Maternal immune activation in late gestation enhances locomotor response to acute but not chronic amphetamine treatment in male mice offspring: Role of the D1 receptor
Research report

Maternal immune activation in late gestation enhances locomotor response to acute but not chronic amphetamine treatment in male mice offspring: Role of the D1 receptor

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ABSTRACT

Exposure to elevated levels of maternal cytokines can lead to functional abnormalities of the dopaminergic system in the adult offspring, including enhanced amphetamine (AMPH)-induced locomotion. Therefore, it seems reasonable to consider that offspring of challenged mothers would behave differently in models of addictive behavior, such as behavioral sensitization. Thus, we sought to evaluate the effects of prenatal exposure to lipopolysaccharide (LPS) on the locomotor response to acute and chronic AMPH treatment in male mice offspring. For this purpose, LPS (Escherichia coli 0127:B8; 120 μg/kg) was administered intraperitoneally to pregnant Swiss mice on gestational day 17. At adulthood, male offspring were studied under one of the following conditions: (1) locomotor response to acute AMPH treatment (2.5 or 5.0 mg/kg) in an open field test; (2) behavioral sensitization paradigm, which consists of a daily injection of AMPH (1.0 mg/kg) for 10 days and observation of locomotion in the open field on days 1, 5, 10 (development phase), 15 and 17 (expression phase). The LPS-stimulated offspring showed enhanced locomotion after an acute AMPH challenge in comparison to baseline and saline pre-treated mice. They also showed development of behavioral sensitization earlier than the saline pre-treated group, although no differences between saline and LPS pre-treated groups were observed on development or expression of locomotor behavioral sensitization to AMPH. Furthermore, there was up-regulation of D1 receptor protein level within striatum in the LPS-stimulated offspring which was strongly correlated with increased grooming behavior. Taken together, our results indicate that maternal immune activation alterations caused by maternal immune activation are restricted to the acute AMPH challenge, mostly due to up-regulation of the D1 receptor within the mesolimbic and nigrostriatal pathways, but no locomotor differences were observed for behavioral sensitization to AMPH.

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1. Introduction

The occurrence of maternal infection during pregnancy is considered an environmental risk factor that can lead to emergence of many neuropsychiatric disorders in later life, including autism [1] and schizophrenia [2]. It is worth noting that many studies have attempted to mimic the long-term consequences of maternal immune activation (MIA) during pregnancy on the subsequent brain development of the offspring. These models include exposure to lipopolysaccharide (LPS) and polyriboinosinic-polyribocytidylic acid (PolyI:C), which mimic gram-negative bacterial infection and viral infection, respectively [for recent reviews, see 3, 4]. LPS is recognized by toll-like receptor (TLR) 2 and TLR4, whereas PolyI:C is recognized primarily by TLR3 [5,6]. Upon binding to TLRs, LPS and PolyI:C both stimulate the production and release of many pro-inflammatory cytokines by immune cells, including interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α [7,8]. Additionally, PolyI:C is a potent inducer of type I interferon (IFN)-α and IFN-β [5,9]. It is now widely accepted that elevated levels of maternal pro-inflammatory cytokines during specific stages of fetal brain development could lead to abnormalities in the adult offspring’s central neurotransmission and behavior.

In this sense, maternal immune activation during pregnancy can lead to functional abnormalities in the adult offspring’s dopaminergic system, including altered expression of tyrosine hydroxylase (TH) in the dorsal and ventral striatum [10,11] and the dopamine (DA) receptors in the prefrontal cortex [11,12]. Disruption of normal dopaminergic development may thus represent a mechanism by which maternal immune challenge during pregnancy can lead to brain and behavioral pathological changes in the offspring. Additionally, behavioral alterations in the offspring exposed...
to inflammatory stimuli include impaired social behavior [13], reduced motor activity after an immune challenge [14], impaired sensorimotor gating [12], and enhanced amphetamine (AMPH)-induced locomotor response [7,11,12,15].

