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RESEARCH ARTICLE

Effect of subchronic exposure to ambient fine and ultrafine particles on rat motor activity and *ex vivo* striatal dopaminergic transmission

María-de-los-Angeles Andrade-Oliva[a], Yazmín Debray-García[b], Guadalupe-Elide Morales-Figueroa[a], Juan Escamilla-Sánchez[a], Omar Amador-Muñoz[c], Raúl V. Díaz-Godoy[d], Michael Kleinman[e], Benjamín Florán[a], José-Antonio Arias-Montaño[a]* and Andrea De Vizcaya-Ruiz[f]*†

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ABSTRACT

Alterations in dopaminergic transmission are associated with neurological disorders, such as depression, autism, and Parkinson's disease. Exposure of rats to ambient fine (FP) or ultrafine (UFP) particles induces oxidative and inflammatory responses in the striatum, a neuronal nucleus with dense dopaminergic innervation and critically involved in the control of motor activity.

Objectives: We used an *ex vivo* system to evaluate the effect of *in vivo* inhalation exposure to FP and UFP on motor activity and dopaminergic transmission.

Materials and Methods: Male adult Wistar rats were exposed to FP, UFP, or filtered air for 8 weeks (subchronic exposure; 5 h/day, 5 days/week) in a particle concentrator. Motor activity was evaluated using the open-field test. Uptake and release of [^{3}H]-dopamine were assessed in striatal synaptosomes, and dopamine D_2 receptor (D_2R) affinity for dopamine was evaluated by the displacement of [^{3}H]-spiperone binding to striatal membranes.

Results: Exposure to FP or UFP significantly reduced spontaneous motor activity (ambulatory distance: FP −25%, UFP −32%; ambulatory time: FP −24%, UFP −22%; ambulatory episodes: FP −22%, UFP −30%), decreased [^{3}H]-dopamine uptake (FP −18%, UFP −24%), and increased, although not significantly, [^{3}H]-dopamine release (113.3 ± 16.3 and 138.6 ± 17.3%). Neither FP nor UFP exposure affected D_2R density or affinity for dopamine.

Conclusions: These results indicate that exposure to ambient particulate matter reduces locomotion in rats, which could be related to altered striatal dopaminergic transmission: UFP was more potent than FP. Our results contribute to the evidence linking environmental factors to changes in brain function that could turn into neurological and psychiatric disorders.

ARTICLE HISTORY

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KEYWORDS

Fine particles; ultrafine particles; dopamine; D_2 receptor; motor activity; striatum; air pollution

HIGHLIGHTS

- Young adult rats were exposed to fine (FP) or ultrafine (UFP) particles for 40 days.
- Exposure to FP or UFP reduced motor activity.
- Exposure to FP or UFP reduced dopamine uptake by striatal synaptosomes.
- Neither D_2R density or affinity for dopamine was affected by FP or UFP.
- UFP was more potent than FP to exert the effects reported.

Abbreviations: D_1R: dopamine D_2 receptor; D_2R: dopamine D_2 receptor; DAT: dopamine transporter; FA: filtered air; FP: fine particles; I_{max}: maximum inhibition; *Ki*: inhibition constant; p*Ki*: $-\text{Log}_{10}$ *Ki*; UFP: ultrafine particles

Introduction

Environmental particle pollution is a persistent problem that has a serious negative impact on human health. Particulate matter (PM) is a complex mixture of organic and inorganic compounds with variable temporal and spatial compositions. Fine particles (FP, aerodynamic diameter ≤2.5 µm; also referred to as $PM_{2.5}$) are the main components of the mass of inhalable particles in the air, while ultrafine particles

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(UFP, aerodynamic diameter $\leq$0.1 μm) are more numerous and exhibit greater surface area as well as higher reactivity, toxicity, and ability to penetrate deeper into the respiratory tract. Both FP and UFP elicit oxidative stress and inflammatory responses in the peripheral and central nervous systems in a size-dependent manner (Gillespie et al. 2013; Guerra et al. 2013; Aztatzi-Aguilar et al. 2015).

The toxic effects of PM on the central nervous system are related to the effects of cytokines produced in peripheral systems, damage to the blood–brain barrier, and translocation of particles into brain tissues (Oberdörster et al. 2002; Peters et al. 2006; Genc et al. 2012; Liu et al. 2015), and these effects appear to be related to the presence of minerals, metals, and carbonaceous and organic species (Guerra et al. 2013; Haghani et al. 2020).

The striatum, a neuronal nucleus critically involved in the planning, execution, and regulation of motor programs (Bolam et al. 2000; Obeso et al. 2008; Obeso and Lanciego 2011) is highly susceptible to *in vivo* PM exposure, which results in increased dopamine turnover, altered glutamate and 5-hydroxytryptamine (5-HT; serotonin) levels (Allen et al. 2014a; Liu et al. 2015) and glial activation (Andrade-Oliva et al. 2018). Furthermore, exposure to $PM_{2.5}$ reduces the number of dopaminergic neurons in mesencephalic neuron-glia cultures (Block et al. 2004).

