

# Parental, Prenatal, and Postnatal Nicotine Exposure Enhances the Induction and Expression of Locomotor Sensitization to Nicotine in Rats

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## Article

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# Abstract

Prenatal and postnatal nicotine exposure affects the development and function of the central nervous system in offspring, altering nicotine-induced dopamine release and increasing nicotine self-administration. Moreover, prenatal nicotine exposure enhances the risk of developing substance use disorder in adolescents. Given that locomotor sensitization is a critical physiological mechanism that enhances salience and motivation toward drug-related stimuli, thereby facilitating drug craving and compulsive drug-seeking behavior. Therefore, this study aimed to determine the effect of prenatal and postnatal nicotine exposure on locomotor sensitization in rats.

Pregnant female Wistar rats were divided into two groups: a 'nicotine pre-exposure group' received daily nicotine (0.4 mg/Kg; s.c.) from gestational day 0 (GD0) to GD21, while a 'saline pre-exposure group' received daily saline. During lactation (postnatal day 0 to PND21), the dams in these groups continued to receive either nicotine or saline, respectively. From the litters of both groups, only male rats were used. Their locomotor activity was recorded during the induction and expression of locomotor sensitization, using different doses of nicotine (0.4, 0.6, and 0.8 mg/Kg/day; i.p.). Assessments were conducted at postnatal ages 30, 60, 90, and 120 days, representing adolescence and adulthood.

The study revealed that prenatal and postnatal nicotine exposure enhanced both locomotor activity and locomotor sensitization in a dose- and age-dependent manner. These findings suggest that prenatal and postnatal nicotine exposure may increase vulnerability to nicotine abuse in young and adult humans.

## 1. Introduction

Clinical studies consistently indicate that tobacco smoking is a serious health problem, linked to the development of various comorbid conditions (Barnett et al., 2025). Maternal smoking is of particular importance in epidemiological reports due to its profound impact on the physical and mental health of offspring (Mihov et al., 2025; Wells and Lotfipour, 2023; Xavier et al., 2022).

Epidemiological reports estimate that maternal smoking during pregnancy accounts for approximately 2% of tobacco users worldwide (Lange et al., 2018). Human studies consistently show that prenatal tobacco exposure, including passive smoking, causes developmental alterations (Karatayev et al., 2024). Prenatal risks include premature birth, stillbirth, neonatal mortality, spontaneous abortion, intrauterine growth restriction, infant morbidity, ectopic pregnancy, and placental abruption (Mihov et al., 2025; Castro et al., 2023; Costa and Pollak, 2023). In early postnatal life, infants may experience sudden infant death syndrome (SIDS), cardiovascular morbidity, increased susceptibility to lower respiratory tract diseases, and immune dysfunction (Nordenstam, 2021; Laviolette, 2021; Hunter et al., 2020). Later, in childhood, adolescence, and adulthood, individuals exposed prenatally face a higher risk of psychological and neurological disorders, type II diabetes, obesity, childhood cancer, cardiovascular disorders, metabolic syndrome, and altered lung function (Alhowail, 2021; Tiesler and Heinrich, 2014).

In experimental animals, prenatal nicotine administration has been shown to alter brain sexual differentiation, cell survival, synaptogenesis, and cholinergic modulation of brain development (Dwyer et al., 2008, 2009; Ernst et al., 2001; Sobrian and Holson, 2011; Mitchell et al., 2012). However, behavioral findings have been contradictory (Sobrian and Holson, 2011; Polli and Kohlmeier, 2020). Some research indicates that prenatal and postnatal nicotine administration increases anxiety-like behaviors and hyperactivity, and improves cognitive task performance, such as increased efficiency in the radial maze (Sobrian et al., 2003; Vaglenova et al., 2004; Levin et al., 1993). Conversely, other studies report that prenatal nicotine exposure induces anxiolytic behaviors, no motor effects, and reduced learning task performance (Tizabi et al., 2000; Levin et al., 1996).

Nicotine, the primary psychoactive component of tobacco, plays a critical role in tobacco dependence (Markou, 2008; Chawla and Garrison, 2018). Its rewarding effects are mediated by acting on nicotinic acetylcholine receptors (nAChRs) and increasing neurotransmission in mesolimbic and nigrostriatal dopaminergic pathways, which induce drug-seeking and drug-use behaviors (Tiwari et al., 2020; Fowler et al., 2020).

Evidence suggests that chronic cigarette smoking during pregnancy increases the risk of adolescent smoking (Hellström-Lindahl and Nordberg, 2002; De Genna et al., 2016) and the likelihood of using other substances like alcohol or marijuana (Karatayev et al., 2024). Furthermore, prenatal nicotine exposure has been linked to increased motivation for nicotine- or methamphetamine-induced reinforcement (Levin et al., 2006; Lacy et al., 2014), potentially due to alterations in the development and functionality of the mesolimbic-cortical dopaminergic system (Olff and Gallardo, 1999).

Previous findings indicate that prenatal nicotine exposure increases the drug-reinforcing effect, evidenced by heightened nicotine self-administration (Levin et al., 2006; Matta and Elberger, 2007). However, whether prenatal nicotine exposure also enhances drug salience, as manifested by an increase in nicotine locomotor sensitization, has been less thoroughly investigated. While few studies have addressed this question, pioneering work revealed that high-dose nicotine exposure during pregnancy potentiated nicotine-induced locomotor sensitization (Sobrian et al., 2008).

For the current study, we employed a prenatal and postnatal drug exposure model, which ensures drug exposure across all stages of brain development (Smith et al., 2010; Dobbing, 1971; Dobbing and Sands, 1981). We have successfully utilized this model in previous work to evaluate the effects of prenatal and postnatal cocaine exposure on cocaine-induced locomotor sensitization in Wistar rats (Barbosa-Méndez and Salazar-Juárez, 2020; 2024). Building on this established methodology, the objective of the present study was to determine the effect of prenatal and postnatal nicotine exposure on locomotor sensitization in adolescent and adult rats.

The nicotine-induced locomotor sensitization paradigm is a critical physiological mechanism implicated in the persistent features of drug abuse. Sensitization enhances the salience of and motivation towards drug-associated stimuli, thereby facilitating drug craving and compulsive drug-seeking behavior (Koob and Le Moal, 2008). Based on this understanding, we hypothesized that prenatal and postnatal exposure

to nicotine would enhance locomotor sensitization, consequently increasing the likelihood of nicotine abuse.

## 2. Materials and Methods

### 2.1 Animals

Nulliparous female Wistar rats, approximately 10–12 weeks old and weighing 225–250 g at the time of mating, were pair-housed in polycarbonate rodent cages (45 cm x 23 cm x 20 cm). The colony room was maintained at a constant temperature ( $21 \pm 2^\circ\text{C}$ ) and humidity (40–50%), on a 12:12-h light/dark cycle (lights on at 7:00 a.m.). Animals had continuous access to rodent chow pellets (Laboratory Rodent Diet 5001; LabDiet; St. Louis, Missouri, USA) and water, except during experimental sessions. All experiments were conducted during the light phase of the cycle (between 9:00 a.m. and 3:00 p.m.). **The Institutional Animal Care Committee and Bioethics Committee of the National Institute of Psychiatry approved all procedures (Protocol # CEI/C/003/IC17056.0/2018). These procedures strictly complied with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH) and were reported in accordance with ARRIVE guidelines.** To reduce the number of animals and adhere to the "three R's" principle, the same animals were used for experiments 1 and 2, experiments 3 and 4, and experiments 5 and 6.

### 2.2 Animal Obtaining Procedure

Two stages comprised the animal acquisition process: (1) a prenatal phase that included mating and pregnancy, and (2) a postnatal phase that included litter acquisition and the random assignment of animals to experimental groups. Both stages followed procedures that our lab had previously reported (Barbosa-Méndez and Salazar-Juárez, 2020; 2024).

In summary, before beginning either saline or nicotine pre-exposure, 192 virgin female Wistar rats and 64 sexually experienced male rats were acclimated to the animal quarters for at least a week. Four experimental phases made up the prenatal phase: a 5-day group assignment phase, a 10-day pre-exposure phase, a 7-day mating phase, and a 21-day pregnant period.

All of the rats were handled and weighed every day for five days throughout the group assignment period to soften them before mating. After that, the 192 female rats were assigned into two groups at random: the saline pre-exposure group ( $n = 96$ ) and the nicotine pre-exposure group ( $n = 96$ ). Females in the nicotine group received ten once-daily injections of nicotine (0.4 mg/kg/day; i.p.) during the next 10-day pre-exposure period, whereas females in the saline group received ten once-daily injections of saline. In order to evaluate estrous activity and forecast maximum fertility, vaginal smears were also obtained during this time (Fig. 1A).

During the same 10-day pre-exposure period, the 64 sexually experienced males were split into two groups: 32 were randomly assigned to a saline pre-exposure group and received 10 once-daily saline

administrations, while the remaining 32 were assigned to a nicotine pre-exposure group and received 10 once-daily nicotine administrations (0.4 mg/kg/day; i.p.) (Fig. 1B).

One male was housed with three females during the seven-day mating period. Pre-exposed females were housed with pre-exposed males in accordance with their respective treatment groups (saline-treated females with saline-treated males; nicotine-treated females with nicotine-treated males). Every day, females were examined for vaginal cytology; the presence of a vaginal plug, which marked "gestation day 0" (GD0), indicated pregnancy. Since not every female became pregnant on the same day, the length of this period differed for every animal. Following confirmation of pregnancy, each pregnant rat was kept separately in plastic cages with Sani-chip<sup>TM</sup> bedding for the duration of the pregnancy and lactation.

Males were put back in their home cages following the breeding season. Every day between 8:30 and 9:00, pregnant dams were weighed. Throughout the pregnancy, daily measurements of the mother's body weight, food intake, and water intake were taken, and the dams were watched for miscarriages. Another laboratory investigation used females who did not become pregnant (Fig. 1A).

Females (225–300 g) in the nicotine pre-exposed group continued to receive once-daily subcutaneous injections of nicotine (0.4 mg/kg/day) during the 21-day pregnancy, whereas the saline pre-exposed group got once-daily injections of isotonic saline (Fig. 1A). To reduce skin injury, injection locations on the back were switched between the two groups. Neither immediately nor later on did the animals exhibit any obvious symptoms of injection-related discomfort or tenderness. Every animal was continuously observed by a staff veterinarian.

In order to minimize the impact of hormonal fluctuations (mostly estradiol release) on nicotine-induced locomotor activity, only male rats were employed in this investigation.

Four stages comprised the offspring assignment process: 1) Obtaining Litters (at birth, one day), 2) Lactation (21 days), 3) Grouping (at weaning, one day), and 4) Group Assignment (at thirty days of age).

1. **Obtaining Litters:** A total of 64 litters were collected, 32 from females preexposed to saline and 32 from females preexposed to nicotine. All pups were weighed, sexed, and had their toes clipped for identification on the day of delivery (usually gestational day 21, also known as postnatal day 1 or PND1). Eight pups—four males and four females—were removed from the litters and placed in typical polycarbonate cages with wooden bedding. Offspring were not kept apart from the nursing mother during this procedure. At PND21, the pups were weaned.
2. **Lactation Phase (PND0 to PND21):** While lactating females in the nicotine pre-exposed group received daily nicotine, those in the saline pre-exposed group got daily saline. During times of nicotine-induced locomotor hyperactivity, the pups were briefly taken out of the nest box and only put back in once the nicotine's effects had worn off in order to prevent moms from hurting them.
3. **Grouping Phase (at weaning):** The male saline pre-exposed group (SALpp) was created by removing all 128 male pups from the saline pre-exposed litters from their mothers and housing them together. The male nicotine pre-exposed group (NICpp) was created by grouping all of the male pups (n = 128)

from the nicotine pre-exposed litters. Before the pups were divided into experimental groups, this process assisted in homogenizing the pup population. Additionally, females from both groups were taken from their mothers and utilized in additional research.