The locomotor response to acute AMPH treatment is routinely used as a behavioral indicator of mesolimbic DA pathway activity [16,17]. Moreover, enhanced susceptibility to dopaminergic stimulation by acute AMPH treatment has been associated with the positive symptoms of schizophrenia [18–20]. Therefore, in light of the fact that sensitization of the DA pathways has been related to schizophrenia symptoms [21], it seems reasonable to consider that oﬀspring exposed to pro-inflammatory stimuli during pregnancy would behave differently in models of addictive behavior, such as behavioral sensitization.

Behavioral sensitization consists of the ability of addictive drugs to increase drug-elicited behavioral responses (locomotion) progressively after repeated exposure in laboratory animals [22]. This has been demonstrated for diﬀerent drugs of abuse, such as cocaine [23], AMPH [24], ethanol [25], morphine [26] and nicotine [27]. Sensitization to drug-induced hyperlocomotion is a powerful tool for the study of drug reinforcement properties and drug-seeking behavior, which promote understanding of the mechanisms of plasticity in the dopaminergic mesolimbic pathway, which is crucial to the establishment of drug addiction and cravings [28–30].

Based on this background, we sought to determine the diﬀerential outcomes of maternal immune activation induced by LPS injection on locomotor response to acute and chronic AMPH treatment (i.e., behavioral sensitization) in male mice oﬀspring. Furthermore, the participation of the D1 dopaminergic receptor was also evaluated.

2. Methods

2.1. Animals

Pregnant Swiss mice from our own colony, weighing 45–60 g each, were used (gestational day (GD) 0: presence of vaginal plug). Dams were individually housed in standard polypropylene cages at a room with controlled temperature (22 ± 2 °C), humidity (65–70%), and artificial lighting (12 h light/12 h dark cycle, lights on at 6:00 am), and the mice were given free access to Nuvilab® rodent chow (Nuvital company, São Paulo, Brazil) and filtered water. Sterilized and residue-free wood shavings were used as animal bedding. The animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo, Brazil (protocol # 1683/2009, FMVZ-USP).

2.2. Drugs

LPS derived from Escherichia coli serotype 0127:B8 (Sigma, USA) was freshly dissolved in 0.9% sterile saline and was administered intraperitoneally (i.p.) to pregnant mice at a dose of 120 μg/kg (approximately 4 μg/animal) on GD 17. This dose and gestational period was chosen based on previous studies in which maternal inflammation was achieved without inducing preterm delivery or inﬂuencing the oﬀspring’s growth and sensory-motor reflex development [31–33].

Amphetamine sulfate (Sigma, USA) was freshly dissolved in 0.9% sterile saline and was administered i.p. at doses of 1.0, 2.5 or 5.0 mg/kg. The doses used and time-lag were chosen according to previous studies in which diﬀerences between prenatal treatments were reported following higher AMPH doses [12,15], whereas behavioral sensitization is achieved after repeated exposure to lower AMPH doses [34].

2.3. Open field test

The open field device consisted of a round wooden arena (40 cm in diameter, 25.5 cm high walls) painted black with an acrylic washable covering. For the observations, each mouse was individually placed in the center of the apparatus, and the total locomotor activity (distance traveled in centimeters) and mean velocity were automatically measured over a period of 10 min. A video camera mounted 100 cm above the arena was used to collect the data, which were analyzed with the Ethovision 2.3 software (Noldus Information Technology, Leesburg, VA) installed on an IBM-compatible computer in an adjacent room. The time spent in grooming behavior was manually scored by an experimenter unaware of the pharmacological treatments. The device was washed with a 5% alcohol/water solution before placement of the animals to eliminate possible biasing eﬀects from odor clues left by the previous subject. Control and experimental mice were intermixed for observations that were performed from 08:00 am to 12:00 pm.