The striatum is densely innervated by the axons of dopaminergic neurons located mainly in the substantia nigra pars compacta (Zhai et al. 2018). The effects of dopamine are mediated by five receptors (D_1–D_5) divided into D_1-like receptors (D_1Rs; D_1 and D_5 subtypes) and D_2-like receptors (D_2Rs; D_2, D_3, and D_4 subtypes). The function of striatal neurons is finely regulated by D_1R- and D_2R-mediated effects of dopamine, and in turn, dopaminergic transmission is controlled by the regulation of neurotransmitter synthesis and release, clearance from the extracellular space by the dopamine transporter (DAT), and receptor signaling (Elsworth and Roth 1997). D_2Rs are located pre- and postsynaptically (Beaulieu et al. 2015; Sulzer et al. 2016), coupled to $G\alpha_{i/o}$ proteins and are major regulators of motor activity (Seeman et al. 2005; Agid et al. 2007).

Oxidative stress and inflammation in the brain have been related to alterations in dopaminergic transmission and thus to neurological disorders, such as Parkinson's disease, autism, depression, and schizophrenia (Chinaglia et al. 1992; Dunlop and Nemeroff 2007; Self 2010; Kim et al. 2020). In humans, PM exposure increases depressive and anxiety symptoms and the risk of cognitive impairment and dementia (Lim et al. 2012; Cacciottolo et al. 2017; Pun et al. 2017), and the exposure of rats to PM *in vivo* has been shown to induce hypoactivity, preference for immediate reward, and depressive- or autism-like behaviors (Fonken et al. 2011; Allen et al. 2013, 2014a; Li et al. 2018).

We previously reported that acute exposure of rats to PM results in decreased striatal D_2R density, whereas subchronic exposure decreases D_2R-mediated G protein activation in membranes (Andrade-Oliva et al. 2018); additionally, we showed that *in vitro* exposure to FP and UFP can alter dopamine uptake and release in isolated rat striatal nerve terminals (synaptosomes) and affect D_2R affinity and signaling in membranes and slices (Andrade-Oliva et al. 2020). Together, this information suggests a link between PM pollution and alterations in dopaminergic transmission. In the present study, we further evaluated the effect of *in vivo* exposure to concentrated FP and UFP on motor activity and *ex vivo* striatal dopaminergic transmission.

Materials and methods

Chemicals

The following chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA): (±)-L-ascorbic acid, butaclamol hydrochloride, desipramine hydrochloride, dopamine hydrochloride, GBR-12909 (1-(2-[bis(4-fluorophenyl)methoxy]ethyl)-4-(3-phenylpropyl)piperazine dihydrochloride), fluoxetine hydrochloride, ketanserin (+)-tartrate salt, and pargyline hydrochloride. [^{3}H]-Dopamine (48 Ci/mmol) and [^{3}H]-spiperone (80.6 Ci/mmol) were obtained from Perkin Elmer (Boston, MA, USA).

Animals

Wistar rats (males; 220–270 g, 8–9 weeks old) were provided in-house from the Animal Production and Experimentation Unit Laboratory (UPEAL, *Unidad de Producción y Experimentación de Animales de Laboratorio*) and divided into three groups (12 animals per group): control, exposed to filtered air (FA), FP exposure and UFP exposure groups. The sample size was estimated based on the experimental design for the *in vitro* experiments. The number of animals per group was established as $n = 12$ for each exposure condition (filtered air, FP, and UFP) because each of these groups was divided later into two subgroups. One subgroup of $n = 6$ was utilized for the obtention of synaptosomes and the other subgroup of $n = 6$ was utilized for the collection of membranes, allowing for statistical comparisons among conditions. Behavioral analyses were performed on 12 animals per condition. To further extend our previous reported work on striatal dopaminergic transmission (Andrade-Oliva et al. 2018, 2020), we performed the hereby presented study, to evaluate the impact of the exposure to FP and UFP on behavior, and we thus consider it relevant to maintain the same experimental model of male rats.

Animals were exposed to FA, FP, or UFP in whole-body exposure chambers, and because lung ventilation depends on sex, age, and weight (Valenza et al. 2011; Peralta et al. 2020), we maintained age and sex as fixed parameters. Therefore, animals were distributed equally into the experimental groups according to their initial weight (FA 244.68 ± 2.79 g, FP 245.20 ± 2.31 g, UFP 245.38 ± 2.26 g; 12 animals per group, $p > 0.05$, one-way ANOVA and Tukey's *post-hoc* test). No statistical differences in body weight were observed at the end of the exposure protocol (FA 417.25 ± 7.52 g, FP 432.52 ± 6.43 g, UFP 429.70 ± 5.35 g).

Animals were maintained under a 12-h dark-light cycle in a free-standing clean room with a changing station

docking port (bioBubble, Fort Collins, CO, USA) throughout the experimental procedures, and food and water were provided *ad libitum*. All the procedures were approved by the Cinvestav Animal Care Committee (protocol 94-14) and were conducted in accordance with the guidelines issued by the National Institutes of Health (NIH, Publications No. 8023, revised 1978) and the Mexican Council for Animal Care and under the guideline NOM-062 ZOO-1999 in compliance with Mexican law.

Exposure to concentrated airborne particles

Animal exposure was performed as described previously (Andrade-Oliva et al. 2018), with a few modifications, employing whole-body chambers associated with a particle concentrator with an inertial particle separator system (cut-off 2.5 µm for FP and 0.18 µm for UFP). Subchronic exposure (January and February of 2018) was performed for 5 h per day and 5 days per week (Monday to Friday) for eight consecutive weeks (40 effective days).

Concentrated aerosols were delivered to whole-body exposure chambers (six rats per chamber) with two chambers per treatment (FA, FP, or UFP). Control animals were exposed to ambient FA through scrubbers containing sodium permanganate and activated carbon and a high-efficiency particle HEPA filter (Mautz 1997). Ambient air was drawn at a flow rate of 150 l/min, and the airflow was set to 2.5 l/min in each chamber. The FP or UFP mass concentration in the exposure chambers was estimated by the determination of the particle mass in Teflon filters, and the concentration in ambient samples was determined with MiniVol samplers (no data for outdoor UFP mass levels were available).