4. **Group Assignment Phase:** Every animal in the NICpp group (n = 128) and SALpp group (n = 128) was randomized at random to a separate experiment. The initial pre-exposure (saline-pp or nicotine-pp) and the particular treatment (nicotine or saline) received throughout each trial were combined to create the names of the experimental groups. While Experiments 5–6 started at different ages, such as PND30, PND60, PND90, or PND120, Experiments 1–4 started on PND30 (Fig. 2).

## 2.3 Drugs

After receiving the necessary regulatory authorization, nicotine (nicotine tartrate salt; CAS No. 65-31-6; Sigma-Aldrich, St. Louis, Missouri, USA) was acquired in accordance with official requirements (COFEPRIS-2016, Mexico).

Sterile saline (0.9% NaCl, CAS No. 7647-14-5; Sigma-Aldrich, St. Louis, Missouri, USA) was used to dissolve nicotine. Before each dose, fresh solutions were made, adjusted to pH 7.4 using 1 M sodium hydroxide (CAS No. 1310-73-2; Sigma-Aldrich, St. Louis, Missouri, USA) and kept in black bottles at 4°C to keep out light. In every experiment, saline (0.9% NaCl) was used as a control. The animals' body weight (BW) in kilograms [BW (Kg)/1 mL] determined the injection volume. In accordance with earlier research conducted in our lab (Barbosa-Méndez and Salazar-Juárez, 2020; 2024), nicotine was given intraperitoneally to all male rats during the experimental phase and subcutaneously to pregnant female rats to prevent injury to the fetus.

Three criteria were used to determine the ideal nicotine dosage: 1) a dose of 0.4 mg/kg/day significantly increases nicotine-induced locomotor activity and behavioral sensitization (Barbosa-Méndez et al., 2017; Barbosa-Méndez and Salazar-Juárez, 2018); 2) this dose does not cause seizures or lethality (Barbosa-Méndez et al., 2017; Barbosa-Méndez and Salazar-Juárez, 2018). and 3) in humans, this dose is equivalent to smoking 2 to 4 cigarettes daily (Benowitz and Henningfield, 1994; Benowitz and Jacob, 1984).

During pregnancy and nursing, nicotine was given. Important phases of organogenesis, neurogenesis, migration, gliogenesis, and the beginning of synaptogenesis are all included in this exposure period (Smith et al., 2010; Dobbing, 1971; Dobbing and Sands, 1981).

Nicotine was given subcutaneously (s.c.) during pregnancy in order to minimize injury to fetuses, lessen treatment stress, and guarantee slow, constant absorption for consistent pharmacokinetics. Nicotine was given intraperitoneally (i.p.) for the duration of the experiment. By avoiding initial hepatic metabolism, this method—which is frequently used in investigations of a similar nature—produces a notable increase in nicotine-induced locomotor activity and behavioral sensitization, enabling quick attainment of high plasma concentrations.

## 2.4 Behavioral Sensitization

Transparent Plexiglas activity chambers (50 x 50 x 30 cm) connected to a PC were used to measure each animal's locomotor activity. A 16x16 photocell beam array was placed 3 cm from the floor in each chamber to monitor activity (OMNIALVA, Instruments, Mexico). Using OABiomed software (1.1), photobeam interruptions—defined as successive breaks in the beams—were automatically measured and then examined (OMNIALVA, Mexico).

A standard methodology that has been used in several research was used to quantify spontaneous locomotor activity (Salazar-Juarez et al. 2016; Barbosa-Mendez et al., 2017). Before being randomized to pharmacological treatment groups, the animals were acclimated to the activity chambers over the course of three 30-minute sessions. Locomotor activity was monitored for 30 minutes in trials 1, 3, and 5. Activity was monitored every 15 minutes for a total of 240 minutes in experiments 2, 4, and 6. Rats were put back in their cages at the end of each session.

Three 10-day stages made up the behavioral sensitization process:

1. **Nicotine-Induction Phase:** While animals in saline-treated groups received daily saline, those in nicotine-treated groups received daily nicotine administration (0.4 mg/kg/day, i.p.).
2. **Nicotine-Withdrawal Phase:** All groups received daily saline after nicotine was stopped.
3. **Nicotine-Expression Phase:** Saline-treated groups got daily saline, while nicotine-treated animals once more received daily nicotine (0.4 mg/kg/day, i.p.).

### 2.3.3 Euthanasia

Animals were mercifully put to death in a different room using an overdose of pentobarbital sodium (Sedal-Vet, 65 mg/ml) at the end of the experimental procedures. The Official Mexican Standard NOM-062-ZOO-1999 (Barbosa-Mendez and Salazar-Juarez, 2024; Official Mexican Standard NOM, 1999) recognizes and approves this technique of euthanasia.

## 2.5. Experimental Procedures

Two approaches were used to reduce bias in treatment group assignment: single-blind treatment administration, where only the lead investigator knew the treatment codes, and random assignment of animals to experimental groups using a Latin square design.

Experiments on locomotor sensitization began on postnatal day 30 (PND30). Six experiments were conducted using a total of 256 male Wistar rats. The following was the experimental design: 32 animals were utilized in four experimental groups (n = 8) in experiments 1 and 2, 96 animals in twenty groups (n = 8) in experiments 3 and 4, and 128 animals in sixteen groups (n = 8) in experiments 5 and 6. A distinct pharmacological therapy was administered to each experimental group.

### **2.5.1. Experiment 1: Prenatal and postnatal nicotine exposure enhanced induction and expression of sensitization produced by nicotine.**

Three 10-day pharmacological periods comprised Experiment 1 (Fig. 3A): Phases I, II, and III are the phases of nicotine induction, withdrawal, and expression, respectively.

During all three phases, saline (0.9% NaCl, i.p.) was administered to the SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups. During the induction and expression phases, nicotine (0.4 mg/kg/day, i.p.) was administered to the SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups. These groups simply got saline every day while nicotine was withheld during the nicotine-withdrawal phase. Each animal's locomotor activity was monitored for 30 minutes following each dose.

### **2.5.2. Experiment 2: Prenatal and postnatal nicotine exposure increased the duration of nicotine-induced locomotor activity.**

In the second experiment, the duration of nicotine-induced locomotor activity was examined in relation to prenatal and postnatal nicotine intake. It included a one-day testing period in addition to the three stages outlined in Experiment 1.

The pharmacological treatments outlined in Experiment 1 were administered to the SAL<sub>pp</sub> + SAL, SAL<sub>pp</sub> + NIC, NIC<sub>pp</sub> + SAL, and NIC<sub>pp</sub> + NIC groups throughout the expression phase. Each animal's locomotor activity was monitored for 30 minutes in activity chambers (Fig. 4A).

To ascertain baseline responses, all rats were kept in the testing box for 30 minutes without any treatment once the expression phase was over. Following the administration of either nicotine or saline to each animal or group, locomotor activity was monitored every 15 minutes for a total of 240 minutes.

### **2.5.3. Experiment 3: Prenatal and postnatal nicotine exposure modified induction and expression of sensitization induced by different nicotine doses.**

Nicotine exposure throughout pregnancy and after birth increased the induction and manifestation of nicotine-induced locomotor sensitization. The procedure outlined in Experiment 1 was followed in this experiment.

In both experimental phases, the following groups were given saline (0.9% NaCl, i.p.): SAL<sub>pp</sub> + SAL<sup>-0.4mg</sup>, SAL<sub>pp</sub> + SAL<sup>-0.6mg</sup>, SAL<sub>pp</sub> + SAL<sup>-0.8mg</sup>, NIC<sub>pp</sub> + SAL<sup>-0.4mg</sup>, NIC<sub>pp</sub> + SAL<sup>-0.6mg</sup>, and NIC<sub>pp</sub> + SAL<sup>-0.8mg</sup>.

During induction and expression, various nicotine doses (0.4, 0.6, and 0.8 mg/kg/day, i.p., respectively) were administered to the SAL<sub>pp</sub> + NIC<sup>-0.4mg</sup>, SAL<sub>pp</sub> + NIC<sup>-0.6mg</sup>, SAL<sub>pp</sub> + NIC<sup>-0.8mg</sup>, NIC<sub>pp</sub> + NIC<sup>-0.4mg</sup>, NIC<sub>pp</sub> + NIC<sup>-0.6mg</sup>, and NIC<sub>pp</sub> + NIC<sup>-0.8mg</sup> groups (Fig. 5A). Nicotine was stopped throughout withdrawal, and all groups were given saline every day.

#### **2.5.4. Experiment 4: Prenatal and postnatal nicotine exposure increased the duration of locomotor activity induced by different nicotine doses.**

In this study, the duration of nicotine-induced locomotor activity was assessed in relation to prenatal and postnatal nicotine intake. It included a one-day testing phase in addition to the three stages outlined in Experiment 3.

The pharmacological treatments outlined in Experiment 3 were administered to the SAL<sub>pp</sub> + SAL<sup>-0.4mg</sup>, SAL<sub>pp</sub> + SAL<sup>-0.6mg</sup>, SAL<sub>pp</sub> + SAL<sup>-0.8mg</sup>, NIC<sub>pp</sub> + SAL<sup>-0.4mg</sup>, NIC<sub>pp</sub> + SAL<sup>-0.6mg</sup>, NIC<sub>pp</sub> + SAL<sup>-0.8mg</sup> and the SAL<sub>pp</sub> + NIC<sup>-0.4mg</sup>, SAL<sub>pp</sub> + NIC<sup>-0.6mg</sup>, SAL<sub>pp</sub> + NIC<sup>-0.8mg</sup>, NIC<sub>pp</sub> + NIC<sup>-0.4mg</sup>, NIC<sub>pp</sub> + NIC<sup>-0.6mg</sup>, NIC<sub>pp</sub> + NIC<sup>-0.8mg</sup> groups during the expression phase. Each animal's locomotor activity was monitored in locomotor activity chambers for thirty minutes (Fig. 6A).

To ascertain baseline responses, all rats were kept in the testing box for 30 minutes without any treatment once the expression phase was over. Following the administration of either nicotine or saline to each animal or group, locomotor activity was monitored every 15 minutes for a total of 240 minutes.

#### **2.5.5. Experiment 5: Prenatal and postnatal nicotine exposure enhanced nicotine sensitization at different ages.**

The expression of nicotine-induced locomotor sensitization during adolescence (30 and 60 days of age) and adulthood (90 and 120 days of age) was increased by prenatal and postnatal nicotine exposure. The technique outlined in Experiment 1 was used to assess the effects.

