMK + ETOH_{SAL}$, Fig. 3C). However, early exposure still interfered with the response to the nicotine challenge, notwithstanding the pattern of effects was distinct from that identified in adolescent mice. While the nicotine challenge failed to result in a hyperlocomotor effect in control females ($VEH_{SAL} = VEH_{NIC}$), early exposure to the drugs of abuse potentiated nicotine effects: Both SMK and SMK + ETOH groups when exposed to acute nicotine, exhibited an hyperlocomotor profile ($VEH_{NIC} < SMK_{NIC}$, $VEH_{NIC} < SMK + ETOH_{NIC}$,

https://doi.org/10.1017/S2040174420001191 Published online by Cambridge University Press

respectively) (Fig. 3C). The 2-d analysis further indicated that the effect of the dual exposure reflected the summation of the effects of tobacco smoke and ethanol (lack of Smoke × Ethanol interaction). These results suggest increased susceptibility to nicotine due to early tobacco smoke and ethanol dual exposure.

In adult males, there were no significant alterations (Fig. 3D).

## Discussion

Despite evidence that pregnant smoking women are frequently intermittent consumers of alcoholic beverages[43,44] and that developmental exposure to either tobacco smoke or ethanol predisposes offspring to subsequent drug use[22,28–30], only limited information exists regarding the consequences of early co-exposure later in life. To the best of our knowledge, this is the first study that investigated the impact of a dual early developmental exposure to tobacco smoke and ethanol on a re-exposure to (challenge with) nicotine during adolescence and adulthood. The assessment of nicotine susceptibility is particularly relevant considering the fast increase of ENDS use among adolescents and young adults[32,33,61]. We showed that even a short-term intermittent exposure to ethanol, limited to the period which, in rodents, roughly corresponds to the third trimester of human gestation, when combined to chronic exposure to tobacco smoke during the period equivalent to the entire human gestation, increases nicotine susceptibility in a sex- and age-dependent manner, with stronger effects in adult females.

### *Body mass, cotinine and ethanol levels*

There is an inverse correlation between maternal cotinine blood levels, cotinine in umbilical cord and birth weight. Ivorra and collaborators[99] described that infants born to moderate smoking mothers have, on average, a 250 g reduction in body mass[99]. In the same direction, in our animal model, pups exposed to tobacco smoke (SMK and SMK + ETOH) weighted less and showed less body mass gain during postnatal exposure when compared to controls (VEH and ETOH). Nicotine is known to increase energy expenditure through sympathomimetic actions and suppress food intake by increasing leptin actions in the hypothalamus[100].

It should also be noted that dams exposed to tobacco smoke were lighter than control ones, which, *per se*, may have impacted the offspring response to nicotine. In this regard, there is evidence that, in rats, dams' undernourishment during the offspring perinatal development leads to an increased response of the mesocorticolimbic dopaminergic pathway to the rewarding effects of cocaine[101] and morphine at adulthood[102]. Despite that, nicotine susceptibility was not increased in adolescent mice[103]. Even though the models of undernutrition used in the aforementioned studies led to more severe effects on body mass than the small to moderate reductions identified in dams exposed to tobacco smoke in the current study, future studies are needed to investigate whether maternal and offspring undernutrition played a role in our results. Despite the deficits in body mass identified in the offspring during postnatal development, at adulthood, SMK males were heavier than controls. This finding mirrors epidemiological findings of increased body mass index in the offspring born to smoking mothers[104], possibly representing a programming effect. In this regard, the pathophysiology of nicotine-elicited obesity has been associated to hypothyroidism at adulthood[105]. While, in ETOH females, a small decrease in body mass was identified at PN90, there were no effects during exposure or at adolescence, which again suggests a programming effect of early exposure.

The assessment of cotinine levels confirmed that the level of exposure to tobacco smoke used in this study is equivalent to that of smokers[73–76]. As for ethanol blood concentrations, they are comparable to those a human fetus would be exposed to after maternal ingestion of a moderate to heavy dose of ethanol[83]. Most importantly, cotinine and ethanol levels did not differ between exposed groups, which indicate that pharmacokinetic interactions do not play a significant role in the outcomes of SMK + ETOH exposure. Interestingly, Lkhagvadorj and collaborators[106] described a male-only increased nicotine metabolism in both neonate and adult mice exposed to tobacco smoke throughout prenatal development[106]. In neonates, this effect was accompanied by a higher Cyp2a5 gene expression, which was correlated with higher DNA methylation. Besides, there is also evidence that hepatic drug metabolism may be altered in low birth weight rats, with reports of both increased and decreased P450 enzymes expression[107–109]. Despite the fact that our data failed to indicate pharmacokinetic interactions between SMK and ETOH by the end of exposure, considering that there is a positive association between nicotine metabolism and nicotine addiction[110,111], whether there are differences in nicotine pharmacokinetics between SMK and SMK + ETOH mice during adolescence and adulthood is worth further investigation.

### *Sex- and age-selective effects of both combined and single tobacco smoke and ethanol developmental exposures*

Studies in animal models suggest a mechanistic link between early developmental exposure to nicotine or ethanol and the offspring's susceptibility to drugs of abuse. The ionotropic nicotinic acetylcholine receptors (nAChRs) are the primary cellular mediators of nicotine's effects and ethanol influences directly the function of various ligand-gated ion channels, including nAChRs[112]. In mice, functional nAChRs are identified as early as the tenth gestational day[113]. Consistent with these receptors roles in many events that occur during the development of the central nervous system, including the modulation of cell proliferation, neuronal differentiation, synapse formation and maturation and neurotransmitter release, there is evidence of significant structural, functional and behavioral alterations evoked by developmental exposures to nicotine/tobacco smoke and ethanol[114–116].