Filters on the particle concentrator (47 mm diameter Teflon filters, PTFE 0.2 µm pore, Pall Tissue quartz filters, 47 mm diameter, 2500QAT-UP; Gelman Science, Ann Arbor, MI, USA) were mounted in an interspersed fashion into one line of an inertial particle separator system, removed at the end of each daily exposure and mounted again at the beginning of the next day's exposure during each week of exposure. The mean particle concentration in the exposure chambers was monitored in real-time using a TSI 3787 condensation particle counter (TSI Incorporated, Minnesota, USA).

PM characterization

Chemical characterization was performed on particles impacted onto quartz filters placed in a particle concentrator and, in parallel, on samples from ambient air obtained with a MiniVol (Airmetrics, Eugene, OR, USA) at a flow rate of 5.0 l/min. Inorganic and organic substances were determined as previously described (Andrade-Oliva et al. 2020). Elemental analysis was performed with GUPIXWIN software and a PIXE System, and organic compounds were analyzed in a gas chromatograph-mass spectrometer (GC–MS 6890–5973N, Agilent Technologies, Santa Clara, CA, USA) as described previously (Andrade-Oliva et al. 2020: Beristain-Montiel et al. 2016).

Evaluation of motor activity

Spontaneous motor activity was evaluated in 36 rats with an Activity Test Chamber (ENV-515s, MED Associates, St Albans, VT, USA). The computer-controlled system consists of opaque acrylic boxes (43.38 × 43.38 × 30.28 cm) equipped with photocells and 16 infrared beams that project through the cages from left to right and 16 rays that project through the cages from front to back.

Spontaneous locomotion activity was determined before and at the end of the exposure protocol. Following the exposure to FA or PM, animals were returned to their cages with water and food ad libitum, and after at least 4 h (to allow for recovery from the exposure) locomotion activity was evaluated. The animals were placed in the center of the open-field activity test arena, and motor activity was recorded for 1 h in 1-min epochs using the software provided by the manufacturer, and later grouped in 5-min periods. Horizontal activity (ambulatory distance, ambulatory time, ambulatory episodes, resting time, and average velocity) was monitored in the dark phase of the waking cycle (6–7 pm).

The behavioral analysis was fully automated; once the animal interrupts the first set of infrared beams, activity recording started and at the end of the recording period values for the behaviors of interest were automatically generated for each animal and analyzed offline. To evaluate the crossing of the central area (delineated by beams 6 and 11 from left to right and front to back; see Figure 1), the density of the trajectory lines was measured using ImageJ 1.47v software (National Institutes of Health, USA).

Synaptosome extraction

Three days after the end of the exposure to PM, animals (six per group; one synaptosomal preparation per animal) were decapitated using a rodent guillotine according to AVMA guidelines, the brain was quickly removed from the skull, and striatal synaptosomes were harvested (Andrade-Oliva et al. 2020). Briefly, the forebrain was dissected and immersed in ice-cold Krebs-Henseleit (KH) solution (in mM: 116 NaCl, 3 KCl, 1 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 11 $_{D}$-glucose; pH 7.4 after saturation with O_2/CO_2, 95:5% v:v), and coronal slices (400 µm thick) were obtained with a vibratome (World Precision Instruments, Sarasota, FL, USA). To reduce excitotoxicity, $CaCl_2$ was not added to the KH solution.

The striatum was dissected from the slices, and the tissue was homogenized in 10 ml of a buffered sucrose solution (0.32 M sucrose, 10 mM HEPES, 1 mg/ml bovine serum albumin (BSA), and 1 mM EDTA, pH 7.4 after adjustment with NaOH) with 10 strokes of a hand-held homogenizer (400 rpm). The homogenate was centrifuged (1500 × *g*, 4 °C, 10 min), the supernatant was collected and centrifuged (14 000 × *g*, 12 min, 4 °C), and the resulting pellet was