2.4. Determination of D1R protein expression

The frontal cortex and striatum from mice were lysed in 1.5-ml Eppendorf tubes by sonication in the presence of an ice-cold buffer containing 120 mM Tris–HCl (pH 6.8), 10% sodium dodecyl sulfate (SDS), 20% glycerol, 1 mM dithio-o-l-threitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), and a protease inhibitor cocktail (Sigma). Extracts were centrifuged at 13,000 × g for 13 min at 4 °C, and the supernatants were collected and stored at −80 °C. Proteins were quantiﬁed by the Bradford method (Bio-Rad protein assay), and 50 μg of total protein was electrophoresed on a 13% SDS-polyacrylamide gel (Bio-Rad system). The proteins were transferred to a nitrocellulose membrane (Invitrogen) using an iBlot gel transfer system (Invitrogen). The membrane was blocked for 1 h in 5% nonfat dry milk and 1% Tween-20 in Tris–buffered saline (pH 8.0). Subsequently, each membrane was probed overnight at 4 °C with monoclonal antibodies against D1R (1:500; Chemicon, USA) or β-actin (1:1000; Sigma), followed by several washes in TBS-T. The membranes were then incubated with an anti-mouse secondary antibody conjugated to hors eradish peroxidase (1:1000) for 120 min and visualized by chemiluminescence (ECL, Amer sham, USA). The bands were quantiﬁed using the software ImageJ.

2.5. Experimental procedure

2.5.1. Experiment 1: locomotor response to acute AMPH challenge

During adulthood (postnatal day 70–90), saline or LPS-treated mice oﬀspring (n = 9/group) were subjected to an injection of saline prior to an open ﬁeld test to deﬁne the baseline locomotion. Immediately after that test, all mice received a single administration of AMPH (2.5 or 5.0 mg/kg) and were subjected to open ﬁeld test 25 min later (AMPH induced locomotion). To prevent possible cumulative eﬀects of the drug and habitation of the open ﬁeld arena, the same subject received only one dose of AMPH.

2.5.2. Experiment 2: behavioral sensitization to repeated AMPH treatment

2.5.2.1. Development phase. During adulthood, saline or LPS-treated mice oﬀspring were initially placed in the open ﬁeld for 5 min without drug administration to evaluate their baseline locomotor response in a novel environment (novelty test). To prevent the influence of reactivity to novelty in treatment outcomes, we equated the different treatment groups according to their baseline locomotor activity. A t-test conﬁrmed that there were no diﬀerences in baseline activity between treatment groups. Five days after the novelty test, mice of each prenatal treatment group received vehicle (n = 7/group) or 1.0 mg/kg of AMPH (n = 13/group) for 10 days. On days 1, 5, and 10, 10 min after vehicle or AMPH administration, the mice were tested for 10 min in the open ﬁeld arena.

2.5.2.2. Expression phase. After a 5 day withdrawal period, all mice received a saline and an AMPH (1.0 mg/kg) challenge on days 15 and 17, respectively. Ten min after administration, locomotor activity was recorded for 10 min. Immediately after the AMPH challenge test (day 17), the animals were decapitated in an adjacent room, and their brains were collected. The cortex and striatum were dissected and stored at −80 °C until analysis of D1 receptor expression.

2.6. Statistical analysis

Values are expressed as the mean ± SEM. For analysis of the acute locomotor response and the locomotion during the expression phase, a two-way analysis of variance (ANOVA) was performed, followed by a Bonferroni post hoc test. For the locomotion during the development phase, a repeated measures ANOVA followed by a Bonferroni test was performed. For protein levels, a one-way ANOVA followed by Dunnnett’s test was performed. Pearson’s correlation coefficient was calculated to compare D1R protein levels in the striatum with time spent in grooming during the AMPH challenge. The results were analyzed with the GraphPad Prism 5.0 software, and the level of statistical signiﬁcance was set at p ≤ 0.05.

3. Results

3.1. Experiment 1: locomotor response to acute AMPH challenge

3.1.1. Total locomotion

A two-way ANOVA revealed an eﬀect of prenatal treatment [F1,11] = 8.3; p = 0.01] and AMPH challenge [F2,22] = 96.455; p = 0.001] on the distance traveled in centimeters. Bonferroni post hoc analyses indicated that the 2.5 mg/kg dose of AMPH increased locomotion in the offspring of LPS-treated dams compared to the baseline locomotion (p ≤ 0.01) and the saline group (p ≥ 0.01). After a 5.0 mg/kg dose, AMPH increased locomotion in the saline group in comparison with baseline (p ≥ 0.01) and AMPH-treated mice
(2.5 mg/kg, p < 0.01). In addition, the LPS pre-treated group presented an increase of the stimulant effect induced by the 5.0 mg/kg dose compared to the baseline (p < 0.01) and the 2.5 mg/kg AMPH (p < 0.05) and the saline pre-treated groups (p < 0.05), as shown in Fig. 1A.