Lasting effects of early exposure either to nicotine or ethanol in the mesocorticolimbic system have been reported[117,118], with potential profound impact in the response of this reward system to a second exposure later in life[38,119]. Nicotine-induced dopamine release in the nucleus accumbens is mediated, at least in part, by nAChRs[120]. Besides, it has been described that ethanol-induced stimulation of locomotor activity and mesolimbic dopamine system also involves nAChR activation[121,122]. Accordingly, inappropriate stimulation of the cholinergic system by exposure to tobacco smoke and ethanol could disrupt the normal course of brain development, particularly the dopamine reward system, leading to alterations in drug susceptibility later in life. To our knowledge, only two studies focused on the combined exposure to nicotine and ethanol and its consequences on nicotine self-administration and on the mesocorticolimbic system. Matta and Elberger[123] demonstrated that co-exposure during the period that corresponds to human pregnancy evokes a more intense nicotine self-administration at adulthood than early exposure to either nicotine or ethanol[123]. Subsequently, Roguski and collaborators[124] suggested that these effects are associated with the disrupted control of ventral tegmental dopaminergic circuitry by N-methyl-D-

https://doi.org/10.1017/S2040174420001191 Published online by Cambridge University Press

aspartate (NMDA) receptors of adolescent animals[124]. Both behavioral and neurochemical analysis of adolescent mice failed to include nicotine- and ethanol-only groups of exposure. Therefore, whether the combined exposure evoked stronger effects than either nicotine or ethanol still needs further investigation.

Our current study demonstrated that adolescent males and females early exposed to either tobacco smoke or ethanol were susceptible to the nicotine challenge; however, the impact of nicotine in SMK + ETOH mice was either equivalent or diminished when compared to that identified in mice early-exposed to either drug. Distinctively, at adulthood, an increased susceptibility to nicotine was identified only in females and only in the experimental groups that were early exposed to tobacco smoke. This effect was more pronounced in mice from the dual exposure group, which provided evidence that even an early short-term intermittent exposure to ethanol when combined to chronic exposure to tobacco smoke aggravates susceptibility to nicotine re-exposure later in life. A recent study from our group used an experimental protocol of early developmental exposure equivalent to the one used in the current study except that instead of tobacco smoke, mice were exposed to nicotine (chronic developmental exposure to nicotine combined with an intermittent ethanol exposure restricted to the brain growth spurt period)[46]. We demonstrated worsened outcomes of the dual exposure for some behavioral and neurochemical variables (locomotor activity, cortical cAMP and cortical and hippocampal cGMP levels) while, for others (memory/learning and hippocampal cAMP levels), the outcomes reflected less-than-additive effects[46]. These results suggest that, in the current study, nicotine present in tobacco smoke, *per se*, play a role in both the worsened and diminished responses in the dual exposure group when compared to either tobacco smoke or ethanol ones. However, the role of nonnicotine components of tobacco smoke cannot be ruled out[49–60] and, even though the large number of substances present in tobacco smoke and the lack of data on their psychoactive properties have been delaying progress in this area, recent evidence indicates that tobacco smoke components may interact with nicotine and/or ethanol in affecting the central nervous system[52,71,72]. In this regard, exposure of adult male rats to a cocktail of tobacco smoke minor alkaloids (nornicotine, cotinine, myosmine, anatabine, and anabasine), β-carbolines (harman and norharman) and acetaldehyde did not significantly enhance nicotine self-administration. However, tranylcypromine, an irreversible inhibitor of MAO-A and MAO-B, increased nicotine self-administration independent of other smoke constituents[125]. Exposure of rats to both ethanol and tobacco-specific nitrosamine synergistically decreased myelinated fiber density in the frontal cortex[126]. Interestingly, exposures restricted to the brain growth spurt resulted in adverse effects on cerebellar development and function; however, tobacco-specific nitrosamine and ethanol exerted independent effects which, in most cases, were not additive[127]. Considering that tobacco smoke contains thousands of components, these results underscore the need of further studies aiming to identify components of tobacco smoke that interact with nicotine and/or ethanol.

Sex differences in response to drugs of abuse, likely emerge as a result of the sexual differentiation of the brain, which begins during prenatal development and extends until the end of adolescence[128]. While the exact mechanisms underlying females' unique sensitivity are poorly understood, there is a growing body of evidence that drugs of abuse impact organizational effects of gonadal hormones on brain cells structure and function[117]. Interestingly, ligand-gated ion channels are influenced by gonadal hormones. Progesterone inhibits function of α4 containing nAChRs[129] and 17β-estradiol increases α7 nAChR subunit expression[130], providing potential mechanisms for both nicotine and ethanol nAChR-mediated sex-dependent effects. Consistent with this possibility, there is evidence that nicotine inhibits aromatase activity in human trophoblast[131] as well as in the brain of male offspring exposed during prenatal development[132,133]. Nicotine direct action on the adrenal gland during the prenatal development of rats leads to increased corticosterone levels, which, in turn, diminishes the pivotal testosterone surge during the perinatal period[132,133]. Increased corticosterone levels were also identified in adolescent females exposed to ethanol during prenatal development, with a positive correlation between corticosterone and estradiol levels[134].

Common targets of nicotine and ethanol suggest that the increased nicotine susceptibility of adult females submitted to a dual tobacco smoke and ethanol exposure during early development is due to the additive effects of these drugs, which have already been demonstrated when other behavioral and neurochemical endpoints were investigated[46]. As discussed above, sex steroid hormones may be important mediators of both tobacco smoke and ethanol differential effects through sex-dependent interactions during critical periods of development, possibly leading to relevant organizational alterations of gonadal hormones on neuronal function in brain regions important to motivation and reward. Future studies are needed to investigate the mechanisms involved. There is evidence that the phase of the estrous cycle could impact behavioral outcomes[135,136]. However, the fact that the age of vaginal opening and of first estrous in small rodents typically occur between PN32 and PN36 indicates that in the current study, it would only be meaningful to verify the phase of the cycle in adult females. In this case, the extra manipulation (to collect the vaginal smears) only of adult females would add an undesirable confounding factor to the analysis. Despite this limitation of the study, there was no evidence that female data variability was higher than that observed for males. In addition, most of our results were female-only. Considering the aforementioned, it seems that any putative variation in behavior associated with the estrous cycle that might have been present in our study was not sufficient to hinder the results from being identified.

## Conclusions

Adult female mice exposed to ethanol during the brain growth spurt and to tobacco smoke during the period equivalent to human gestation are more susceptible to nicotine re-exposure at adulthood, representing a worsened outcome from the dual exposure. In spite of the fact that generalizations based on the results from preclinical studies should be considered with care, our data suggest that if a similar effect occurs in humans, smoking and drinking alcoholic beverages during gestation may predispose the use/abuse of nicotine later in life. In this regard, future studies that aim to investigate whether maternal exposure to these drugs of abuse during gestation contributes to the burst of ENDS use worldwide are warranted.