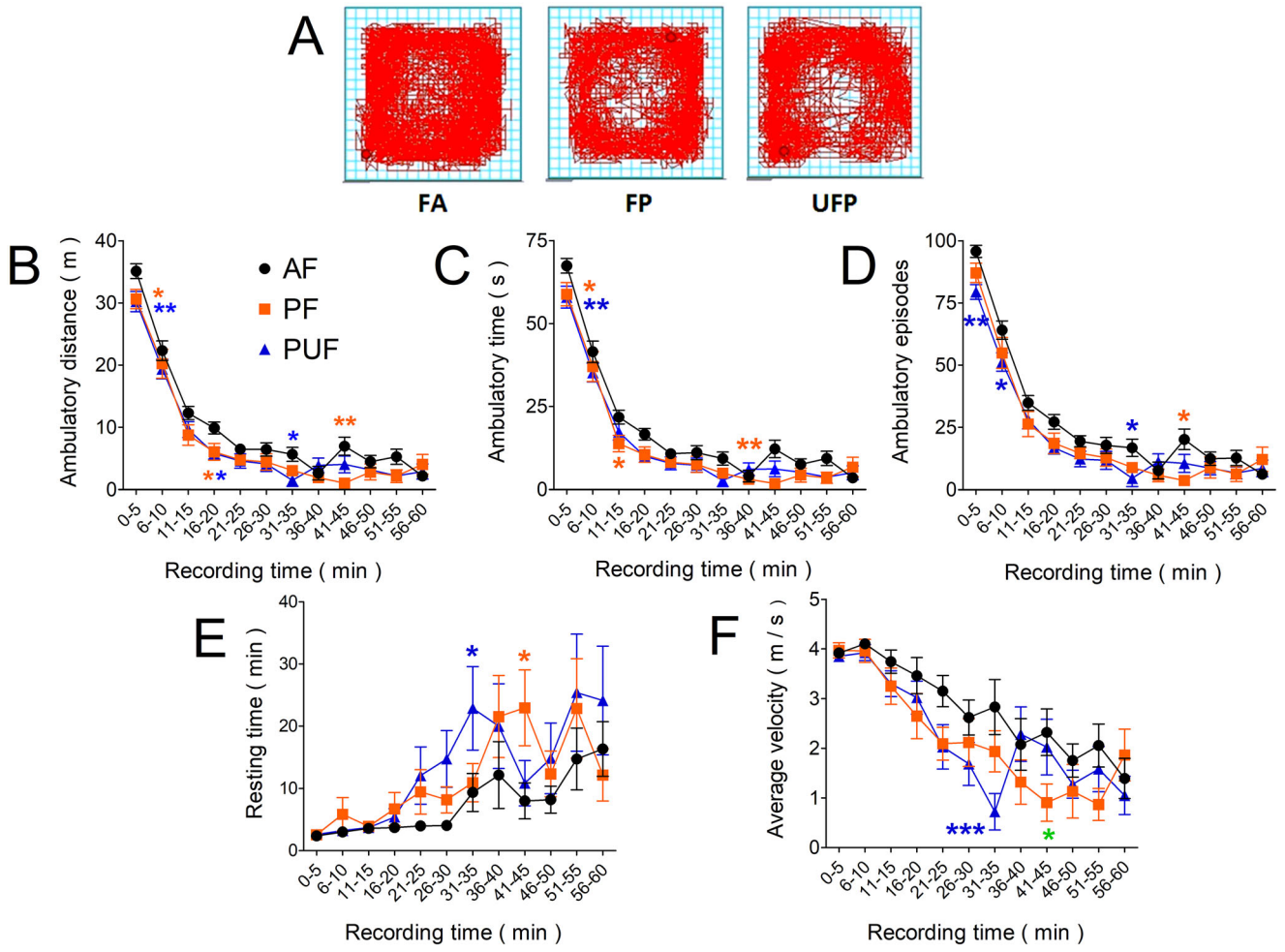

Figure 1. Effect of subchronic exposure to FP and UFP on spontaneous motor activity. (A) Representative graphs of motor activity. Images were generated by the MED activity motor program. (B–F) Time course of ambulatory distance (B), ambulatory time (C), ambulatory episodes (D), resting time (E), and average velocity (F). Values are means ± SEM of 5-min epochs (six measurements per minute) from 12 animals per group. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* the control group (square, FP, triangle, UFP); RM two-way ANOVA followed by Tukey's test.

resuspended in 5 ml of a Percoll solution (45% in a modified Krebs-HEPES solution; in mM: 140 NaCl, 10 HEPES, 5 D-glucose, 4.7 KCl, and 1 EDTA; pH 7.4). After centrifugation at 14 000 × g (2 min, 4 °C), the upper phase was collected and adjusted to a volume of 20 ml with Krebs-Ringer-HEPES solution (KRH; in mM: 113 NaCl, 25 $NaHCO_3$, 4.7 KCl, 1.2 $MgCl_2$, 1.2 KH_2PO_4, 1.8 $CaCl_2$, 15 D.glucose and 20 HEPES; pH 7.4). The suspension was centrifuged (20 000 × g, 20 min, 4 °C), and the pellet (synaptosomes) was resuspended in KRH solution. Half the volume of the synaptosomal suspension was used for [^{3}H]-dopamine uptake assessment, and the other half was used for [^{3}H]-dopamine release assays, and these tests were performed in parallel.

[^{3}H]-dopamine uptake by synaptosomes

Striatal synaptosomes (one preparation per animal, triplicate determinations for total and non-specific uptake in parallel, six samples per preparation) were incubated in 200 µl of KRH solution (8 min, 37 °C) in the presence of [^{3}H]-dopamine (final concentration 50 nM). The reaction was terminated by adding 1 ml ice-cold KRH buffer, and samples were filtered through Whatman glass fiber paper GF/B presoaked in 0.3% polyethylenimine for 2 h. Filters were washed three times with ice-cold KRH solution and immersed in 3 ml of scintillation liquid, and the tritium content was determined by scintillation counting. Non-specific uptake was determined in the presence of 10 µM GBR 12909, a selective DAT inhibitor, and specific [^{3}H]-dopamine uptake was calculated by subtracting non-specific uptake from total uptake.

Depolarization-evoked [^{3}H]-dopamine release from striatal synaptosomes

This assay was performed as previously reported (Andrade-Oliva et al. 2020), with minor modifications. Briefly, synaptosomes from individual animals were suspended in 2 ml KRH solution supplemented with 50 nM [^{3}H]-dopamine and 10 µM pargyline/200 µM ascorbic acid (to prevent neurotransmitter degradation and oxidation, respectively).