3.1.2. Mean velocity

An ANOVA test showed that changes in the mean velocity were observed for both prenatal treatment [F(1,11) = 6.8; p = 0.05] and AMPH challenge [F(2,22) = 89.2; p = 0.001]. The post hoc test indicated that the 2.5 mg/kg dose of AMPH increased the mean velocity of the offspring of LPS-treated dams compared to the baseline locomotion (p < 0.01) and the saline group (p < 0.01). After a 5.0 mg/kg dose, AMPH increased velocity in the saline group in comparison with the baseline (p < 0.01) and AMPH-treated mice (2.5 mg/kg, p < 0.01). In addition, the LPS pre-treated group presented an increase in the stimulant effect induced by the 5.0 mg/kg dose compared to the baseline (p < 0.01) and the 2.5 mg/kg AMPH (p < 0.05) and the saline pre-treated groups (p < 0.05), as shown in Fig. 1B.

3.2. Experiment 2: behavioral sensitization to chronic AMPH treatment

3.2.1. Development phase

A repeated measures ANOVA revealed an effect of the groups [F(3,27) = 6.1; p = 0.01] and a group x time interaction [F(6,54) = 2.7; p = 0.02] on the total locomotion during the development phase of behavioral sensitization, as illustrated in Fig. 2. The post hoc test indicated that the Sal-AMPH group presented an increase in locomotion on day 10 compared to day 1 (p < 0.05) and to the Sal-Sal group (p < 0.05). On the other hand, the groups pre-treated with LPS showed increased locomotion in response to daily AMPH administration earlier, on day 5, in relation to the LPS-Sal group (day 5, p < 0.05). On day 10, the LPS-AMPH group presented a locomotion pattern significantly different from that observed on day 1 (p < 0.001) and in the LPS-Sal group (p < 0.05).

3.2.2. Expression phase

The total locomotion analysis in the open-field after the saline challenge presented a significant treatment effect [F(1,29) = 12.8; p = 0.001], as indicated by a two-way ANOVA (Fig. 3A). For both prenatal treatments, mice that received daily AMPH injections showed increased locomotion after the withdrawal period compared to the respective saline group (p < 0.05). The total time in grooming behavior after the saline challenge presented a significant pre-treatment [F(1,27) = 4.99; p = 0.03] and treatment [F(1,27) = 6.30; p = 0.01] effects, as indicated by a two-way ANOVA (Fig. 3C). The grooming was marked increased in the LPS-saline group (p < 0.01) based on Bonferroni post hoc test.

Regarding the locomotor response to AMPH challenge, a two-way ANOVA revealed a significant treatment effect [F(1,29) = 13.8; p = 0.001]. As indicated by a post hoc test, locomotion of the AMPH-sensitized mice was significantly enhanced by the AMPH challenge for both prenatal treatments (saline, p < 0.05; and LPS, p < 0.01; Fig. 3B). The total time in grooming behavior after the AMPH challenge presented a significant pre-treatment [F(1,22) = 6.58; p = 0.01] and interaction [F(1,22) = 5.84; p = 0.02] effects, as indicated by a two-way ANOVA (Fig. 3D). The grooming was marked increased in the LPS-saline group (p < 0.05) based on Bonferroni post hoc test.

3.2.3. D1 receptor protein level

All of the protein expression analyses were performed by normalizing the densitometric values for each experiment to those obtained from the Sal-Sal group (control) from each brain region, which were defined as an optical density of 100%. The other measurements were expressed as percentages of the Sal-Sal group, with positive percentages indicating D1R up-regulation and negative percentages indicating down-regulation.