**Supplementary material.** To view supplementary material for this article, please visit https://doi.org/10.1017/S2040174420001191

**Acknowledgements.** The authors are thankful to Ulisses Risso for animal care.

**Financial Support.** This work was supported by fellowships from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-BRAZIL); Conselho Nacional de Desenvolvimento Científico e Tecnológico

(CNPq-BRAZIL) and Sub-Reitoria de Pós-Graduação e Pesquisa da Universidade do Estado do Rio de Janeiro (SR2-UERJ) and grants from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ-BRAZIL, E-26/110.211/2013 and E-26/201.455/2014 to YA-V) and CNPq-BRAZIL (304332/2016-0 to YA-V).

**Conflicts of Interest.** None.

**Ethical standards.** The authors assert that all procedures contributing to this work comply with the ethical standards of the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health and has been approved by the Animal Care and Use Committee of the Universidade do Estado do Rio de Janeiro (CEUA0312012).

## References

1. Mullen PD. How can more smoking suspension during pregnancy become lifelong abstinence? Lessons learned about predictors, interventions, and gaps in our accumulated knowledge. *Nicotine Tob Res*. 2004; 6(Suppl. 2). doi: 10.1080/14622200410001669150
2. Herzberg J, Barrier B, Sprague DJ, Vinson DC. Substance use in women of reproductive age. *Mo Med*. 2016; 113(3), 182–186.
3. Denny CH, Tsai J, Floyd RL, Green PP. Alcohol use among pregnant and nonpregnant women of childbearing age – United States, 1991–2005. *Morb Mortal Wkly Rep*. 2009; 58(19), 529–532.
4. Reitan T, Callinan S. Changes in smoking rates among pregnant women and the general female population in Australia, Finland, Norway, and Sweden. *Nicotine Tob Res*. 2017; 19(3), 282–289. doi: 10.1093/ntr/ntw188
5. McHugh RK, Wigderson S, Greenfield SF. Epidemiology of substance use in reproductive-age women. *Obstet Gynecol Clin North Am*. 2014; 41(2), 177–189. doi: 10.1016/j.ogc.2014.02.001
6. Hammer JH, Parent MC, Spiker DA, World Health Organization. Global status report on alcohol and health 2018. Vol. 65; 2018. doi:10.1037/cou0000248
7. Meschke LL, Holl JA, Messelt S. Assessing the risk of fetal alcohol syndrome: understanding substance use among pregnant women. *Neurotoxicol Teratol*. Published online 2003. doi: 10.1016/j.ntt.2003.07.004
8. Pauly JR, Slotkin TA. Maternal tobacco smoking, nicotine replacement and neurobehavioural development. *Acta Paediatr Int J Paediatr*. 2008; 97(10), 1331–1337. doi: 10.1111/j.1651-2227.2008.00852.x
9. Bruin JE, Gerstein HC, Holloway AC. Long-term consequences of fetal and neonatal nicotine exposure: a critical review. *Toxicol Sci*. 2010; 116(2), 364–374. doi: 10.1093/toxsci/kfq103
10. Tong VT, Dietz PM, Morrow B, *et al.* Trends in smoking before, during, and after pregnancy – pregnancy risk assessment monitoring system, United States, 40 sites, 2000-2010. *MMWR Surveill Summ*. 2013; 62(6), 1–19.
11. Mendelsohn C, Gould GS, Oncken C. Management of smoking in pregnant women. *Aust Fam Physician*. 2014; 43(1), 46–51.
12. Curtin SC, Matthews TJ. Smoking prevalence and cessation before and during pregnancy: data from the birth certificate, 2014. *Natl Vital Stat Rep*. 2016; 65(1), 1–14.
13. Senecky Y, Weiss N, Shalev SA, *et al.* Alcohol consumption during pregnancy among women in israel. *Can J Clin Pharmacol*. 2011; 18(2), 261–272.
14. Balachova T, Bonner B, Chaffin M, *et al.* Women's alcohol consumption and risk for alcohol-exposed pregnancies in Russia. *Addiction*. 2012; 107(1), 109–117. doi: 10.1111/j.1360-0443.2011.03569.x
15. Balachova T, Shaboltas A, Nasledov A, *et al.* Alcohol and HIV risk among Russian women of childbearing age. *AIDS Behav*. 2017; 21(7), 1857–1867. doi: 10.1007/s10461-016-1542-3
16. Spohr HL, Willms J, Steinhausen HC. Fetal alcohol spectrum disorders in young adulthood. *J Pediatr*. Published online 2007. doi: 10.1016/j.jpeds.2006.11.044
17. Welch-Carre E. The neurodevelopmental consequences of prenatal alcohol exposure. *Adv Neonatal Care*. 2005; 5(4), 217–229. doi: 10.1016/j.adnc.2005.04.007
18. Kuja-Halkola R, D'Onofrio BM, Larsson H, Lichtenstein P. Maternal smoking during pregnancy and adverse outcomes in offspring : genetic and environmental sources of covariance. *Behav Genet*. 2014; 44(5), 456–467. doi: 10.1007/s10519-014-9668-4
19. Eskenazf B, Trupln LS. Passive and active maternal smoking during pregnancy, as measured by Serum Cotinine, and postnatal smoke exposure. II. Effects on neurodevelopment at age 5 years. *Am Joumal Epidemiol*. 1995; 142(9).
20. Fergusson D, Woodward L, Horwood J. Maternal smoking during pregnancy and psychiatric adjustment in youngf adulthood. *Arch Gen Psychiatry*. 1998; 55(8), 721–727.
21. Burd L, Klug MG, Martsolf JT, Kerbeshian J. Fetal alcohol syndrome: Neuropsychiatric phenomics. *Neurotoxicol Teratol*. 2003; 25(6), 697–705. doi: 10.1016/j.ntt.2003.07.014
22. Rasmussen C. Executive functioning and working memory in fetal alcohol spectrum disorder. *Alcohol Clin Exp Res*. 2005; 29(8), 1359–1367. doi: 10.1097/01.alc.0000175040.91007.d0
23. 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. doi: 10.1016/j.neubiorev.2016.11.013
24. Marquardt K, Brigmana JL. The impact of prenatal alcohol exposure on social, cognitive and affective behavioral domains: insights from rodent models. *Physiol Behav*. 2016; 51(1), 1–15. doi: 10.1016/j.physbeh.2017.03.040
25. Sabzalizadeh M, Afarinesh MR, Mafi F, *et al.* Alcohol and nicotine co-Administration during pregnancy and lactation periods alters sensory discrimination of adult NMRI mice offspring. *Physiol Behav*. 2020; 213(October 2019). doi: 10.1016/j.physbeh.2019.112731
26. Stephen JM, Ph D, Kodituwakku PW, *et al.* Delays in auditory processing identified in preschool children with FASD Julia. *Alcohol Clin Exp Res*. 2012; 36(10), 1720–1727. doi: 10.1111/j.1530-0277.2012.01769.x.Delays
27. Mccartney JS, Fried PA, Watkinson B. Central auditory processing in school-age children prenatally exposed to cigarette smoke. *Neurotoxicol Teratol*. 1994; 16(3), 269–276. doi: 10.1016/0892-0362(94)90048-5
28. Kandel DB, Wu P, Davies M. Maternal smoking during pregnancy and smoking by adolescent daughters. *Am J Public Health*. 1994; 84(9), 1407–1413. doi: 10.2105/AJPH.84.9.1407
29. Buka SL, Shenassa ED, Niaura R. Elevated risk of tobacco dependence among offspring of mothers who smoked during pregnancy: A 30-year prospective study. *Am J Psychiatry*. 2003; 160(11), 1978–1984. doi: 10.1176/appi.ajp.160.11.1978
30. Oncken C, McKee S, Krishnan-Sarin S, O'Malley S, Mazure C. Gender effects of reported in utero tobacco exposure on smoking initiation, progression and nicotine dependence in adult offspring. *Nicotine Tob Res*. 2004; 6(5), 829–833. doi: 10.1080/1462220042000282555
31. Wang TW, Gentzke A, Sharapova S, Cullen KA, Ambrose BK, Jamal A. Tobacco use among middle and high school students - United States, 2011-2016 Morbidity and Mortality Weekly Report Tobacco. *Morb Mortal Wkly Rep*. 2018; 66(28), 765.
32. Gentzke AS, Creamer M, Cullen KA, *et al.* Vital signs: tobacco product use among middle and high school students – United States, 2011–2018. *Morb Mortal Wkly Rep*. 2019; 68(6), 157–164. doi: 10.15585/mmwr.mm6839a2
33. Miliano C, Scott ER, Murdaugh LB, *et al.* Modeling drug exposure in rodents using e-cigarettes and other electronic nicotine delivery systems. *J Neurosci Methods*. 2019; 330, 108458. doi: 10.1016/j.jneumeth.2019.108458
34. Kaleta D, Niedzin M, Jankowska A, Polańska K. Predictors of e-cigarette use susceptibility—A study of young people from a socio-economically disadvantaged rural area in Poland. *Int J Environ Res Public Health*. 2019; 16(20), 1–11. doi: 10.3390/ijerph16203935
35. Baer JS, Barr HM, Bookstein FL, Sampson PD, Streissguth AP. Prenatal alcohol exposure and family history of alcoholism in the etiology of adolescent alcohol problems. *J Stud Alcohol*. 1998; 59(5), 533–543. doi: 10.15288/jsa.1998.59.533
36. Baer JS, Sampson PD, Barr HM, Connor PD, Streissguth AP. A 21-year longitudinal analysis of the effects of prenatal alcohol exposure on young