Desipramine and fluoxetine (1 µM each) were added to prevent uptake by noradrenergic or serotonergic terminals, respectively. After 30 min at 37 °C, the synaptosomal suspension was apportioned between the chambers of a superfusion apparatus (12 chambers in parallel: four replicates for each of three synaptosomal preparations from one animal per condition, i.e. FA, FP, and UFP) and perfused (1 ml/min) with KH medium supplemented with 10 µM pargyline and 200 µM ascorbic acid. After 20 min, two basal fractions were collected before [^{3}H]-dopamine release was stimulated by perfusion (1 min) with a KRH solution supplemented with 40 mM K^+ (KCl substituted equimolarly for NaCl) and returned to normal KH solution for a 6-min incubation.

The collected fractions were mixed with 4 ml of scintillation liquid to measure the tritium content by scintillation counting. The content of [^{3}H]-dopamine remaining in the synaptosomal tissue was determined by treatment with 0.5 ml 1 M HCl for 30 min before the addition of scintillation liquid. The [^{3}H]-dopamine efflux was calculated as a fraction of the tissue content at the onset of the collection period, and fractional values were then transformed to a percentage of that value of fraction 1. For statistical comparisons, the area under the curve after K^+ stimulation was determined and compared among the different groups.

Collection of striatal membranes

The striata from six rats per group (one membrane preparation per animal) were dissected and homogenized in lysis buffer (10 mM Tris-HCl, 1 mM EGTA, pH 7.4, 4 °C) using 10 strokes of a hand-held homogenizer. The homogenate was centrifuged at 800 × g (10 min, 4 °C), and the supernatant was centrifuged at 20 000 × g for 20 min at 4 °C. The resulting pellet (which contained the membranes) was resuspended in an incubation buffer, aliquoted, and stored at −70 °C until use. The composition of the incubation buffer was 50 mM Tris-HCl, 10 mM KCl, 1 mM $MgCl_2$, and 2 mM EDTA, pH 7.4, at 4 °C.

[^{3}H]-spiperone binding

For the competition assay, membranes (one preparation per animal) were incubated for 90 min at 25 °C in incubation buffer containing 2 nM [^{3}H]-spiperone and increasing concentrations of dopamine (eight concentrations, 10^{-9} to 10^{-4} M, triplicate determinations). Non-specific binding was determined in the presence of 100 nM (±)-butaclamol. The incubation buffer was supplemented with 100 nM ketanserin (to prevent [^{3}H]-spiperone from binding to 5-HT_2 receptors) and 10 µM pargyline/200 µM ascorbic acid to prevent dopamine degradation. Incubations were terminated by rapid filtration through GF/B filters presoaked in 0.3% polyethylenimine for 2 h. Filters were soaked in 3 ml of scintillation liquid, and the tritium content was determined by scintillation counting. The protein content was determined by a bicinchoninic acid assay (BCA; Pierce, Rockford, IL, USA) with BSA as a standard.

Inhibition curves were fitted to a logistic (Hill) equation by non-linear regression (GraphPad Prism 6; GraphPad Software, San Diego, CA, USA), and values for the inhibition constants (*Ki*) were calculated according to the equation (Cheng and Prusoff 1973):

$$Ki = IC_{50}/1 + \{[D]/K_d\}$$

where [D] is the concentration of [^{3}H]-spiperone present in the assay (2 nM) and K_d is the mean value for the equilibrium dissociation constant obtained from saturation analysis (0.84 nM; Andrade-Oliva et al. 2018).

Statistical analysis

The values are expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism 6.0. The data were compared using one- or two-way ANOVA and Tukey's test (comparisons between different groups). *p*-Values <0.05 were considered statistically significant.

Results

Particulate matter characterization and exposure

Rats were exposed to fine particles (FP), ultrafine particles (UFP), or filtered air (FA) in whole-body chambers using a particle concentrator for 5 h per day, 5 days per week (Monday to Friday) for 8 consecutive weeks during the winter (January and February) of 2018. Table 1 shows the FP concentration in ambient air during the exposure period, which was 29.5 ± 1.6 µg/m^3. We achieved a 3.91-fold enrichment in the exposure chambers (115.5 ± 7.1 µg/m^3). Data for outdoor UFP mass levels were not available; however, the concentration in the exposure chambers was 74.8 ± 5.6 µg/m^3. Control rats were exposed to FA (<1 µg/m^3 of PM). It is worth mentioning that a good agreement between FP mass and particle number efficiency was observed (Table 1).

The results of the chemical analysis and element quantification of FP and UFP are presented in Table 2. Total components per mass were 2.09-fold higher in FP than in UFP, and the content of elemental substances was higher than that of organic compounds for both FP and UFP (76/24% and 67/33%, respectively). The main inorganic elements present were calcium, sulfur, iron, potassium, zinc, and nickel, and the main organic compounds were benzo[*ghi*]perylene and benzylbutyl phthalate.

Effect of PM exposure on motor activity

Figure 1(A) shows representative graphs of motor activity after exposure to FA, FP, or UFP. The red line shows the movement of the animal during the recording period, and line density is therefore proportional to motor activity.

The analysis of activity showed that the main significant changes in exploratory activity occurred within the first 5 min of recording (Figures 1(B–D)). Exposure to either FP or UFP resulted in a significant decrease in the total

Table 1. Ambient fine and ultrafine particle concentration over the exposure period.