No statistical differences were found in the optical density of bands from the frontal cortical tissue among the different experimental groups (p > 0.05). In the striatum, however, D1R protein level showed significant variations among groups [F(1,8) = 5.86; p = 0.05], as depicted in Fig. 4A. The D1 receptor protein level was increased in the LPS-saline group (p < 0.05) based on Dunnnett’s post hoc test. Moreover, D1R protein level in the striatum correlated strongly with the time spent in grooming behavior during AMPH challenge (r = 0.98, p < 0.01; Fig. 4B).
4. Discussion

In the present study, we report that exposure to LPS during late gestation affects the developing brain dopaminergic system in a specific manner. On one hand, the locomotor response induced by a stimulant dose of AMPH is enhanced in the offspring of LPS-treated dams. On the other hand, the emergence of dopaminergic and behavioral disturbances provoked by maternal immune activation were not observed in the behavioral sensitization paradigm, which is a model of addictive behavior. In this sense, the current data indicate that disturbances in the maternal cytokine balance during specific stages of fetal development modulate the dopaminergic and behavioral outcomes in the adult offspring.

Emerging literature from epidemiologic, clinical, and preclinical investigations has brought attention to the association between maternal infection and the emergence of psychiatric disorders. For example, the occurrence of infections during pregnancy with Influenza, Toxoplasma gondii, Rubella and Herpes Simplex Virus Type 2 is believed to increase the risk of schizophrenia in children [for review, see 35]. Although the mechanisms underlying this epidemiological relationship remain unclear, the maternal cytokine-associated inflammatory response to infection is considered a crucial link, as the identity of the pathogen seems irrelevant. Given the complexity of schizophrenia, an approach to the development of relevant animal models relies on focusing on specific signs or symptoms associated with schizophrenia, rather than mimicking the entire syndrome. In such cases, specific observations that have been identified in schizophrenic patients provide a focus of study in experimental animals.

It is worth noting that many studies using animal models have attempted to correlate the emergence of dopaminergic disturbances induced by maternal immune activation to symptoms of schizophrenia, such as impaired sensorimotor gating [12,36,37], deficits in social interaction [13] and working memory [38], and enhanced behavioral sensitivity to acute treatment with the indirect DA receptor agonist AMPH and the NMDA-receptor antagonist MK-801 [11,12].

In this sense, our results are in agreement with previously published studies demonstrating that prenatal exposure to pro-inflammatory cytokines enhances the offspring’s locomotor response to acute AMPH treatment, an indicator of increased
dopaminergic neurotransmission in the mesolimbic pathway [11,12]. In fact, it has been demonstrated that this outcome could be prevented by early antipsychotic treatment, which is a well-established treatment for schizophrenic patients [39].

Nevertheless, it is worth noting that the majority of studies have focused on PolyI:C exposure on GD 9 to evaluate this specific outcome. In this sense, the main feature of our study is that the stimulating effect is elicited even when the immune insult is LPS and applied on GD 17, demonstrating that the emergence of altered DA transmission is evidently independent of the gestation window of immune activation. In addition, early/middle pregnancy (GD 9) and late pregnancy (GD 17) in the mouse roughly correspond to the middle/end of the first trimester and to the middle of the second trimester of human pregnancy, respectively, with respect to developmental biology and the percentage of the gestational time in mice and humans.

The acute psychopharmacological actions of AMPH have been relatively well characterized. In particular, the ability to produce psychomotor activation and reward are thought to be primarily due to their capability to increase DA neurotransmission through the stimulation of DA release by presynaptic vesicles. The acute administration of psychostimulants, such as AMPH, elicits a typical dose–effect curve in rodents: whereas low doses stimulate locomotor and exploratory behavior, higher doses produce compulsive and stereotyped behaviors (such as licking and gnawing), with no increase or even a reduction in locomotion and exploration. The locomotor stimulation has been classically attributed to the psychostimulant-induced DA release in the ventral striatum, whereas compulsive and highly stereotyped behavior is related to DA release within the dorsal striatum [40]. Nevertheless, it is important to stress that the enhancement of the locomotor stimulant effect of AMPH only took place in mice treated with the highest dose (5.0 mg/kg), as the lowest dose (2.5 mg/kg) did not show the stimulant effect in saline-treated offspring in our experimental conditions. This discrepancy with the results of previous studies may be due to differences in the strain of mice used herein, which can account for distinct resistance to DA stimulation.