The SAL<sub>pp</sub> + SAL<sup>-30d</sup>, SAL<sub>pp</sub> + SAL<sup>-60d</sup>, SAL<sub>pp</sub> + SAL<sup>-90d</sup>, SAL<sub>pp</sub> + SAL<sup>-120d</sup>, NIC<sub>pp</sub> + SAL<sup>-30d</sup>, NIC<sub>pp</sub> + SAL<sup>-60d</sup>, NIC<sub>pp</sub> + SAL<sup>-90d</sup>, NIC<sub>pp</sub> + SAL<sup>-120d</sup>, SAL<sub>pp</sub> + NIC<sup>-30d</sup>, SAL<sub>pp</sub> + NIC<sup>-60d</sup>, SAL<sub>pp</sub> + NIC<sup>-90d</sup>, SAL<sub>pp</sub> + NIC<sup>-120d</sup>, NIC<sub>pp</sub> + NIC<sup>-30d</sup>, NIC<sub>pp</sub> + NIC<sup>-60d</sup>, NIC<sub>pp</sub> + NIC<sup>-90d</sup>, and NIC<sub>pp</sub> + NIC<sup>-120d</sup> groups were administered the treatments described in Experiment 1 (Fig. 7A). Each animal's locomotor activity was monitored for 30 minutes following each dose.

#### **2.5.6. Experiment 6: Prenatal and postnatal nicotine exposure increased the duration of nicotine-induced locomotor activity at different ages.**

This experiment had a one-day testing phase in addition to the three stages outlined in Experiment 5.

During the expression phase, the SAL<sub>pp</sub> + SAL<sup>-30d</sup>, SAL<sub>pp</sub> + SAL<sup>-60d</sup>, SAL<sub>pp</sub> + SAL<sup>-90d</sup>, SAL<sub>pp</sub> + SAL<sup>-120d</sup>, NIC<sub>pp</sub> + SAL<sup>-30d</sup>, NIC<sub>pp</sub> + SAL<sup>-60d</sup>, NIC<sub>pp</sub> + SAL<sup>-90d</sup>, NIC<sub>pp</sub> + SAL<sup>-120d</sup>, SAL<sub>pp</sub> + NIC<sup>-30d</sup>, SAL<sub>pp</sub> + NIC<sup>-60d</sup>, SAL<sub>pp</sub> + NIC<sup>-90d</sup>, SAL<sub>pp</sub> + NIC<sup>-120d</sup>, NIC<sub>pp</sub> + NIC<sup>-30d</sup>, NIC<sub>pp</sub> + NIC<sup>-60d</sup>, NIC<sub>pp</sub> + NIC<sup>-90d</sup>, and NIC<sub>pp</sub> + NIC<sup>-120d</sup> groups received the pharmacological treatments described in Experiment 5. In the testing box, each animal's locomotor activity was monitored for thirty minutes (Fig. 8A).

To ascertain baseline responses, all rats were kept in the testing box for 30 minutes without any treatment once the expression phase was over. Following the administration of either nicotine or saline to each animal or group, locomotor activity was monitored every 15 minutes for a total of 240 minutes.

## 2.6. Statistical Analysis

Beam breaks during testing were used to evaluate locomotor activity, and the results were presented as mean  $\pm$  S.E.M.

Using a linear general model with litter, prenatal and postnatal treatment (saline and nicotine), and experimental phases (induction and expression) as the between-subjects factor and groups (saline and nicotine), nicotine doses (0.4, 0.6, and 0.8 mg/Kg/day), or age (30, 60, 90, and 120 days) as the intra-subjects factor, we examined the locomotor activity in each group for experiments 1, 3, and 5. We used the Bonferroni test to perform another post hoc analysis of group differences when the interaction had a significant F value.

Regarding Experiments 2, 4, and 6, data were analyzed using two-way repeated measures ANOVA, where time (minutes) was the repeated measure, starting on treatment day 1, in order to ascertain the duration of effect (the amount of time it takes for locomotor activity to recover to its basal level). The locomotor activity in the SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups was the baseline value for Experiments 2, 4, and 6 because the two-way repeated measures ANOVA did not reveal any variations over time between these groups. When the post hoc test revealed no significant differences between nicotine-induced locomotor activity and that observed in the SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups, we determined that nicotine-induced locomotor activity had returned to its baseline level. The threshold for statistical significance was fixed at  $p < 0.05$ . SPSS statistics program 21 was used to perform statistical analysis.

## 3. Results

### 3.1. Experiment 1: Prenatal and postnatal nicotine exposure enhanced induction and expression of sensitization produced by nicotine.

The SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups did not significantly increase locomotor activity ( $p = 0.99$ ), as seen in Figs. 3B and 3C. Rather, compared to the SAL<sub>pp</sub> + SAL ( $P < 0.001$ ) and NIC<sub>pp</sub> + SAL ( $P < 0.001$ ) groups, the administration of nicotine (0.4 mg/kg/day) to the SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups significantly increased locomotor activity during induction and expression (prenatal and postnatal treatment X experimental phase X litter X groups interaction;  $F(1, 325) = 6253.18$   $P < 0.001$ ). Additionally, the post-hoc test showed that the NIC<sub>pp</sub> + NIC group had significantly higher mean locomotor activity during induction ( $P < 0.002$ ) and expression ( $P < 0.001$ ) than the SAL<sub>pp</sub> + NIC group. It also found that the SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups had different locomotor activity during the induction phase compared to the expression phase (Table 1A; Fig. 3C). This result implies that exposure to nicotine throughout pregnancy and the postnatal period increased both the nicotine-induced locomotor sensitization and locomotor activity during the induction and expression phases.

## 3.2. Experiment 2: Prenatal and postnatal nicotine exposure increased the duration of nicotine-induced locomotor activity.

As previously indicated, daily saline administration had no effect on baseline locomotor activity ( $P = 0.99$ ), but in the  $SAL_{pp} + NIC$  and  $NIC_{pp} + NIC$  groups, a dose of nicotine (0.4 mg/Kg) significantly increased locomotor activity duration (Fig. 4B) (two-way repeated measures ANOVA;  $F = (38,285) 125.69, p < 0.0001$ ).

Locomotor activity peaked 30 minutes after drug administration in the  $SAL_{pp} + NIC$  group and 15 minutes after drug administration in the  $NIC_{pp} + NIC$  group. Although the  $NIC_{pp} + NIC$  group's locomotor activity was higher than that of the  $SAL_{pp} + NIC$  group, the  $SAL_{pp} + NIC$  group's locomotor activity declined much more quickly (150 minutes after the injection) than the  $NIC_{pp} + NIC$  groups. 240 minutes after injection, the latter group returned to baseline levels ( $P = 0.94$ ).

In comparison to the  $SAL_{pp} + NIC$  group ( $P < 0.0002$ ), our statistical analysis revealed a substantial increase in locomotor activity duration in the  $NIC_{pp} + NIC$  group (prenatal and postnatal treatment X experimental period X litter X group interaction  $F(1, 325) = 1358.65, P < 0.001$ ) (Table 1B; Fig. 4C). This implies that nicotine exposure throughout pregnancy and after birth lengthened the duration of nicotine-induced locomotor activity.

## 3.3. Experiment 3: Prenatal and postnatal nicotine exposure modified induction and expression of sensitization induced by different nicotine doses.

The rats in the groups  $SAL_{pp} + NIC_{0.4mg}$ ,  $SAL_{pp} + NIC_{0.6mg}$ ,  $SAL_{pp} + NIC_{0.8mg}$ ,  $NIC_{pp} + NIC_{0.4mg}$ ,  $NIC_{pp} + NIC_{0.6mg}$ , and  $NIC_{pp} + NIC_{0.8mg}$ , compared to those in the  $SAL_{pp} + SAL_{0.4mg}$ ,  $SAL_{pp} + SAL_{0.6mg}$ ,  $SAL_{pp} + SAL_{0.8mg}$  ( $P < 0.0001$ ),  $NIC_{pp} + SAL_{0.4mg}$ ,  $NIC_{pp} + SAL_{0.6mg}$ , and  $NIC_{pp} + SAL_{0.8mg}$  ( $P < 0.0001$ ) experienced a dose-dependent increase in locomotor activity during the induction and expression phases (prenatal and postnatal treatment X experimental phase X group X nicotine dose interaction X litter,  $F = (2, 972) 143.28, P < 0.0001$ ; Figs. 5B to 5D).

The post hoc test identified differences between the nicotine-treated groups during the induction and expression stages. In other words, comparing  $SAL_{pp} + NIC_{0.4mg}$  and  $NIC_{pp} + NIC_{0.4mg}$  ( $P < 0.0001$ );  $SAL_{pp} + NIC_{0.6mg}$  and  $NIC_{pp} + NIC_{0.6mg}$  ( $P < 0.0001$ ); and  $SAL_{pp} + NIC_{0.8mg}$  and  $NIC_{pp} + NIC_{0.8mg}$  (Table 2; Fig. 5E).

Additionally, the Bonferroni test showed differences between the groups receiving 0.4 mg/kg/day nicotine ( $SAL_{pp} + NIC_{0.4mg}$  and  $NIC_{pp} + NIC_{0.4mg}$ ) and those receiving 0.6 mg/kg/day ( $SAL_{pp} + NIC_{0.6mg}$ ,  $P < 0.002$ ;  $NIC_{pp} + NIC_{0.6mg}$ ,  $P < 0.002$ ); or 0.8 mg/kg/day ( $SAL_{pp} + NIC_{0.8mg}$ ,  $P < 0.003$ ;  $NIC_{pp} + NIC_{0.8mg}$ ,  $P < 0.002$ ) nicotine. In addition, we also found differences between  $SAL_{pp} + NIC_{0.6mg}$  and

NIC<sub>pp</sub> + NIC<sub>-0.6mg</sub> and groups treated with 0.8 mg/kg/day (SAL<sub>pp</sub> + NIC<sub>-0.8mg</sub>,  $P < 0.0002$ ; NIC<sub>pp</sub> + NIC<sub>-0.8mg</sub>,  $P < 0.0002$ ; Table 2; Fig. 5E). This suggested that prenatal and postnatal exposure to nicotine enhanced locomotor activity as induced by different nicotine doses.

### **3.4. Experiment 4: Prenatal and postnatal nicotine exposure increased the duration of the locomotor activity induced by different nicotine doses.**

Figures 6B to 6D show that prenatal and postnatal exposure of animals to nicotine resulted in a dose-dependent increase (two-way repeated measures ANOVA;  $F = (38, 298) 108.64$   $p < 0.0001$ ) in the duration of nicotine-induced locomotor activity compared to the SAL<sub>pp</sub> + SAL<sub>-0.4mg</sub>, SAL<sub>pp</sub> + SAL<sub>-0.6mg</sub>, SAL<sub>pp</sub> + SAL<sub>-0.8mg</sub> ( $P < 0.0001$ ), and the NIC<sub>pp</sub> + SAL<sub>-0.4mg</sub>, NIC<sub>pp</sub> + SAL<sub>-0.6mg</sub>, and NIC<sub>pp</sub> + SAL<sub>-0.8mg</sub> ( $P < 0.0001$ ) groups.

In the group of animals dosed with 0.4 mg/kg/day of nicotine, locomotor activity peaked at 30 minutes after drug administration, whereas at a dose of 0.6 or 0.8 mg/kg/day of nicotine, locomotor activity peaked at 45 or 60 minutes after administration, respectively.