	Chamber concentrations		Ambient concentrations	
Exposure group	PM (μg/m^3)	Particle number (#/cm^3)	PM (μg/m^3)	Particle number (#/cm^3)
Filtered air	<1.0	0.02 ± 0.01	–	–
FP	115.5 ± 7.1	80 100 ± 3284	29.5 ± 1.6	21 800 ± 1.014
UFP	74.8 ± 5.6	61 000 ± 2001	N/A	N/A
FP concentration factor	3.9	3.7		

Values are means ± SEM from eight determinations. Particle counting refers to determinations (one min) at the beginning, the middle, and the end of the daily exposure (40 effective days, Monday to Friday, 5 h per day). PM concentration was obtained by calculating the difference in weight of the Teflon filters placed every third day of the week rendering by air flow and time of exposure.

Table 2. Chemical characterization of ambient fine and ultrafine particles.

	FP	UFP
Inorganic elements		
Major elements (ng/m^3)		
Fe	12 700	7484
Ca	7570	1165
S	5047	2303
K	3282	1501
Trace elements (ng/m^3)		
Zn	1979	1406
Cr	579	777
Ti	567	ND
Ni	791	865
Mn	289	245
Cu	516	222
V	88	ND
TOTAL Inorganic elements	33 408	15 968
Organic compounds		
Alkanes (ng/m^3)		
n-Dodecane	36.79	ND
n-Tetradecane	25.25	ND
n-Hexadecane	ND	31.41
n-Octadecane	15.67	29.63
n-Eicosane	77.03	85.99
n-Hentricontane	41.53	52.59
n-Pentatriacontane	20.33	22.10
n-Tricosane	ND	70.68
n-Hexacosane	ND	55.44
n-Octacosane	ND	50.50
n-Nonacosane	60.95	114.82
n-Triacontane	19.33	48.19
n-Tritricontane	41.87	ND
Polycyclic aromatic hydrocarbons (PAHs, ng/m^3)		
Phytane	37.86	35.82
Phenanthrene	1.18	1.89
Pyrene	2.23	2.51
Naphthalene	2.02	3.39
Retene	0.38	0.46
Cyclopenta[*cd*]pyrene	1.80	3.25
Chrysene	2.25	4.52
Benzo[*b*]fluoranthene	1.87	4.07
Benz[*a*]anthracene	1.12	3.65
Benzo[*k*]fluoranthene	2.67	6.09
Benzo[*e*]pyrene	2.10	4.21
Benzo[*a*]pyrene	2.22	6.41
Perylene	0.36	1.18
Indeno[1,2,3-*cd*]pyrene	2.37	5.60
Benzo[*ghi*]perylene	4.68	8.43
Fluoranthene	1.66	1.66
Phthalates (ng/m^3)		
Dimethyl phthalate	3.98	5.11
Bis[2-ethyl-hexyl] phthalate	3289.20	1978.24
Diethyl phthalate	794.55	473.71
Diisobutyl phthalate	152.59	110.38
Di-*n*-butylphthalate	669.19	664.77
Benzylbutyl phthalate	58.85	7.67
Diciclohexyl phthalate	21.69	30.51
Total organic compounds	5395.57	3924.88

Compounds were determined from aerosol concentrator filters during the exposure time. ND: not detected. Inorganic compounds have emissions as anthropogenic sources, industrial emissions, mobile parts of vehicles, and dust. Organic compounds have road traffic and industrial emissions as sources.

ambulatory distance (FP, −25%; UFP, −32%; Figures 1(B) and 2(A)), ambulatory time (FP, −24%; UFP, −22%; Figures 1(C) and 2(B)), and ambulatory episodes (FP, −22%; UFP, −30%; Figures 1(D) and 2(C)). There was a modest but non-significant increase in resting time, with no effect on average velocity (Figures 1(E,F) and 2(D,E)). Before exposure to PM, there were no differences among the three groups (data not shown). Furthermore, Figure 2(F) shows that rats exposed to FP or UFP showed less movement in the center of the arena than control rats, as evaluated by the density of trajectory lines (Figure 1(A)); this effect of UFP was significantly stronger than that of FP (FP, −19.4%; UFP, −39.2%; Figure 2(F)).

These data indicate that the reduction in motor activity of rats exposed to FP and UFP was mainly due to decreased ambulatory episodes, resulting in the reduced traveled distance, ambulatory time, and ambulatory episodes. Overall, we observed fewer exploratory movements and lower activity after exposure to PM, with more marked effects of UFP than FP, suggesting a depression-like behavior (see Discussion).

Effect of PM exposure on [^{3}H]-dopamine uptake and release in striatal synaptosomes

The uptake of [^{3}H]-dopamine by the synaptosomes from animals exposed to FP or UFP was significantly reduced (−18 and −24%, respectively) in comparison with that by the synaptosomes from control animals (Figure 3(A); in pmol/mg protein: FA, 9.72 ± 0.53; FP, 7.99 ± 0.45; UFP, 7.38 ± 0.39).

Depolarization-evoked [^{3}H]-dopamine release showed an increasing trend in the synaptosomes from animals exposed to FP or UFP (113.3 ± 16.3 and 138.6 ± 17.3% of control values, respectively; Figure 3(B,C)); however, the effect was not statistically significant.

These results indicate that PM exposure can alter striatal dopaminergic transmission at the synaptic level by reducing neurotransmitter reuptake.