adult drinking. *Arch Gen Psychiatry*. 2003; 60(4), 377–385. doi:10.1001/archpsyc.60.4.377\r60/4/377[pii]
37. Yates WR, Cadoret RJ, Troughton EP, Stewart M, Giunta TS. Effect of fetal alcohol exposure on adult symptoms of nicotine, alcohol, and drug dependence. *Alcohol Clin Exp Res*. 1998; 22(4), 914–920. doi: 10.1111/j.1530-0277.1998.tb03889.x
38. Levin ED, Lawrence S, Petro A, Horton K, Seidler FJ, Slotkin TA. Increased nicotine self-administration following prenatal exposure in female rats. *Pharmacol Biochem Behav*. 2006; 85(3), 669–674. doi: 10.1016/j.pbb.2006.11.006
39. Schneider S, Schutz J. Who smokes during pregnancy? A systematic literature review of population-based surveys conducted in developed countries between 1997 and 2006. *Eur J Contracept Reprod Heal Care*. 2008; 13(2), 138–147. doi: 10.1080/13625180802027993
40. Chotro MG, Arias C, Laviola G. Increased ethanol intake after prenatal ethanol exposure: Studies with animals. *Neurosci Biobehav Rev*. 2007; 31(2), 181–191. doi: 10.1016/j.neubiorev.2006.06.021
41. Slater ME, Haughwout SP, Castle IP. Trends in substance use among reproductive-age females in the United States, 2002–2013. 2015.
42. Delgado-Lobete L, Montes-Montes R, Vila-Paz A, *et al.* Individual and environmental factors associated with tobacco smoking, alcohol abuse and illegal drug consumption in university students: A mediating analysis. *Int J Environ Res Public Health*. 2020; 17(9), 4–7. doi: 10.3390/ijerph17093019
43. Dierker L, Lloyd-Richardson E, Stolar M, *et al.* The proximal association between smoking and alcohol use among first year college students. *Drug Alcohol Depend*. Published online 2006. doi: 10.1016/j.drugalcdep.2005.05.012
44. Weitzman ER, Chen YY. The co-occurrence of smoking and drinking among young adults in college: national survey results from the United States. *Drug Alcohol Depend*. Published online 2005. doi: 10.1016/j.drugalcdep.2005.05.008
45. Walker MJ, Al-Sahab B, Islam F, Tamim H. The epidemiology of alcohol utilization during pregnancy: an analysis of the Canadian Maternity Experiences Survey (MES). *BMC Pregnancy Childbirth*. 2011; 11(1), 52. doi: 10.1186/1471-2393-11-52
46. Abreu-Villaça Y, Carvalho-Graca AC, Skinner G, *et al.* Hyperactivity and memory/learning deficits evoked by developmental exposure to nicotine and/or ethanol are mitigated by cAMP and cGMP signaling cascades activation. *Neurotoxicology*. 2018; 66(2010), 150–159. doi: 10.1016/j.neuro.2018.04.003
47. Ribeiro-Carvalho A, Lima CS, Medeiros AH, *et al.* Combined exposure to nicotine and ethanol in adolescent mice: effects on the central cholinergic systems during short and long term withdrawal. *Neuroscience*. 2009; 162(4), 1174–1186. doi: 10.1016/j.neuroscience.2009.05.032
48. Maggio SE, Saunders MA, Nixon K, *et al.* An improved model of ethanol and nicotine co-use in female P rats: Effects of naltrexone, varenicline, and the selective nicotinic α6β2* antagonist r-bPiDI. *Drug Alcohol Depend*. 2018; 193, 154–151. doi: 10.1016/j.drugalcdep.2018.09.008
49. Abreu-Villaça Y, Filgueiras CC, Guthierrez M, *et al.* Exposure to tobacco smoke containing either high or low levels of nicotine during adolescence: Differential effects on choline uptake in the cerebral cortex and hippocampus. *Nicotine Tob Res*. 2010; 12(7), 776–780. doi: 10.1093/ntr/ntq075
50. Abreu-Villaça Y, Filgueiras CC, Correa-Santos M, *et al.* Tobacco smoke containing high or low levels of nicotine during adolescence: effects on novelty-seeking and anxiety-like behaviors in mice. *Psychopharmacology (Berl)*. 2015; 232(10), 1693–1703. doi: 10.1007/s00213-014-3801-1
51. Abreu-Villaça Y, Correa-Santos M, Dutra-Tavares AC, *et al.* A ten fold reduction of nicotine yield in tobacco smoke does not spare the central cholinergic system in adolescent mice. *Int J Dev Neurosci*. 2016; 52, 93–103. doi: 10.1016/j.ijdevneu.2016.06.002
52. Abreu-Villaça Y, Guimarães VMS, Nunes-Freitas A, *et al.* Tobacco smoke and ethanol during adolescence: Both combined- and single-drug exposures lead to short- and long-term disruption of the serotonergic system in the mouse brain. *Brain Res Bull*. 2019; 146(October 2018), 94–103. doi: 10.1016/j.brainresbull.2018.12.007