In light of the fact that maternal immune activation promotes enhanced acute AMPH-induced DA release within the mesolimbic pathway and consequentially increased locomotion, we questioned whether this enhancing property would be evident in a paradigm of repeated exposure, such as behavioral sensitization. In this sense, low doses of psychostimulants evoke or exacerbate psychotic symptoms among schizophrenic patients [41], and drugs used to treat both idiopathic and psychostimulant-induced psychosis act primarily as DA receptor antagonists [42]. In light of these shared characteristics, investigators have suggested that sensitization to psychostimulants may also be a useful animal model of schizophrenia [43].

As reviewed by Vanderschuren and Kalivas [44], behavioral sensitization to psychostimulants has two distinct stages according to drug-elicted behavior and neuroadaptations within dopaminergic pathways. Notably, the development phase is characterized by a progressive and persistent increase in locomotor activity after repeated drug administration and is correlated with an increased firing rate in the ventral tegmental area (VTA), and synaptic DA concentration in the nucleus accumbens is then increased. As a consequence, after a withdrawal period, increases in the expression and activity of the DA transporter and vesicular monoamine transporter may contribute to the enhanced release of DA during the expression phase (drug re-exposure). In response to DA overflow through the synapse, up-regulation of postsynaptic D1 receptors within the ventral striatum is also reported. In summary, the VTA plays an important role in the development of behavioral sensitization, while the NAcc is mainly related to its expression.

Although much of the research regarding sensitization to psychostimulants is focused on mesolimbic dopaminergic transmission, the emerging role of the mesocortical DA system, particularly in the medial prefrontal cortex, has gained particular attention [45]. Yet, it has been shown that a challenge with drug-inducing sensitization yields a marked increase in the DA levels within the dorsal and ventral striatum than in animals receiving the drug for the first time [46,47] or in animals exposed to the same drug but in a different environment [48–50].

In this sense, although mice treated with a repeated low dose of AMPH injection presented progressively increased locomotion during the development phase, both prenatal treatments produced similar motor stimulation in our experimental conditions, indicating that maternal treatment with LPS did not affect the offspring’s drug-induced locomotion in the behavioral sensitization paradigm. Nevertheless, our results indicate that LPS-treated mice elicits behavioral sensitization earlier than the Sal group. Additionally, the expression of behavioral sensitization after drug re-exposure (AMPH challenge) was evident in AMPH-sensitized mice regardless of prenatal treatment, demonstrating that prenatal exposure to LPS did not intensify the expression of the offspring’s behavioral sensitization.

Despite the fact that acute motor stimulation is enhanced in the offspring exposed to inflammatory stimuli, which is suggestive of increased dopaminergic transmission within the mesolimbic pathway, it seems intriguing that this susceptibility did not occur in the behavioral sensitization paradigm. However, it is essential to remember that the neuroadaptation inherent to behavioral sensitization is a much more complex process and involves extensive changes in expression of DA-related genes and proteins, such as the DA receptors and DA transporter.

This result contrasts with previous findings in which early-life factors are directly involved in the propensity of adult drug addiction. On one hand, insults during specific developmental stages (i.e., the perinatal period), such as stress [51], cocaine [52] and lead [53] exposure, high fat diet [54], and local inflammation [55], are known to alter the development of behavioral sensitization to psychostimulants. On the other hand, the exact mechanism by which this alteration arises is still unknown. These two main hypotheses have been proposed to identify factors involved on dopaminergic modulation in response to maternal immune activation, maternal hypoferrremia and fetal hypoxia [55–57].