Effect of PM exposure on D_2R density and affinity

Figure 4(A) shows curves of the inhibition by dopamine of [^{3}H]-spiperone binding to striatal D_2Rs, and Table 3 presents the corresponding values for receptor affinity (inhibition constant, *K*i, and Log_{10} of *K*i, p*K*i) and maximal inhibition (I_{max}). Neither the affinity nor I_{max} was

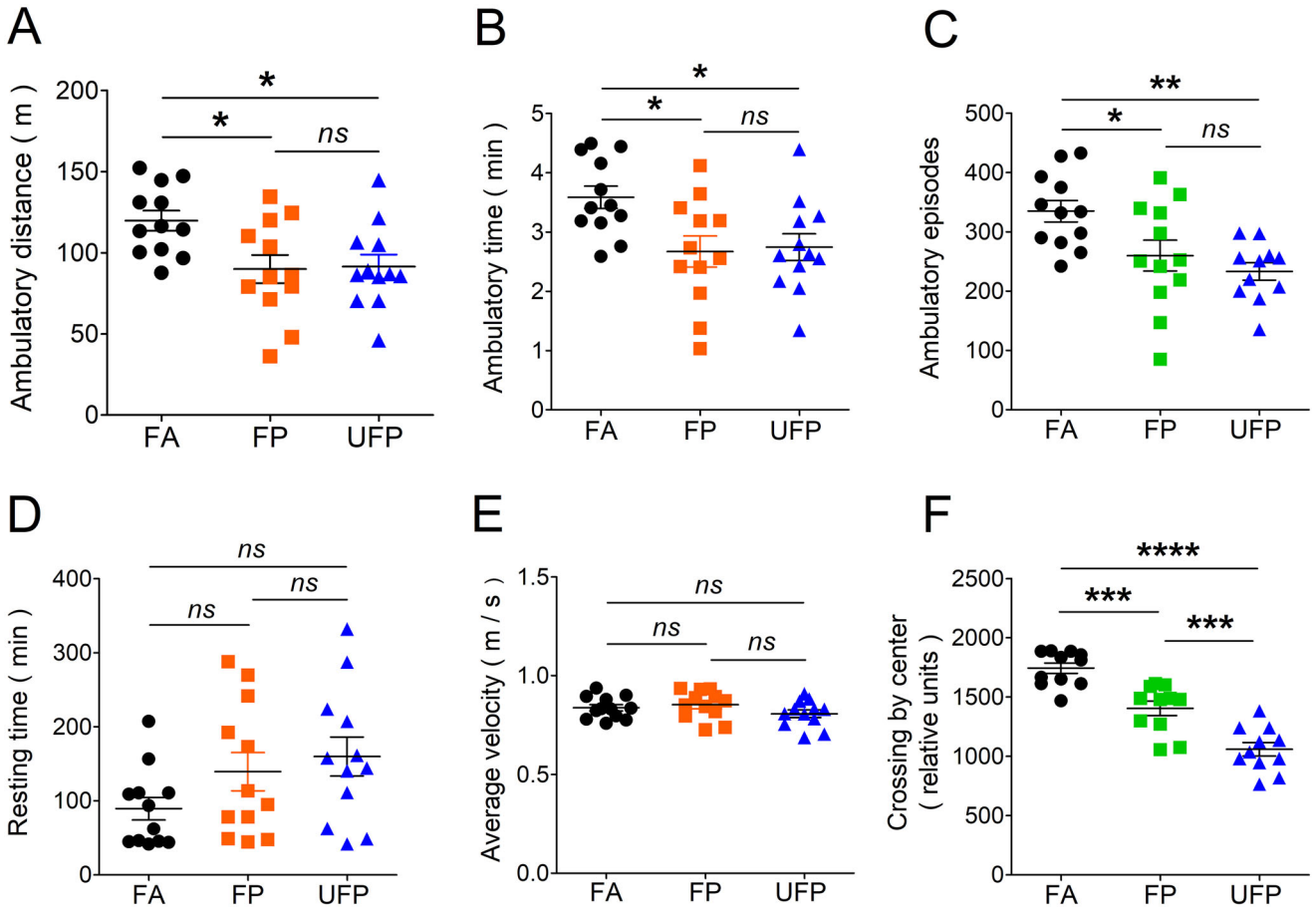

Figure 2. Quantitative analysis of the effect of sub-chronic exposure to FP and UFP on spontaneous motor activity. (A) Ambulatory distance; (B) Ambulatory time; (C) Ambulatory episodes; (D) Resting time; (E) Average velocity. Values are means ± SEM of the cumulative data (1 h) presented in Figure 1(B–E) for 12 animals per group. (F) Analysis of movement in the central area (5 × 5 squares in Figure 1(A)). Values were obtained with the ImageJ program and are means ± SEM from 11 animals for each condition. For all parameters evaluated, "ns" non-significant, * $p < 0.05$, ** $p < 0.01$, * $p < 0.001$, **$p < 0.0001$, the statistical analysis was performed with one-way ANOVA and Tukey's test.

significantly different between the groups, indicating that PM exposure does not affect D_2R affinity for its endogenous agonist.

In accordance with previous work (Andrade-Oliva et al. 2018), D_2R density in striatal membranes was determined *ex vivo*, and it was not affected by *in vivo* exposure to FP or UFP (Figure 4(B); 321.6 ± 13.2, 323.5 ± 11.6 and 321.0 ± 18.9 fmol/mg protein for the FA, FP, and UFP groups, respectively; $p > 0.05$, one-way ANOVA and Tukey's test).