Activity in the NIC<sub>pp</sub> + NIC<sub>-0.4mg</sub>, NIC<sub>pp</sub> + NIC<sub>-0.6mg</sub>, and NIC<sub>pp</sub> + NIC<sub>-0.8mg</sub> groups were significantly higher than in the SAL<sub>pp</sub> groups treated with nicotine (SAL<sub>pp</sub> + NIC<sub>-0.4mg</sub>,  $P < 0.001$ ; SAL<sub>pp</sub> + NIC<sub>-0.6mg</sub>,  $P < 0.001$ ; and SAL<sub>pp</sub> + NIC<sub>-0.8mg</sub>,  $P < 0.002$ ). Further, locomotor activity in the SAL<sub>pp</sub> + NIC<sub>-0.4mg</sub>, SAL<sub>pp</sub> + NIC<sub>-0.6mg</sub>, and SAL<sub>pp</sub> + NIC<sub>-0.8mg</sub> groups decreased significantly faster (between 100–220 minutes after the injection) than in the NIC<sub>pp</sub> groups (NIC<sub>pp</sub> + NIC<sub>-0.4mg</sub>, NIC<sub>pp</sub> + NIC<sub>-0.6mg</sub>, and NIC<sub>pp</sub> + NIC<sub>-0.8mg</sub>), where activity returned to baseline values between 225–240 minutes after the injection, according to the dose of nicotine (Figs. 6B to 6D).

Our statistical analysis also found a significant increase (prenatal and postnatal treatment X experimental phase X Group X nicotine dose interaction X litter,  $F (2, 972) = 1589.27$   $P < 0.0001$ ) in locomotor activity duration in the NIC<sub>pp</sub> + NIC<sub>-0.4mg</sub>, NIC<sub>pp</sub> + NIC<sub>-0.6mg</sub>, and NIC<sub>pp</sub> + NIC<sub>-0.8mg</sub> groups compared to the SAL<sub>pp</sub> + NIC<sub>-0.4mg</sub>,  $P < 0.001$ ; SAL<sub>pp</sub> + NIC<sub>-0.6mg</sub>,  $P < 0.003$ ; and SAL<sub>pp</sub> + NIC<sub>-0.8mg</sub>,  $P < 0.0001$ , groups (Fig. 6E). This suggests that prenatal and postnatal nicotine exposure enhanced the duration of the locomotor activity induced by different doses of nicotine.

### **3.5. Experiment 5: Prenatal and postnatal nicotine exposure enhanced nicotine sensitization at different ages.**

As shown in Figs. 7B to 7E, 0.4 mg/kg/day nicotine induced an increase in locomotor activity during induction and expression (prenatal and postnatal treatment X experimental phase X Group X age interaction X litter,  $F = (3, 2386) 1438.75$   $p < 0.0002$ ), compared to the SAL<sub>pp</sub> + SAL (SAL<sub>pp</sub> + SAL<sub>-30d</sub>, SAL<sub>pp</sub> + SAL<sub>-60d</sub>, SAL<sub>pp</sub> + SAL<sub>-90d</sub>, and SAL<sub>pp</sub> + SAL<sub>-120d</sub>;  $P < 0.0001$ ) and the NIC<sub>pp</sub> + SAL (NIC<sub>pp</sub> + SAL<sub>-30d</sub>, NIC<sub>pp</sub> + SAL<sub>-60d</sub>, NIC<sub>pp</sub> + SAL<sub>-90d</sub>, and NIC<sub>pp</sub> + SAL<sub>-120d</sub>;  $P < 0.0001$ ) groups. The post hoc test revealed a significant increase in mean locomotor activity in the NIC<sub>pp</sub> + NIC<sub>-30d</sub>, NIC<sub>pp</sub> + NIC<sub>-60d</sub>, NIC<sub>pp</sub> +

NIC<sup>-90d</sup>, and NIC<sub>pp</sub> + NIC<sup>-120d</sup> groups compared to the SAL<sub>pp</sub> + NIC<sup>-30d</sup>,  $P < 0.0003$ ; SAL<sub>pp</sub> + NIC<sup>-60d</sup>,  $P < 0.0003$ ; SAL<sub>pp</sub> + NIC<sup>-90d</sup>,  $P < 0.0002$ ; and SAL<sub>pp</sub> + NIC<sup>-120d</sup>,  $P < 0.0001$  groups during induction and expression phase (Fig. 7F), at 30, 60, 90, and 120 days of age.

The post hoc test found differences in locomotor activity in the SAL<sub>pp</sub> + NIC groups (SAL<sub>pp</sub> + NIC<sup>-30d</sup>, SAL<sub>pp</sub> + NIC<sup>-60d</sup>, SAL<sub>pp</sub> + NIC<sup>-90d</sup>, and SAL<sub>pp</sub> + NIC<sup>-120d</sup>) and the NIC<sub>pp</sub> + NIC groups (NIC<sub>pp</sub> + NIC<sup>-30d</sup>, NIC<sub>pp</sub> + NIC<sup>-60d</sup>, NIC<sub>pp</sub> + NIC<sup>-90d</sup>, and NIC<sub>pp</sub> + NIC<sup>-120d</sup>) between the induction and the expression phases, in animals aged 30 ( $P < 0.0002$ ), 60 ( $P < 0.0001$ ), 90 ( $P < 0.0001$ ) and 120 ( $P < 0.0001$ ) days (Table 3; Fig. 7F).

However, our statistical test did not find any differences in locomotor activity between the SAL<sub>pp</sub> + NIC<sup>-30d</sup>, SAL<sub>pp</sub> + NIC<sup>-60d</sup>, SAL<sub>pp</sub> + NIC<sup>-90d</sup>, and SAL<sub>pp</sub> + NIC<sup>-120d</sup> groups and between the NIC<sub>pp</sub> + NIC<sup>-30d</sup>, NIC<sub>pp</sub> + NIC<sup>-60d</sup>, NIC<sub>pp</sub> + NIC<sup>-90d</sup>, and NIC<sub>pp</sub> + NIC<sup>-120d</sup> groups in the induction ( $P = 0.98$ ) or the expression ( $P = 0.98$ ) phase. This finding suggests that prenatal and postnatal nicotine exposure produced an age-dependent increase in nicotine-induced locomotor sensitization.

### **3.6. Experiment 6: Prenatal and postnatal nicotine exposure increased the duration of nicotine-induced locomotor activity at different ages.**

Figures 8B to 8E show that the animals exposed to nicotine prenatally and postnatally had an increase (two-way repeated measures ANOVA;  $F(38,852) = 133.42$ ,  $P < 0.0001$ ) in the duration of nicotine-induced locomotor activity. At all ages, the nicotine-induced locomotor activity peaked 30 minutes after administration.

Activity in the NIC<sub>pp</sub> + NIC groups (NIC<sub>pp</sub> + NIC<sup>-30d</sup>, NIC<sub>pp</sub> + NIC<sup>-60d</sup>, NIC<sub>pp</sub> + NIC<sup>-90d</sup>, and NIC<sub>pp</sub> + NIC<sup>-120d</sup>) was significantly higher than in the SAL<sub>pp</sub> + NIC groups (SAL<sub>pp</sub> + NIC<sup>-30d</sup>,  $P < 0.002$ ; SAL<sub>pp</sub> + NIC<sup>-60d</sup>,  $P < 0.002$ ; SAL<sub>pp</sub> + NIC<sup>-90d</sup>,  $P < 0.0001$ ; and SAL<sub>pp</sub> + NIC<sup>-120d</sup>,  $P < 0.002$ ). Locomotor activity in the SAL<sub>pp</sub> + NIC groups (SAL<sub>pp</sub> + NIC<sup>-30d</sup>, SAL<sub>pp</sub> + NIC<sup>-60d</sup>, SAL<sub>pp</sub> + NIC<sup>-90d</sup>, and SAL<sub>pp</sub> + NIC<sup>-120d</sup>) decreased significantly faster (130–160 minutes after the injection) than in the NIC<sub>pp</sub> + NIC groups (NIC<sub>pp</sub> + NIC<sup>-30d</sup>, NIC<sub>pp</sub> + NIC<sup>-60d</sup>, NIC<sub>pp</sub> + NIC<sup>-90d</sup>, and NIC<sub>pp</sub> + NIC<sup>-120d</sup>), which reached baseline values 240 minutes after the injection (Figs. 8B to 8E).

Our statistical analysis found a significant increase (prenatal and postnatal treatment X group X age X litter interaction,  $F(3, 2742.59) = 2057.88$ ,  $P < 0.001$ ) in locomotor activity duration in the NIC<sub>pp</sub> + NIC groups (NIC<sub>pp</sub> + NIC<sup>-30d</sup>, NIC<sub>pp</sub> + NIC<sup>-60d</sup>, NIC<sub>pp</sub> + NIC<sup>-90d</sup>, and NIC<sub>pp</sub> + NIC<sup>-120d</sup>), compared to the SAL<sub>pp</sub> + NIC groups (SAL<sub>pp</sub> + NIC<sup>-30d</sup>,  $P < 0.001$ , SAL<sub>pp</sub> + NIC<sup>-60d</sup>,  $P < 0.001$ , SAL<sub>pp</sub> + NIC<sup>-90d</sup>,  $P < 0.001$ , and SAL<sub>pp</sub> + NIC<sup>-120d</sup>,  $P < 0.001$ ) (Table 3; Fig. 8F). This suggests that prenatal and postnatal nicotine exposure age-dependent enhanced in the duration of nicotine-induced locomotor activity.

## **4. Discussion**

## Experiment 1

According to this study, nicotine exposure throughout pregnancy and after birth increased nicotine-induced locomotor activity as well as the induction and expression of locomotor sensitization at 30 days of age. In particular, it prolonged the duration of this nicotine-induced motor effect and caused a dose- and age-dependent increase in nicotine-induced locomotor activity during the induction and expression phases of locomotor sensitization.

Prenatal nicotine exposure has been shown to increase nicotine self-administration in animals (Levin et al., 2006; Roguski et al., 2014). Additionally, research by Harrod et al. showed that exposure to nicotine during pregnancy increased sensitivity to the reinforcing effects of methamphetamines (Lacy et al., 2014; Harrod et al., 2012). These results imply that exposure to nicotine during pregnancy may increase adult offspring's sensitivity to nicotine and other medications as well as nicotine reinforcing. Given these effects, whether prenatal nicotine exposure increases nicotine locomotor sensitization—a known behavioral indicator of drug salience—is an important concern in the context of maternal smoking disease. One important physiological mechanism impacting enduring aspects of drug misuse is the nicotine-induced locomotor sensitization paradigm. According to Koob and Le Moal (2008), sensitization facilitates drug desire and compulsive drug-seeking behavior by increasing the salience and incentive associated with drug-related activity.

Our research showed that nicotine exposure during pregnancy and after birth improved the induction and expression of nicotine-induced locomotor sensitization. To the best of our knowledge, this is the first study to demonstrate that exposure during pregnancy and after birth increases locomotor sensitization to nicotine.

Prenatal nicotine administration boosted the induction of behavioral sensitization in male rats, according to a groundbreaking work by Sobrian et al. (Sobrian et al., 2008). This conclusion is supported by our findings, which showed that nicotine increased the induction of locomotor sensitization. This implies that the neurobehavioral, synaptic, and anatomical alterations that define the induction phase of locomotor sensitization are amplified by nicotine exposure throughout pregnancy and after birth (Robinson and Berridge, 1993).

Prenatal nicotine exposure has been shown in rodent studies to modify critical brain development processes, such as brain sexual differentiation, cell survival, and synaptogenesis (Dwyer et al., 2009; Mitchell et al., 2012; Vaglenova et al., 2004; Levin et al., 1993; Dwyer et al., 2008; Ernst et al., 2001; Sobrian and Holson, 2011). Cholinergic control of brain growth is probably affected by these changes (Azam et al., 2007; Oliff and Gallardo, 1999; Gold et al., 2009). Therefore, it is likely that the morphological basis for the increased induction and expression of nicotine locomotor sensitization is formed by changes in different brain developmental processes brought about by prenatal nicotine exposure, along with changes in brain structure brought about by nicotine during the induction phase of locomotor sensitization.