53. Anthenelli RM. Recent advances in the treatment of tobacco dependence. *Clin Neurosci Res*. 2005; 5(2–4), 175–183. doi: 10.1016/j.cnr.2005.08.014
54. Belluzzi JD, Wang R, Leslie FM. Acetaldehyde enhances acquisition of nicotine self-administration in adolescent rats. *Neuropsychopharmacology*. 2005; 30(4), 705–712. doi: 10.1038/sj.npp.1300586
55. Brennan KA, Putt F, Truman P. Nicotine-, tobacco particulate matter- and methamphetamine-produced locomotor sensitisation in rats. *Psychopharmacology (Berl)*. 2013; 228(4), 659–672. doi: 10.1007/s00213-013-3071-3
56. Bruijnzeel AW, Ford J, Rogers JA, *et al.* Blockade of CRF1 receptors in the central nucleus of the amygdala attenuates the dysphoria associated with nicotine withdrawal in rats. *Pharmacol Biochem Behav*. 2012; 101(1), 62–68. doi: 10.1016/j.pbb.2011.12.001
57. Hall BJ, Wells C, Allenby C, *et al.* Differential effects of non-nicotine tobacco constituent compounds on nicotine self-administration in rats. *Pharmacol Biochem Behav*. 2014; 120, 103–108. doi: 10.1016/j.pbb.2014.02.011
58. Hoffman AC, Evans SE. Abuse potential of non-nicotine tobacco smoke components: acetaldehyde, nornicotine, cotinine, and anabasine. *Nicotine Tob Res*. 2013; 15(3), 622–632. doi: 10.1093/ntr/nts192
59. Pickworth WB, Fant R V., Nelson RA, Rohrer MS, Henningfield JE. Pharmacodynamic effects of new de-nicotinized cigarettes. *Nicotine Tob Res*. 1999; 1(4), 357–364. doi: 10.1080/14622299050011491
60. Villégier AS, Gallager B, Heston J, Belluzzi JD, Leslie FM. Age influences the effects of nicotine and monoamine oxidase inhibition on mood-related behaviors in rats. *Psychopharmacology (Berl)*. 2010; 208(4), 593–601. doi: 10.1007/s00213-009-1760-8
61. Chadi N, Hadland SE, Harris SK. Understanding the implications of the "vaping epidemic" among adolescents and young adults: a call for action. *Subst Abus*. 2019; 40(1), 7–10. doi: 10.1080/08897077.2019.1580241
62. Cavallo DA, Krishnan-Sarin S. Nicotine use disorders in adolescents. *Pediatr Clin North Am*. 2019; 66(6), 1053–1062. doi: 10.1016/j.pcl.2019.08.002
63. Kandel ER, Kandel DB. A molecular basis for nicotine as a gateway drug. *N Engl J Med*. 2014; 371(10), 932–943. doi: 10.1056/NEJMsa1405092
64. Schramm-Sapyta NL, Walker QD, Caster JM, Levin ED, Kuhn CM. Are adolescents more vulnerable to drug addiction than adults? Evidence from animal models. *Psychopharmacology (Berl)*. 2009; 206(1), 1–21. doi: 10.1007/s00213-009-1585-5
65. Yuan M, Cross SJ, Loughlin SE, Leslie FM. Nicotine and the adolescent brain. *J Physiol*. 2015; 593(16), 3397–3412. doi: 10.1113/JP270492
66. Keyes KM, Grant BF, Hasin DS. Evidence for a closing gender gap in alcohol use, abuse, and dependence in the United States population. *Drug Alcohol Depend*. 2008; 93(1-2), 21–29. doi: 10.1016/j.drugalcdep.2007.08.017
67. Dazzi L, Seu E, Cherchi G, Barbieri PP, Matzeu A, Biggio G. Estrous cycle-dependent changes in basal and ethanol-induced activity of cortical dopaminergic neurons in the rat. *Neuropsychopharmacology*. 2007; 32(4), 892–901. doi: 10.1038/sj.npp.1301150
68. Lynch WJ. Sex differences in vulnerability to drug self-administration. *Exp Clin Psychopharmacol*. 2006; 14(1), 34–41. doi: 10.1037/1064-1297.14.1.34
69. Lynch WJ, Sofuoglu M. Role of progesterone in nicotine addiction: evidence from initiation to relapse. *Exp Clin Psychopharmacol*. 2010; 18(6), 451–461. doi: 10.1037/a0021265.Role
70. Tanapat P, Hastings NB, Reeves AJ, Gould E. Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. *J Neurosci*. 1999; 19(14), 5792–5801. doi: 10.1523/JNEUROSCI.19-14-05792.1999
71. Abreu-Villaça Y, Cavina CC, Ribeiro-Carvalho A, *et al.* Combined exposure to tobacco smoke and ethanol during adolescence leads to short- and long-term modulation of anxiety-like behavior. *Drug Alcohol Depend*. 2013; 133(1), 52–60. doi: 10.1016/j.drugalcdep.2013.05.033
72. Abreu-Villaça Y, de Carvalho Graça AC, Ribeiro-Carvalho A, de Freitas Naiff V, Manhães AC, Filgueiras CC. Combined exposure to tobacco smoke and ethanol in adolescent mice elicits memory and learning deficits