Furthermore, our results pointed to the occurrence of contextual conditioned behavior during saline challenge, regardless of prenatal treatment. Notably, animals who received chronic AMPH injection throughout the 10 days of the development phase exhibited increased locomotion even when they received saline after the withdrawal period, showing that the pairing drug × open field was sufficient to increase a drug-elicited behavior even in a drug-free condition. Because a distinct context is repetitively paired with the drug, it is often believed that contextual sensitized effects are modulated by Pavlovian conditioning processes [58,59]. To that end, the set of contextual cues acquires the ability to produce conditioned drug-like effects (i.e., enhanced dopaminergic transmission and consequently increased locomotion), which add progressively onto the continuous pharmacological action of the drug as a consequence of repeated administration during the development phase. This phenomenon has been described for many drugs that have specific patterned behavioral effects, including AMPH, apomorphine, cocaine, methamphetamine, morphine, and ethanol [58,60–62].

Regarding the D1 receptor, immunoblotting of the striatum revealed an up-regulation of the D1 receptor protein levels in the offspring exposed to LPS during pregnancy (LPS-Sal), which in turn was down-regulated by AMPH-induced behavioral sensitization (LPS-AMPH). Curiously, despite the absence of D1 up-regulation
in AMPH-sensitized mice, which was an expected finding based on previous molecular studies, our data from experiment 2 corroborate our behavioral results from experiment 1. In this sense, the LPS-exposed offspring seems much more prone to up-regulation of dopaminergic receptors and consequently to stimulant effects of acute AMPH administration mostly due to up-regulation of the D1 receptor within the mesolimbic pathway. Moreover, the immunoblotting revealed three bands, representing the different isoforms of D1R detected in all groups. The different isoforms may correspond to the different levels of post translational modifications of D1 such as glycosylation, palmitoylation and/or phosphorylation. Indeed, these post translational processes are involving in the anchoring of the D1R at the plasma membrane [63]. As we have shown in the western blotting, the total amount of D1R protein are increased in the striatum of the LPS saline group, but decreased after repeated AMPH treatment. However, it seems that the two highest band corresponding to the partially and fully glycosylate forms of D1R are more evident in the striatum of LPS saline group than in the saline-treated group, meaning that there is more D1R at the plasma membrane in the LPS-Sal group. Notwithstanding, the necessity for an autoradiographic study to determine the binding between DA and its receptors, as well as immunolocalization of these receptors, is currently acknowledge.

In addition, grooming behavior during the expression phase was found dramatically increased in the LPS-exposed offspring which were not submitted to chronic AMPH treatment, indicating a marked correlation between D1R expression and stereotyped behavior. Curiously, both the D1R receptor expression and grooming behavior were prevented by previous chronic AMPH administration in the LPS-AMPH group, probably due to activation of negative feedback of dopaminergic receptors provoked by continuous AMPH-induced DA release. In this way, a hyper sensibility of the post synaptic nicotinic of the striatum may enhance the grooming response via the thalamo-cortico-thalamic pathway.

It was already shown that acute LPS administration can dramatically decrease DA turnover within the first hours [64]. Nevertheless, the present study provides additional evidence that DA function can be programmed by immune activation during the perinatal period as well, particularly during gestation, and has a relevant impact upon developmental neuropsychiatric research since its consequences lasts until adulthood and correlates with psychotic-like behaviors, such as enhanced susceptibility to psychostimulants and increased stereotyped behavior. In conclusion, our findings indicate that maternal immune activation in late gestation enhances the locomotor response to acute AMPH challenge and increases grooming behavior in adult male mice offspring. However, neither development nor expression of locomotor sensitization to AMPH was affected by prenatal treatment with LPS. Additionally, up-regulation of the D1 receptor in the striatum was found in offspring of mothers exposed to LPS, which was significantly correlated with stereotyped behavior. Our results indicate that maternal immune activation elicits distinct outcomes upon development of dopaminergic pathways, as locomotor responses were increased in response to acute challenge but not to chronic treatment with AMPH.

Author contributions

AZ and JPN conceived and designed the experiments. AZ and GM performed the experiments. AZ analyzed the data. JPN and GM contributed reagents, materials, and analysis tools. AZ, GM and JPN wrote the paper.

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Competing interests

The authors have declared that no competing interests exist.

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