However, a number of studies have demonstrated that exposure to nicotine during pregnancy hinders the development of the dopaminergic system in the brain, which could provide a neurochemical foundation for increased nicotine locomotor sensitization (Zhang et al., 2014; Dwyer et al., 2019; Alkam et al., 2017; Pinheiro et al., 2015). According to these studies, nicotine uses during pregnancy reduced the amount of dopamine neurons, tyrosine hydroxylase (TH), and dopamine transporter (DAT) in the nucleus accumbens and medial prefrontal cortex (mPFC) (Pinheiro et al., 2015; Alkam et al., 2017; Kazemi et al., 2020). Additionally, changes in the number of D<sub>2</sub> dopamine receptors (D<sub>2</sub>R), changes in the function of D<sub>2</sub> and D<sub>3</sub> receptors in the ventral striatum, and changes in catechol-O-methyltransferase (COMT)-dependent dopamine turnover have been noted (Dwyer et al., 2019). There have also been reports of a decrease in dopamine release in the mPFC (Alkam et al., 2017).

In this work, we also assessed the impact of nicotine exposure during and after pregnancy on the duration of nicotine-induced locomotor activity. In line with earlier findings that nicotine exposure during pregnancy and after birth increases nicotine-induced locomotor activity, we discovered that nicotine administration during brain development increased this activity (Tizabi et al., 1997; 2000; Newman et al., 1999; Pauly et al., 2004; Lacy et al., 2016; Penman et al., 2024). Nevertheless, our research also revealed that exposure to nicotine during pregnancy prolonged the duration of nicotine-induced locomotor activity; we were unable to uncover comparable findings in the literature.

According to several authors, in naive rats, the behavioral response to nicotine (0.4 mg/kg, i.p.) usually peaks at 30 minutes and returns to baseline levels within 140 minutes after injection (Clarke and Kumar, 1983; Ksir et al., 1985). Similar trends were seen in our control rats, which were treated to saline both before and after birth. These rats displayed maximal motor activity 30 minutes after nicotine administration, with a mean duration of 150 minutes. On the other hand, rats exposed to nicotine during pregnancy reached their maximum locomotor activity 15 minutes after the drug was administered, accelerating the peak of maximal activity. After 240 minutes of treatment, this activity gradually dropped and returned to baseline.

Studies using microdialysis have demonstrated a correlation between enhanced dopamine release and behavioral sensitization. In particular, nicotine markedly increased extracellular dopamine in the ventral striatum, peaking 20–30 minutes after injection and going back to baseline in 120 minutes (Imperato et al., 1986; Di Chiara and Imperato, 1988). In contrast, Kane et al. (2004) found that in mice exposed to nicotine during pregnancy, peak dopamine levels were reached within 10 to 15 minutes and recovered to baseline within 60 minutes.

The expression of  $\alpha_2$ ,  $\alpha_4$ ,  $\alpha_7$ , and  $\beta_2$  subunits, as well as the quantity and function of nAChRs in adolescent and adult rats, have been shown to be persistently altered by prenatal nicotine exposure (Gold et al., 2009; Huang et al., 2004; Tizabi et al., 1997). This suggests changes in nAChR density may account for the prolonged duration of nicotine-induced locomotor activity in addition to the reduced density of inhibitory D<sub>2</sub>R and increased dopamine release brought on by prenatal nicotine exposure.

In conclusion, exposure to nicotine during pregnancy causes changes in the dopaminergic system's morphology and neurochemistry. An increase in nicotine self-administration, which suggests a strengthening of the drug's reinforcing effects, may be explained by these modifications. enhanced nicotine-induced locomotor activity, a longer duration of nicotine-induced hyperactivity, and nicotine locomotor sensitization—a marker of enhanced drug salience—are also explained by them. In the end, these consequences may make young people more susceptible to the reinforcing effects of other illegal substances.

## **Experiment 2**

Increased nicotine-induced locomotor activity and persistent hyperactivity were the hallmarks of our earlier research, which showed a dose-dependent link between nicotine and subsequent locomotor sensitization (Barbosa-Méndez et al., 2017; Barbosa-Méndez and Salazar-Juárez, 2022). Prenatal and postnatal nicotine treatment also produced a dose-dependent increase in locomotor activity during both the induction and expression stages of sensitization, as well as in the duration of nicotine-induced locomotor activity, which is consistent with these findings.

These new discoveries are especially important because they may contribute to the explanation of clinical observations that children of pregnant mothers who smoke have a higher chance of developing early-onset smoking (Hellström-Lindahl and Nordberg, 2002; De Genna et al., 2016), possibly even if their initial exposure was mainly passive.

As was previously mentioned, early nicotine exposure increases the sensitivity of kids to nicotine, both during pregnancy and after birth. Changes in the mesolimbocortical system, specifically dopamine neurotransmission, are probably responsible for this increased sensitivity (Zhang et al., 2014; Dwyer et al., 2019). Additional neurotransmitter systems, such as the cholinergic (Gold et al., 2009; Azam et al., 2007; Chen et al., 2005), GABAergic (Eliassen et al., 2020), and glutamatergic (Polli et al., 2018; 2019; McNair and Kohlmeier, 2015) systems, have been demonstrated to be disrupted during pregnancy. When taken as a whole, these extensive neurobiological alterations may result in a condition of perpetual yearning and elevated nicotine salience, increasing susceptibility to nicotine dependence in adulthood.

## **Experiment 3**

Research has demonstrated that exposure to nicotine during pregnancy and after birth causes long-term behavioral abnormalities in the offspring as well as neuronal impairments in the fetal brain (Wang et al., 2018; Mitchell et al., 2012). To the best of our knowledge, however, neither the long-term consequences nor the importance of prenatal nicotine exposure on locomotor sensitization have been shown.

In this work, we found that during nicotine-induced locomotor sensitization, there was an age-dependent increase in locomotor activity. According to earlier studies (Sobrian et al., 2008; Yochum et al., 2014; Pauly et al., 2004), nicotine-induced locomotor activity increases with age, particularly at PND 30, PND 40, PND 60, and PND 120 (Newman et al., 1999; Tizabi et al., 1997; 2000; Lacy et al., 2016). Additionally,

recent research has demonstrated that exposure to nicotine during pregnancy increases nicotine-induced locomotor activity and nicotine locomotor sensitization at later ages (PND 180 and PND 450) (Pauly et al., 2004; Yochum et al., 2014; Sobrian et al., 2008). According to this, exposure to nicotine during pregnancy and after birth alters the morphology and neurochemistry of the brain over time, making the brain more sensitive to nicotine and improving the reaction to it even in later life (PND 450; Sobrian et al., 2008).

In conclusion, our findings show that nicotine exposure throughout pregnancy and after delivery increases nicotine reinforcement and salience. This improvement may highlight the behavioral underpinnings of adolescent and adult humans' heightened susceptibility to nicotine dependence.

## **Declarations**

### **Data availability statement**

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

### **Ethics statement**

The study was approved by the Animal Care and Bioethics Committee of the National Institute of Psychiatry in Mexico City, in accordance with the Principles of Laboratory Animal Care, as outlined by the National Institutes of Health in the USA. The study was conducted in accordance with the local legislation and institutional requirements.

### **Author contributions**

**SB:** Investigation, Methodology, Project administration, Supervision.

**AS-J:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing—original draft, Writing—review & editing.

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### **Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

### **Competing Interests**

The authors declare no competing interests.

## Generative AI statement

The author(s) declared that no Generative AI was used in the creation of this manuscript.

## Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Tables

Tables 1 to 3 are available in the supplementary files section.

Figures

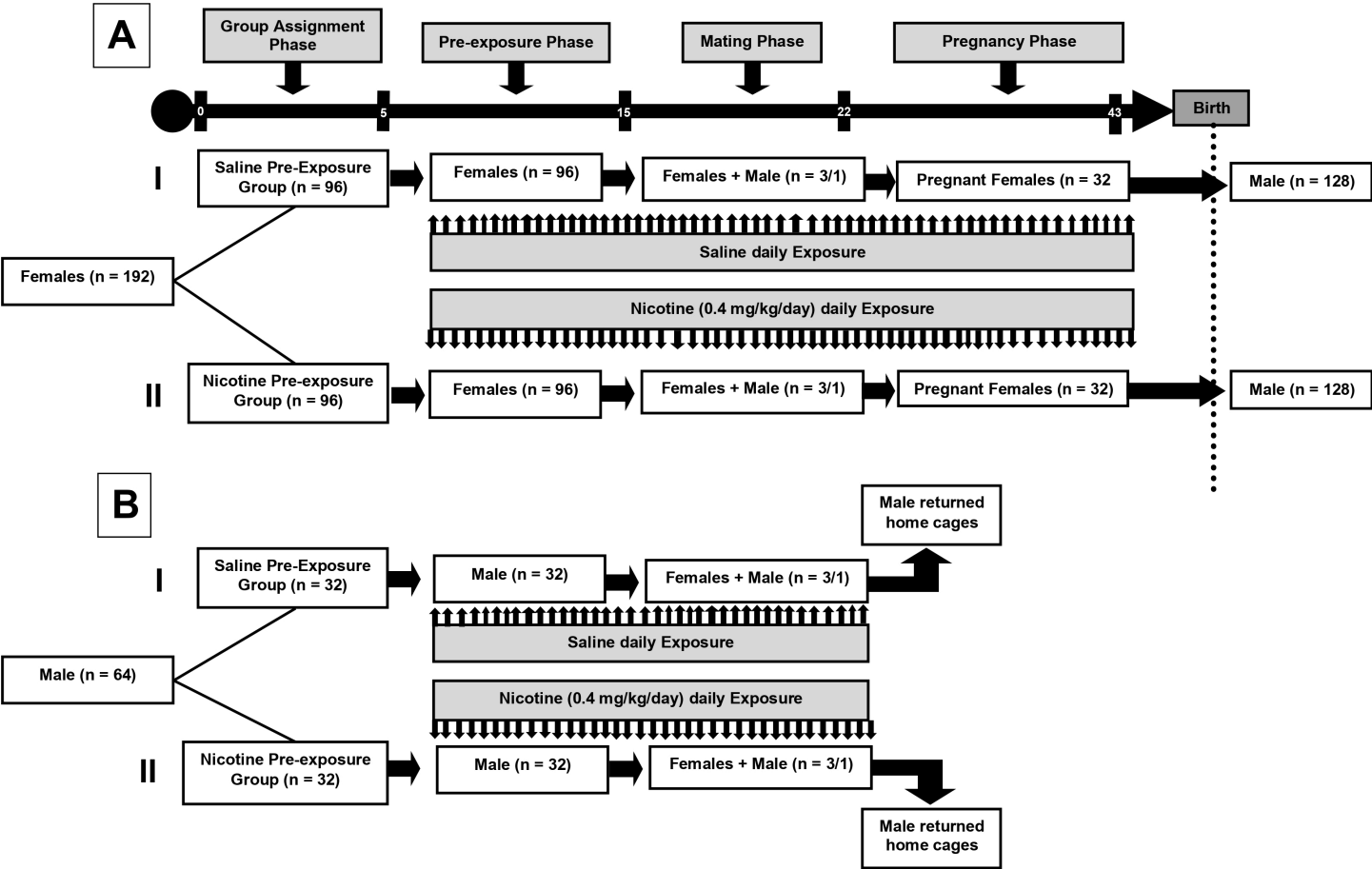


Figure 1

Figure 1

Experimental procedures. A flowchart illustrates the phases of the animal mating procedure. A) In the group assignment phase, 192 virgins female Wistar rats were divided into two groups: I) saline pre-exposed (n = 96) and II) nicotine pre-exposed (n = 96). During the pre-exposure phase, females in the saline group received 10 once-daily saline injections, while those in the nicotine group received 10 once-daily nicotine injections. For the mating phase, 3 females were housed with one sexually experienced male Wistar rat. During the pregnancy phase, pregnant females from the saline pre-exposed group received daily saline administration, and those from the nicotine pre-exposed group received daily nicotine administration. B) Separately, 32 males were assigned to a saline pre-exposed group and 32 males to a nicotine pre-exposed group. In their respective pre-exposure phases, these males received daily administration of saline or nicotine. At the end of the mating phase, males returned to their home cages.