both during exposure and withdrawal. *Nicotine Tob Res*. 2013; 15(7), 1211–1221. doi: 10.1093/ntr/nts250
73. Christensen LH, Høyer BB, Pedersen HS, *et al.* Prenatal smoking exposure, measured as maternal serum cotinine, and children's motor developmental milestones and motor function: A follow-up study. *Neurotoxicology*. 2016; 53, 236–245. doi: 10.1016/j.neuro.2016.02.007
74. Król MK, Florek E, Piekoszewski W, Bokiniec R, Kornacka MK. The impact of intrauterine tobacco exposure on the cerebral mass of the neonate based on the measurement of head circumference. *Brain Behav*. 2012; 2(3), 243–248. doi: 10.1002/brb3.49
75. Mamsen LS, Jönsson BAG, Lindh CH, *et al.* Concentration of perfluorinated compounds and cotinine in human foetal organs, placenta, and maternal plasma. *Sci Total Environ*. 2017; 596–597, 97–105. doi: 10.1016/j.scitotenv.2017.04.058
76. Wrześniak M, Królik M, Kepinska M, Milnerowicz H. The influence of maternal smoking on transferrin sialylation and fetal biometric parameters. *Environ Toxicol Pharmacol*. 2016; 47, 100–107. doi: 10.1016/j.etap.2016.09.008
77. Livy DJ, Maier SE, West JR. Fetal alcohol exposure and temporal vulnerability: Effects of binge-like alcohol exposure on the ventrolateral nucleus of the thalamus. *Alcohol Clin Exp Res*. 2001; 25(5), 774–780. doi: 10.1111/j.1530-0277.2001.tb02278.x
78. Nunes F, Ferreira-Rosa K, Pereira M dos S, *et al.* Acute administration of vinpocetine, a phosphodiesterase type 1 inhibitor, ameliorates hyperactivity in a mice model of fetal alcohol spectrum disorder. *Drug Alcohol Depend*. 2011; 119(1–2), 81–87. doi: 10.1016/j.drugalcdep.2011.05.024
79. Tokunaga S, Silvers JM, Matthews DB. Chronic intermittent ethanol exposure during adolescence blocks ethanol-induced inhibition of spontaneously active hippocampal pyramidal neurons. *Alcohol Clin Exp Res*. 2006; 30(1), 1–6. doi: 10.1111/j.1530-0277.2006.00020.x
80. White AM, Ghia AJ, Levin ED, Scott Swartzwelder H. Binge pattern ethanol exposure in adolescent and adult rats: Differential impact on subsequent responsiveness to ethanol. *Alcohol Clin Exp Res*. 2000; 24(8), 1251–1256. doi: 10.1111/j.1530-0277.2000.tb02091.x
81. Filgueiras CC, Krahe TE, Medina AE. Phosphodiesterase type 1 inhibition improves learning in rats exposed to alcohol during the third trimester equivalent of human gestation. *Neurosci Lett*. 2010; 473(3), 202–207. doi: 10.1016/j.neulet.2010.02.046
82. Eckardt MJ, File SE, Gessa GL, *et al.* Effects of moderate alcohol consumption on the central nervous system. *Alcohol Clin Exp Res*. 1998; 22(5), 998–1040. doi: 10.1111/j.1530-0277.1998.tb03695.x
83. Clancy B, Finlay BL, Darlington RB, Anand K. Extrapolating brain development from experimental species to humans. *Neurotoxicology*. 2007; 28(5), 931–937. doi: 10.1016/j.neuro.2007.01.014.EXTRAPOLATING
84. Quinn GE. The "ideal" management of retinopathy of prematurity. *Eye*. 2005; 19(10), 1044–1049. doi: 10.1038/sj.eye.6701960
85. Bandeira F, Lent R, Herculano-Houzel S. Changing numbers of neuronal and non-neuronal cells underlie postnatal brain growth in the rat. *Proc Natl Acad Sci U S A*. 2009; 106(33), 14108–14113. doi: 10.1073/pnas.0804650106
86. Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum Dev*. 1979; 3(1), 79–83. doi: 10.1016/0378-3782(79)90022-7
87. Dwyer JB, McQuown SC, Leslie FM. The dynamic effects of nicotine on the developing brain. *Pharmacol Ther*. 2009; 122(2), 125–139. doi: 10.1038/jid.2014.371
88. Ethen MK, Ramadhani TA, Scheuerle AE, *et al.* Alcohol consumption by women before and during pregnancy. *Matern Child Heal J*. 2009; 13(2), 274–285. doi: 10.1007/s10995-008-0328-2
89. Gil-Mohapel J, Boehme F, Kainer L, Christie BR. Hippocampal cell loss and neurogenesis after fetal alcohol exposure: Insights from different rodent models. *Brain Res Rev*. 2010; 64(2), 283–303. doi: 10.1016/j.brainresrev.2010.04.011
90. Olney JW, Tenkova T, Dikranian K, Qin YQ, Labruyere J, Ikonomidou C. Ethanol-induced apoptotic neurodegeneration in the developing C57BL/6 mouse brain. *Dev Brain Res*. 2002; 133(2), 115–126. doi: 10.1016/S0165-3806(02)00279-1
91. Thomas JD, Fleming SL, Riley EP. MK-801 can exacerbate or attenuate behavioral alterations associated with neonatal alcohol exposure in the rat, depending on the timing of administration. *Alcohol Clin Exp Res*. 2001; 25(5), 764–773. doi: 10.1111/j.1530-0277.2001.tb02277.x
92. Tzschentke TM. Measuring reward with the conditioned place preference paradigm: A comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol*. 1998; 56(6), 613–672. doi: 10.1016/S0301-0082(98)00060-4
93. Yararbas G, Keser A, Kanit L, Pogun S. Nicotine-induced conditioned place preference in rats: Sex differences and the role of mGluR5 receptors. *Neuropharmacology*. 2010; 58(2), 374–382. doi: 10.1016/j.neuropharm.2009.10.001
94. Mineur YS, Brunzell DH, Grady SR, *et al.* Localized low-level re-expression of high-affinity mesolimbic nicotinic acetylcholine receptors restores nicotine-induced locomotion but not place conditioning. *Genes, Brain Behav*. 2009; 8(3), 257–266. doi: 10.1111/j.1601-183X.2008.00468.x
95. Museo E, Wise RA. Sensitization of locomotion following repeated ventral tegmental injections of cytisine. *Pharmacol Biochem Behav*. 1994; 48(2), 521–524. doi: 10.1016/0091-3057(94)90563-0
96. Abreu-Villaça Y, Medeiros AH, Lima CS, Faria FP, Filgueiras CC, Manhães AC. Combined exposure to nicotine and ethanol in adolescent mice differentially affects memory and learning during exposure and withdrawal. *Behav Brain Res*. 2007; 181(1), 136–146. doi: 10.1016/j.bbr.2007.03.035
97. Slotkin TA, Skavicus S, Ko A, Levin ED, Seidler FJ. Corrigendum to: The developmental neurotoxicity of tobacco smoke can be mimicked by a combination of nicotine and benzo[a]pyrene: Effects on cholinergic and serotonergic systems (Toxicological Sciences (2019) 167, 1 (293-304) DOI: 10.1093/toxsci/kfy241). *Toxicol Sci*. 2019; 168(1), 280. doi: 10.1093/toxsci/kfy304
98. Wainwright PE. Issues of design and analysis relating to the use of multiparous species in developmental nutritional studies. *J Nutr*. 1998 Mar; 128(3), 661–3. doi: 10.1093/jn/128.3.661
99. Ivorra C, García-Vicent C, Ponce F, Ortega-Evangelio G, Fernández-Formoso JA, Lurbe E. High cotinine levels are persistent during the first days of life in newborn second hand smokers. *Drug Alcohol Depend*. 2014; 134(1), 275–279. doi: 10.1016/j.drugalcdep.2013.10.017
100. Audrain-Mcgovern J, Benowitz NL. Cigarette smoking, nicotine, and body weight. *Clin Pharmacol Ther*. 2011; 90(1), 164–168. doi: 10.1038/clpt.2011.105
101. Valdomero A, Bussolino DF, Orsingher OA, Cuadra GR. Perinatal protein malnutrition enhances rewarding cocaine properties in adult rats. *Neuroscience*. 2006; 137(1), 221–229. doi: 10.1016/j.neuroscience.2005.08.055
102. Velazquez EE, Valdomero A, Orsingher OA, Cuadra GR. Perinatal undernutrition facilitates morphine sensitization and cross-sensitization to cocaine in adult rats: a behavioral and neurochemical study. *Neuroscience*. 2010; 165(2), 475–484. doi: 10.1016/j.neuroscience.2009.10.061
103. Dutra-Tavares AC, Manhães AC, Silva JO, *et al.* Locomotor response to acute nicotine in adolescent mice is altered by maternal undernutrition during lactation. *Int J Dev Neurosci*. 2015; 47(Pt B), 278–285. doi: 10.1016/j.ijdevneu.2015.10.002
104. Goldani MZ, Haeffner LSB, Agranonik M, Barbieri MA, Bettiol H, da Silva AAM. Do early life factors influence body mass index in adolescents? *Brazilian J Med Biol Res*. 2007; 40(9), 1231–1236. doi: 10.1590/S0100-879X2006005000131
105. Oliveira E, Moura EG, Santos-Silva AP, *et al.* Short- and long-term effects of maternal nicotine exposure during lactation on body adiposity, lipid profile, and thyroid function of rat offspring. *J Endocrinol*. 2009; 202(3), 397–405. doi: 10.1677/JOE-09-0020
106. Lkhagvadorj K, Meyer KF, Verweij LP, *et al.* Prenatal smoke exposure induces persistent *Cyp2a5* methylation and increases nicotine metabolism in the liver of neonatal and adult male offspring. *Epigenetics*. 2020; 23, 1–16. doi: 10.1080/15592294.2020.1782655
107. Ni S, Wang X, Wang J, *et al.* Effects of intrauterine undernutrition on the expression of CYP3A23/3A1, PXR, CAR and HNF4alpha in neonate rats. *Biopharm Drug Dispos*. 2008; 29(9), 501–510. doi: 10.1002/bdd.635
108. Soo JY, Wiese MD, Berry MJ, McMillen IC, Morrison JL. Intrauterine growth restriction may reduce hepatic drug metabolism in the early