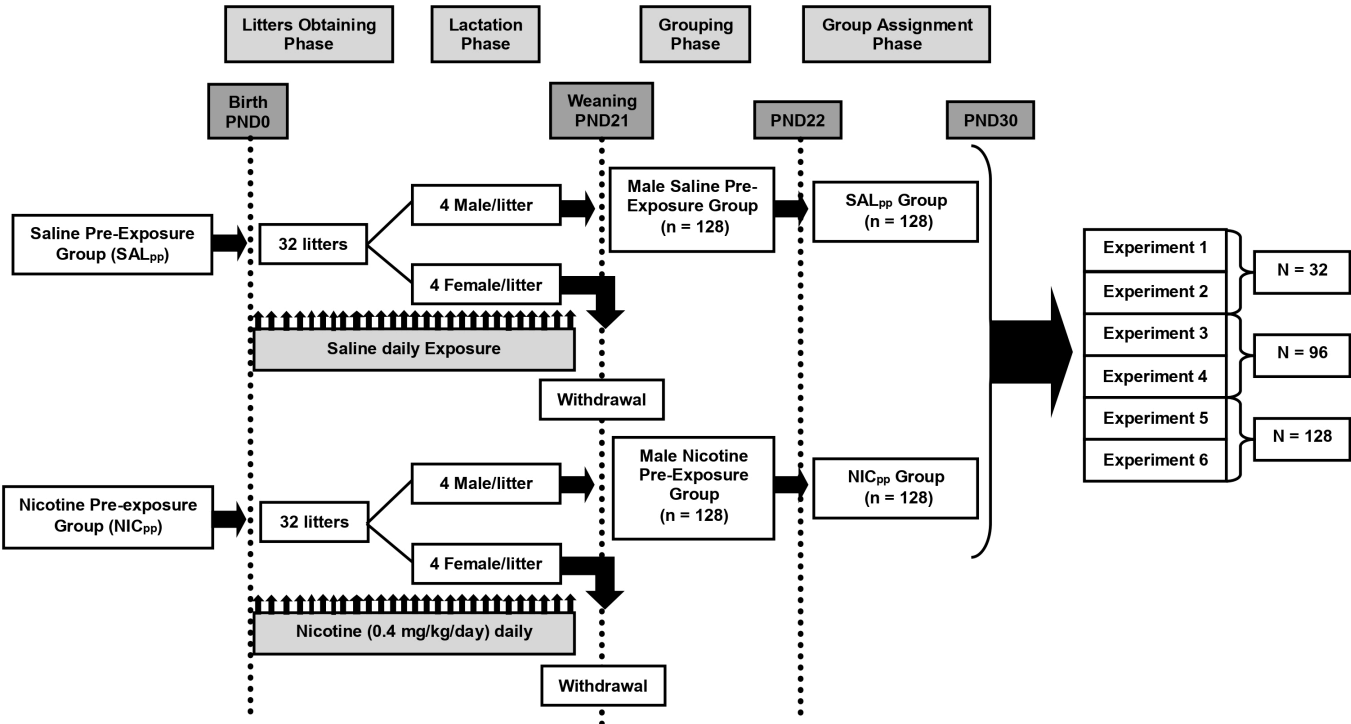


Figure 2

Figure 2

Experimental procedures. A flowchart also details the offspring assignment process. A total of 32 litters were obtained from saline pre-exposed females and 32 litters from nicotine pre-exposed females. Each

litter consisted of 8 pups (4 females, 4 males). During the lactation phase (PND0 to PND21), lactating females from the saline pre-exposed group received once-daily saline, while those from the nicotine pre-exposed group received once-daily nicotine. Male pups from litters of the saline pre-exposed group were designated the male saline pre-exposed group (SAL<sub>pp</sub>; n = 128). Similarly, male pups from litters of the nicotine pre-exposed group formed the male nicotine pre-exposed group (NIC<sub>pp</sub>; n = 128). The 'pp' designation indicates the prenatal/postnatal exposure group of origin for the animals. Finally, animals from the SAL<sub>pp</sub> and NIC<sub>pp</sub> groups were assigned to the various experiments.

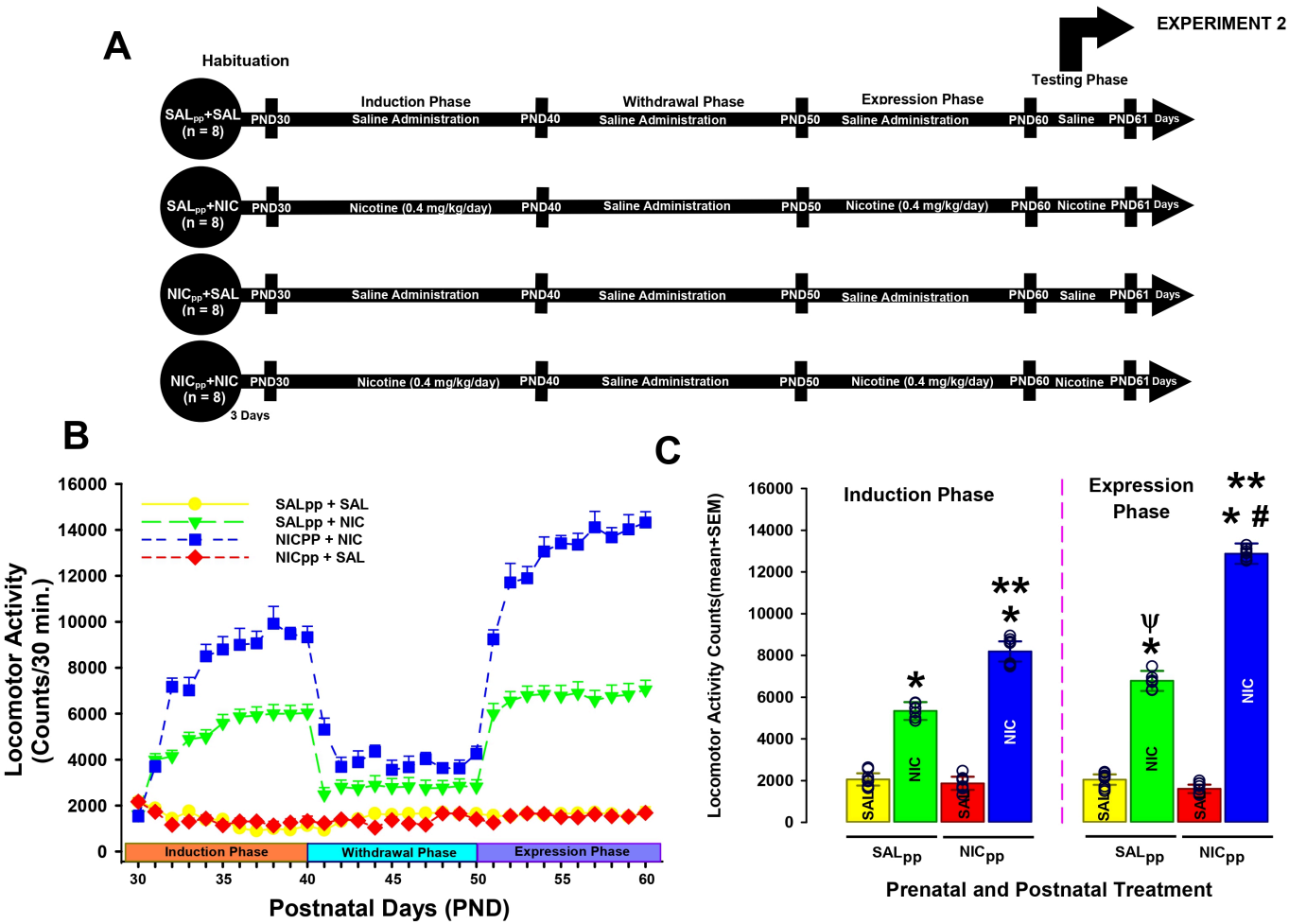


Figure 3

Figure 3

Prenatal and postnatal nicotine exposure enhanced the induction and expression of nicotine-induced locomotor sensitization. (A) Experiment Timeline: Following habituation (Ha), during the induction and expression phases, SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups received saline, while SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups received nicotine (0.4 mg/kg/day). (B) Prenatal and postnatal nicotine exposure enhanced the induction and expression of nicotine-induced locomotor sensitization. (C) Each bar represents the

mean locomotor activity ( $\pm$  S.E.M.) for each group ( $n = 8$  animals/group) during the 10-day induction and 10-day expression phases of locomotor sensitization. Phases are denoted as 'I' for induction and 'II' for expression. \* $p < 0.01$ : significant effects between SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups compared to SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups. \*\* $p < 0.01$ : significant effects between SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups. # $p < 0.01$ : significant effects of nicotine treatment between induction and expression in NIC<sub>pp</sub> + NIC groups. ‡ $p < 0.01$ : significant effects of nicotine treatment between induction and expression in SAL<sub>pp</sub> + NIC groups, as determined by a linear general model followed by Bonferroni tests.

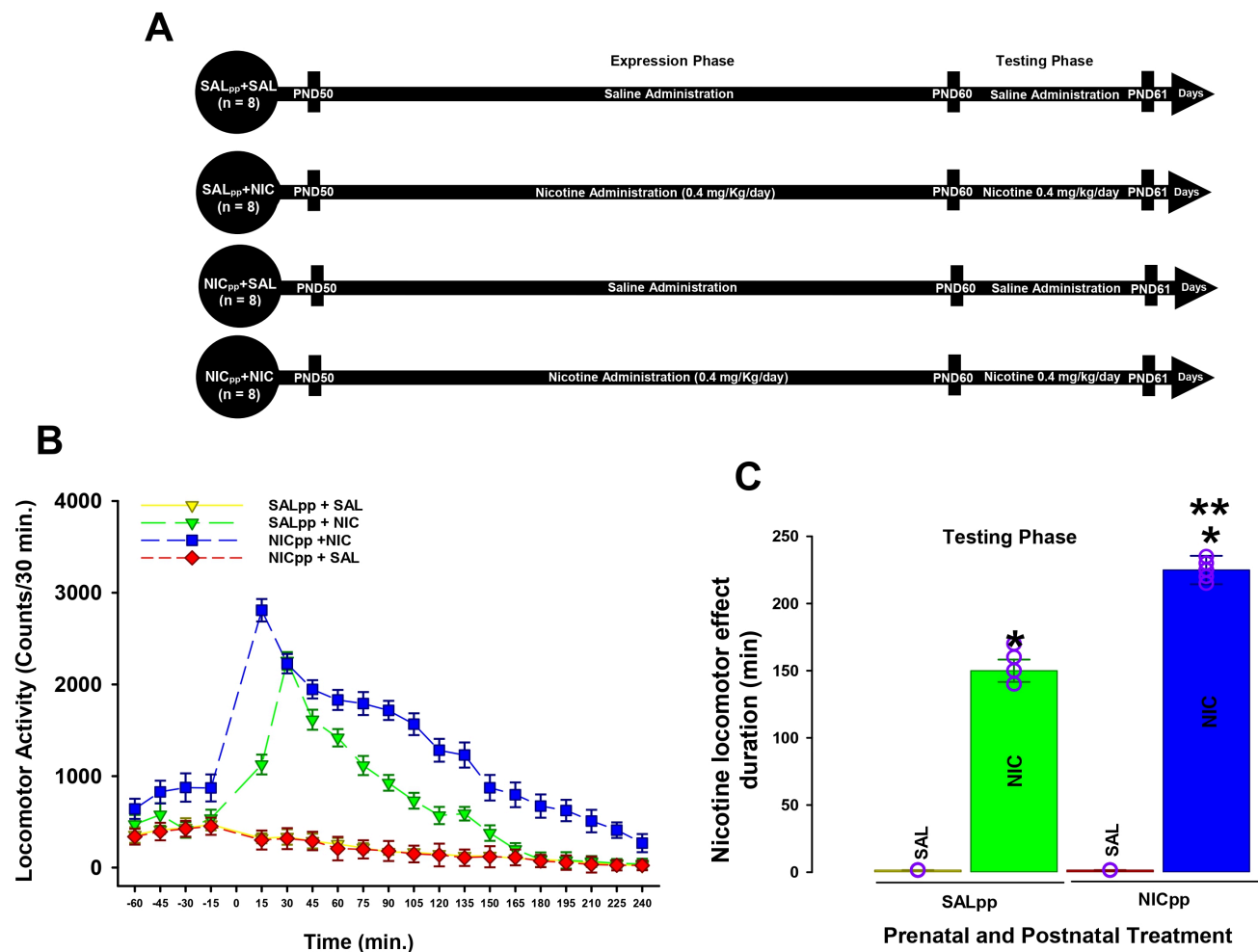


Figure 4

Figure 4

Timeline of Experiment: The SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL received saline, and the SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC rats received nicotine. (A) Time profile of the effect of treatment administration on locomotor activity (B). Prenatal and postnatal nicotine exposure (0.4 mg/Kg/day) enhanced the duration of the nicotine-induced locomotor effect. Mean duration ( $\pm$  S.E.M.) of the locomotor effect induced by the treatments (C). \* $p < 0.01$  significant effects between the SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups

compared to the SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups. \*\*p < 0.01 significant effects between the SAL<sub>pp</sub> + NIC and the NIC<sub>pp</sub> + NIC groups, as determined by linear general model followed by Bonferroni tests.

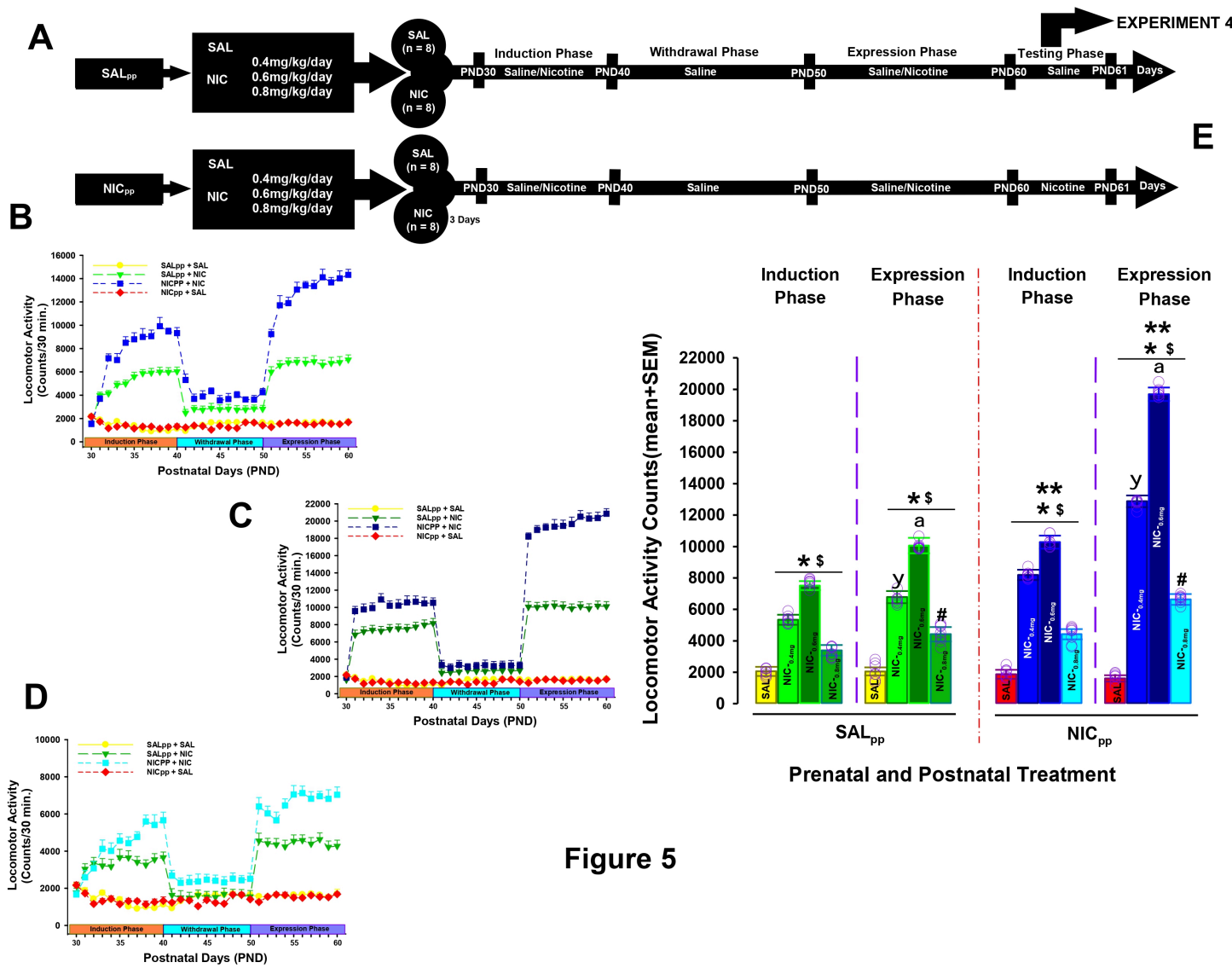


Figure 5

Figure 5

Prenatal and postnatal nicotine exposure enhanced locomotor activity induced by different nicotine doses during induction and expression of locomotor sensitization. (A) Timeline of Experiment: After habituation (Ha), during induction and expression, the SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL received saline, and the SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC animals received different nicotine doses. Prenatal and postnatal nicotine exposure enhanced locomotor activity as induced by administration of (B) 0.4, (C) 0.6, and (D) 0.8 mg/Kg/day of nicotine during induction and expression of locomotor sensitization. (E) Each bar represents the mean locomotor activity (± S.E.M.) per group (n = 8 animals per group), during the 10-day duration of the induction and the 10-day duration of the expression of the locomotor sensitization. “I” represents the induction phase, and “II” represents the expression phase. \*p < 0.01 significant effects

between the SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups compared to the SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups. \*\*p < 0.01 significant effects between the SAL<sub>pp</sub> + NIC and the NIC<sub>pp</sub> + NIC groups. \$p < 0.01 significant effects of the nicotine treatment between induction and expression in the NIC<sub>pp</sub> + NIC groups and between induction and expression in the SAL<sub>pp</sub> + NIC groups. yp < 0.01 significant effects of the 0.4 mg/Kg nicotine dose between induction and expression. ap < 0.01 significant effects of the 0.6 mg/Kg nicotine dose between induction and expression. #p < 0.01 significant effects of the 0.8 mg/Kg nicotine dose between induction and expression, as determined by linear general model followed by Bonferroni tests.

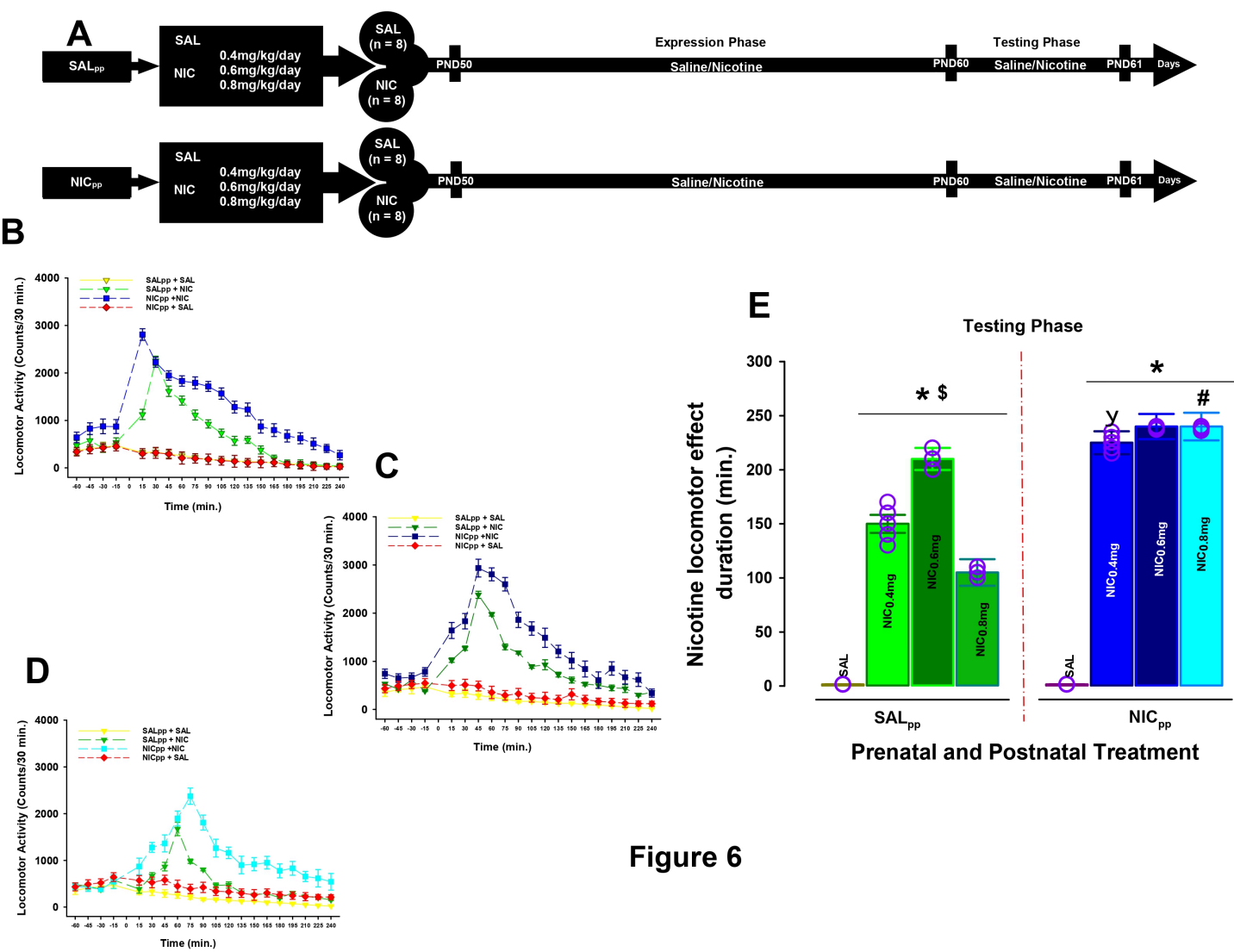


**Figure 6**

Timeline of Experiment: SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups received saline, whereas the SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups received different doses of nicotine. (A) Time profile of the effects of (B) 0.4, (C) 0.6, and (D) 0.8 mg/Kg/day of nicotine. Prenatal and postnatal nicotine exposure enhanced the duration of the nicotine-induced locomotor effects. Mean duration ( $\pm$  S.E.M.) of the locomotor effects produced

by the treatments (G). \* $p < 0.01$  significant effects between the SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups compared to the SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups. \*\* $p < 0.01$  significant effects between the SAL<sub>pp</sub> + NIC and the NIC<sub>pp</sub> + NIC groups. § $p < 0.01$  significant effects of the nicotine treatment between induction and expression in the NIC<sub>pp</sub> + NIC groups and between induction and expression in the SAL<sub>pp</sub> + NIC groups.  $\gamma p < 0.01$  significant effects of the 0.4 mg/Kg nicotine dose between induction and expression.  $\alpha p < 0.01$  significant effects of the 0.6 mg/Kg nicotine dose between induction and expression. # $p < 0.01$  significant effects of the 0.8 mg/Kg nicotine dose between induction and expression, as determined by linear general model followed by Bonferroni tests.