neonatal period. *Pharmacol Res*. 2018; 134, 68–78. doi: 10.1016/j.phrs.2018.06.003
109. Zhu ZW, Ni SQ, Wang XM, Wang J, Zeng S, Zhao ZY. Hepatic CYP3A expression and activity in low birth weight developing female rats. *World J Pediatr*. 2013; 9(3), 266–272. doi: 10.1007/s12519-013-0429-x
110. Benowitz NL. Nicotine addiction. *N Engl J Med*. 2010; 362(24), 2295–2303. doi: 10.1056/NEJMra0809890
111. Ho MK, Mwenifumbo JC, Al Koudsi N, *et al.* Association of nicotine metabolite ratio and CYP2A6 genotype with smoking cessation treatment in African-American light smokers. *Clin Pharmacol Ther*. 2009; 85(6), 635–643. doi: 10.1038/clpt.2009.19
112. Grant KA. Emerging neurochemical concepts in the actions of ethanol at ligand-gated ion channels. *Behav Pharmacol*. 1994; 5(4–5), 383–404. doi: 10.1097/00008877-199408000-00003
113. Abreu-Villaça Y, Filgueiras CC, Manhães AC. Developmental aspects of the cholinergic system. *Behav Brain Res*. 2011; 221(2), 367–378. doi: 10.1016/j.bbr.2009.12.049
114. Abreu-Villaça Y, Manhães AC, Krahe TE, Filgueiras CC, Ribeiro-Carvalho A. Tobacco and alcohol use during adolescence: Interactive mechanisms in animal models. *Biochem Pharmacol*. 2017; 144, 1–17. doi: 10.1016/j.bcp.2017.06.113
115. Levin ED, Abreu-Villaça Y. Developmental neurotoxicity of nicotine and tobacco. In *Handbook of Developmental Neurotoxicology*, Vol 1, 2nd ed., (eds. William Slikker J, Paule MG, Wang C), 2018; 439–452. Academic Press. doi: 10.1016/C2015-0-04830-4
116. Lotfullina N, Khazipov R. Ethanol and the developing brain: inhibition of neuronal activity and neuroapoptosis. *Neuroscientist*. 2018; 24(2), 130–141. doi: 10.1177/1073858417712667
117. Carneiro LMV, Diógenes JPL, Vasconcelos SMM, *et al.* Behavioral and neurochemical effects on rat offspring after prenatal exposure to ethanol. *Neurotoxicol Teratol*. 2005; 27(4), 585–592. doi: 10.1016/j.ntt.2005.06.006
118. Oliff HS, Gallardo KA. [Frontiers in Bioscience 4, d883-897, December 1, 1999] the effect of nicotine on developing brain catecholamine systems Heather S. Oliff and Kathy A. Gallardo. Published online 1999, 883–897.
119. Klein LC, Stine MM, Pfaff DW, Vandenbergh DJ. Maternal nicotine exposure increases nicotine preference in periadolescent male but not female C57B1/6J mice. *Nicotine Tob Res*. 2003; 5(1), 117–124. doi: 10.1080/14622200307257
120. Fu Y, Matta SG, Gao W, Sharp BM. Local α-bungarotoxin-sensitive nicotinic receptors in the nucleus accumbens modulate nicotine-stimulated dopamine secretion in vivo. *Neuroscience*. 2000; 101(2), 369–375. doi: 10.1016/S0306-4522(00)00371-7
121. Blomqvist O, Söderpalm B, Engel JA. Ethanol-induced locomotor activity: involvement of central nicotinic acetylcholine receptors? *Brain Res Bull*. 1992; 29(2), 173–178. doi: 10.1016/0361-9230(92)90023-Q
122. Söderpalm B, Ericson M, Olausson P, Blomqvist O, Engel JA. Nicotinic mechanisms involved in the dopamine activating and reinforcing properties of ethanol. *Behav Brain Res*. 2000; 113(1–2), 85–96. doi: 10.1016/S0166-4328(00)00203-5
123. Matta SG, Elberger AJ. Combined exposure to nicotine and ethanol throughout full gestation results in enhanced acquisition of nicotine self-administration in young adult rat offspring. *Psychopharmacology (Berl)*. 2007; 193(2), 199–213. doi: 10.1007/s00213-007-0767-2
124. Roguski EE, Sharp BM, Chen H, Matta SG. Full-gestational exposure to nicotine and ethanol augments nicotine self-administration by altering ventral tegmental dopaminergic function due to NMDA receptors in adolescent rats. *J Neurochem*. 2014; 128(5), 701–712. doi: 10.1111/jnc.12504
125. Smith TT, Schaff MB, Rupprecht LE, *et al.* Effects of MAO inhibition and a combination of minor alkaloids, β-carbolines, and acetaldehyde on nicotine self-administration in adult male rats. *Drug Alcohol Depend*. 2015; 155, 243–252. doi: 10.1016/j.drugalcdep.2015.07.002
126. Papp-Peka A, Tong M, Kril JJ, De La Monte SM, Sutherland GT. The Differential Effects of Alcohol and Nicotine-Specific Nitrosamine Ketone on White Matter Ultrastructure. *Alcohol Alcohol*. 2017; 52(2), 165–171. doi: 10.1093/alcalc/agw067
127. Zabala V, Silbermann E, Re E, *et al.* Potential co-factor role of tobacco specific nitrosamine exposures in the pathogenesis of fetal alcohol spectrum disorder. *Gynecol Obstet Res*. 2016; 2(5), 112–125. doi: 10.17140/GOROJ-2-125
128. Cross SJ, Linker KE, Leslie FM. Sex dependent effects of nicotine on the developing brain. *J Neurosci Res*. 2017; 95(1–2), 422–436. doi: 10.1002/jnr.23878
129. Valera S, Ballivet M, Bertrand D. Progesterone modulates a neuronal nicotinic acetylcholine receptor. *Proc Natl Acad Sci U S A*. 1992; 89(20), 9949–9953. doi: 10.1073/pnas.89.20.9949
130. Romero-grimaldi C, Moreno-lo B. Age-dependent effect of Nitric Oxide on Subventricular Zone and Olfactory Bulb. *J Comp Neurol*. 2008; 346(October 2007), 339–346. doi: 10.1002/cne
131. Barbieri RL, Gochberg J, Ryan KJ. Nicotine, cotinine, and anabasine inhibit aromatase in human trophoblast in vitro. *J Clin Invest*. 1986; 77(6), 1727–1733. doi: 10.1172/JCI112494
132. von Ziegler NI, Schlumpf M, Lichtensteiger W. Prenatal nicotine exposure selectively affects perinatal forebrain aromatase activity and fetal adrenal function in male rats. *Dev Brain Res*. 1991; 62(1), 23–31. doi: 10.1016/0165-3806(91)90186-M
133. Sarasin A, Schlumpf M, Müller M, Fleischmann I, Lauber ME, Lichtensteiger W. Adrenal-mediated rather than direct effects of nicotine as a basis of altered sex steroid synthesis in fetal and neonatal rat. *Reprod Toxicol*. 2003; 17(2), 153–162. doi: 10.1016/S0890-6238(02)00119-3
134. Comeau WL, Lee K, Anderson K, Weinberg J. Prenatal alcohol exposure and adolescent stress increase sensitivity to stress and gonadal hormone influences on cognition in adult female rats. *Physiol Behav*. 2015; 148, 157–165. doi: 10.1016/j.physbeh.2015.02.033
135. Marques AA, Bevilaqua MC, da Fonseca AM, Nardi AE, Thuret S, Dias GP. Gender differences in the neurobiology of anxiety: focus on adult hippocampal neurogenesis. *Neural Plast*. 2016; 2016, 5026713. doi: 10.1155/2016/5026713
136. Songtachalert T, Roomruangwong C, Carvalho AF, Bourin M, Maes M. Anxiety disorders: sex differences in serotonin and tryptophan metabolism. *Curr Top Med Chem*. 2018; 18(19), 1704–1715. doi: 10.2174/1568026618666181115093136