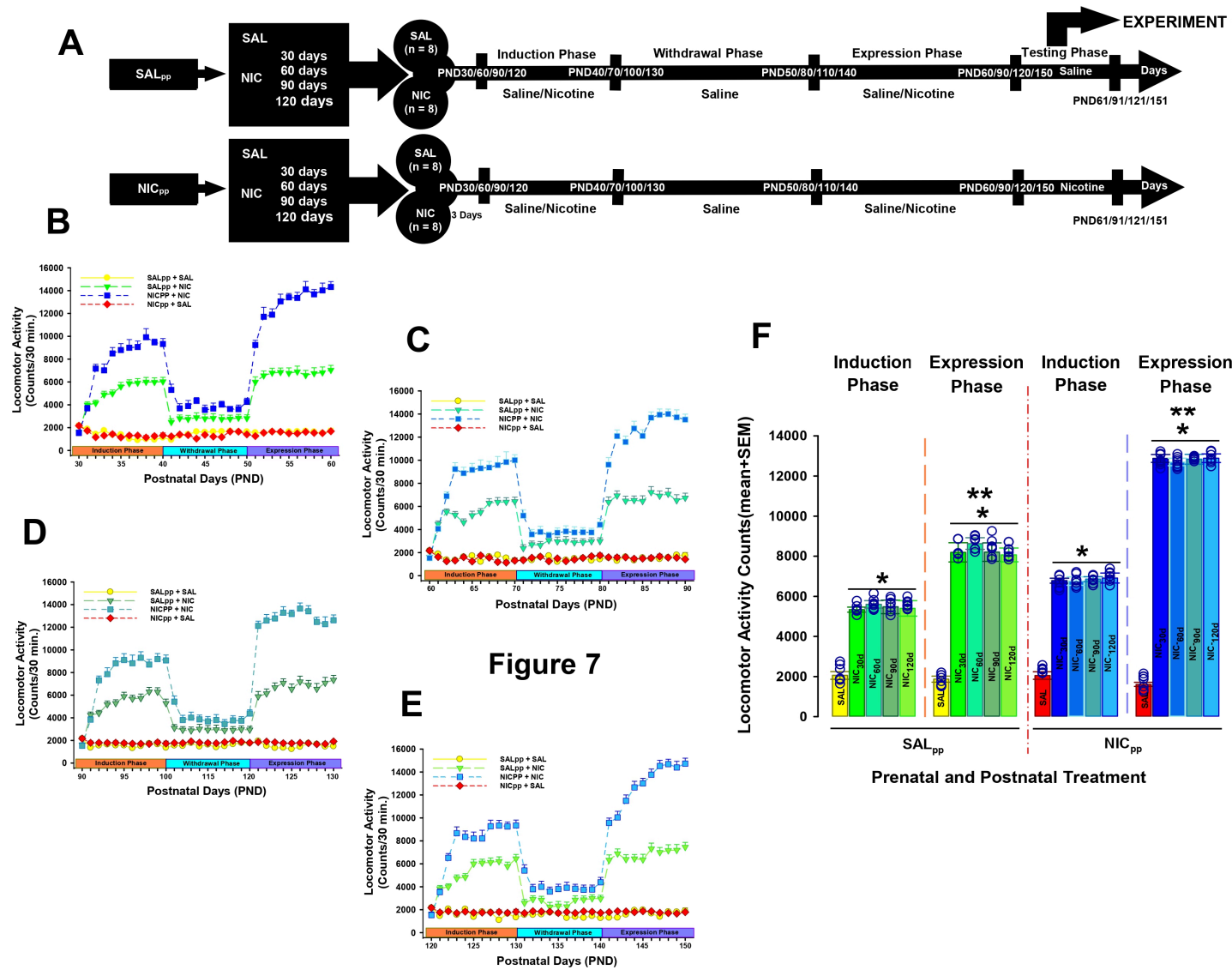


Figure 7

Prenatal and postnatal nicotine exposure enhanced the induction and expression of nicotine-induced locomotor sensitization at different ages. (A) Timeline of Experiment: After habituation (Ha), during induction and expression, the SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups received saline, whereas the SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups received nicotine (0.4 mg/kg/day). (B) Prenatal and postnatal nicotine

exposure enhanced the induction and expression of nicotine-induced locomotor sensitization at 30 (B), 60 (C), 90 (D), and 120 (E) days of age. (F) Each bar represents the mean locomotor activity ( $\pm$  S.E.M.) per group ( $n = 8$  animals per group), during the 10-day duration of the induction and the 10-day duration of the expression of the locomotor sensitization. "I" represents the induction phase, and "II" represents the expression phase. \* $p < 0.01$  significant effects between the SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups compared to the SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups. \*\* $p < 0.01$  significant effects between the SAL<sub>pp</sub> + NIC and the NIC<sub>pp</sub> + NIC groups, as determined by linear general model followed by Bonferroni tests.

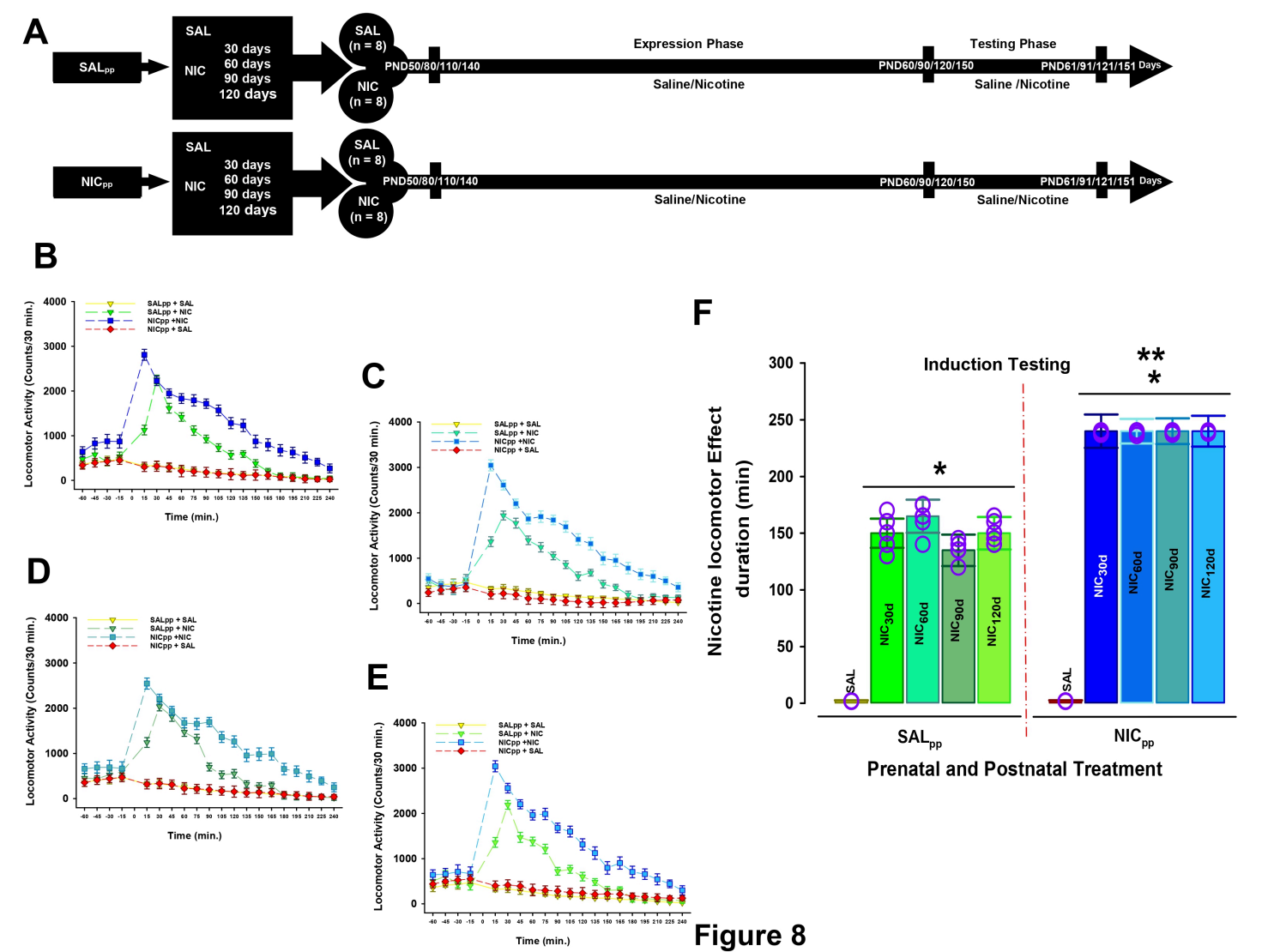


Figure 8

Timeline of Experiment: SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups received saline, whereas the SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups were administered nicotine at different ages. (A) Time profile of the effects at (B) 30, (C) 60, (D) 90, and (E) 120 days of age. Prenatal and postnatal nicotine exposure, age-dependent, enhanced the duration of the nicotine-induced locomotor effects. Mean duration ( $\pm$  S.E.M.) of the locomotor effects induced by the treatments (G). \* $p < 0.01$  significant effects between the SAL<sub>pp</sub> + NIC and NIC<sub>pp</sub> + NIC groups compared to the SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups. \*\* $p < 0.01$  significant effects between the SAL<sub>pp</sub> + NIC and the NIC<sub>pp</sub> + NIC groups, as determined by linear general model followed by Bonferroni tests.

and NIC<sub>pp</sub> + NIC groups compared to the SAL<sub>pp</sub> + SAL and NIC<sub>pp</sub> + SAL groups. \*\*p < 0.01 significant effects between the SAL<sub>pp</sub> + NIC and the NIC<sub>pp</sub> + NIC groups, as determined by linear general model followed by Bonferroni tests.

## Supplementary Files

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