Discussion

The striatum is critically involved in the planning, execution, and learning of motor programs, and its function is modulated by the effects of dopamine on D_1Rs and D_2Rs (Bolam et al. 2000; Obeso et al. 2008; Obeso and Lanciego 2011). These receptors are targets for PM toxicity, which results in oxidative stress, inflammation, astrocyte activation, changes in dopamine content, and altered D_2R density and function (Guerra et al. 2013; Andrade-Oliva et al. 2018, 2020). *In vivo* exposure of rats to FP and UFP resulted in decreased motor activity; *ex vivo* assays indicated a significant reduction in dopamine uptake and a tendency to enhance dopamine release by striatal synaptosomes.

Effect of subchronic exposure to PM on motor activity

PM pollution is a serious health concern, and according to the World Health Organization, 91% of the world's population inhabits areas where $PM_{2.5}$ levels exceed the recommended limits for health (10 μg/m^3 annual mean and 25 μg/m^3 24-h mean; WHO 2021); the highest values are reported for Delhi, Dhaka, Kabul and Beijing (Visual 2020). Considering the average human ventilation parameters, a 24-h exposure, and the mean daily concentration of $PM_{2.5}$ in Mexico City (24 μg/m^3, annual concentration during 2018), in this study, animals were exposed subchronically (40 days) to relevant ambient concentrations (115.5 ± 7.1 μg/m^3) of PM, equivalent to a 24 h average exposure of 23 μg/m^3.

PM can reach the distal parts of the respiratory system, and PM components can thus enter pulmonary circulation and potentially affect distal organs. FP exposure disrupts the blood–brain barrier and can therefore allow toxic components to gain access to the brain (Li et al. 2015; Liu et al. 2015; Chu et al. 2019). Particle size is an important

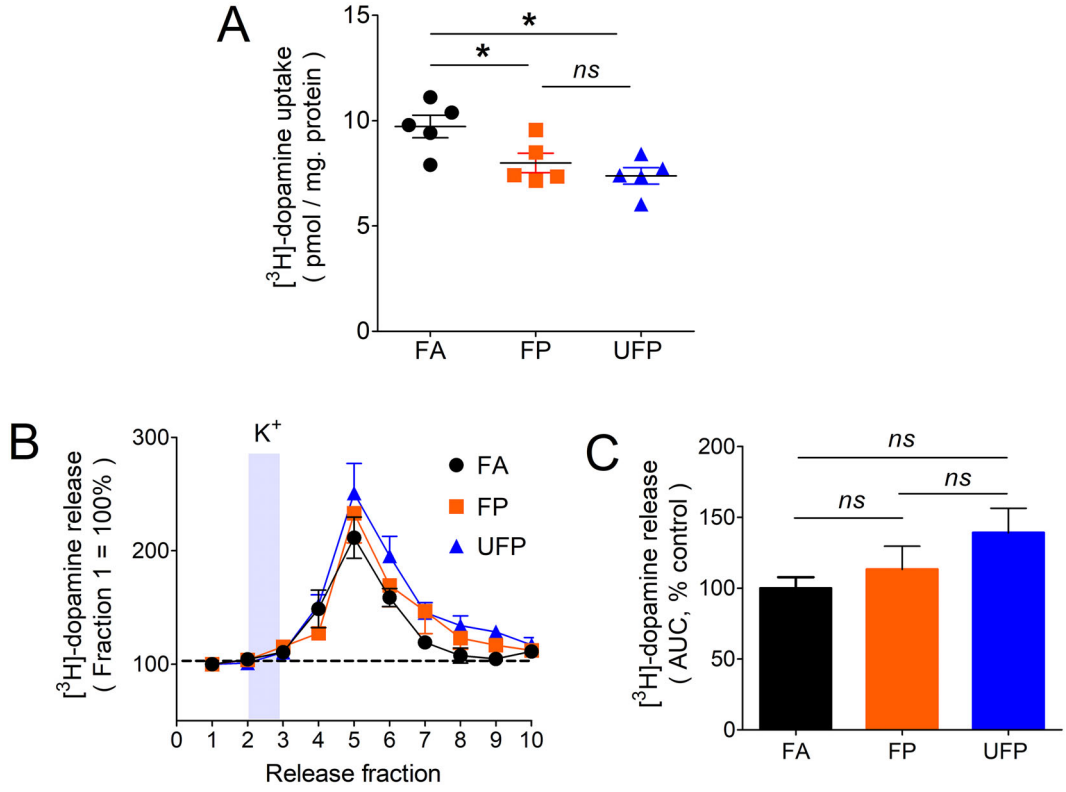

Figure 3. Effect of the subchronic exposure to FP and UFP on [^{3}H]-dopamine uptake and release in striatal synaptosomes. (A) Specific [^{3}H]-dopamine uptake by striatal synaptosomes. Values are means of $n = 5 \pm$ SEM of individual assays (one per animal) performed with triplicate determinations. (B) Representative experiment of depolarization-evoked [^{3}H]-dopamine release. Striatal synaptosomes (one preparation per animal) were labeled with [^{3}H]-dopamine (50 nM) as described in Materials and methods, the suspension was apportioned in four chambers for each preparation, and release was evoked by raising the K^+ concentration in the perfusion medium from 4 to 40 mM for the period indicated by the gray bar. Values are expressed as a percentage of [^{3}H]-dopamine release in fraction 1 and represent the means ± SEM from four replicates. (C) Statistical analysis of 5 experiments. The area under the curve (AUC) for [^{3}H]-dopamine release was calculated after basal subtraction and expressed as a percentage of the release in the corresponding sample from control animals. Values are means ± SEM from five determinations (one per animal, mean from four replicates). For (A,C), the statistical analysis was performed with one-way ANOVA followed by Tukey's test; *ns*: non-significant difference; $^*p < 0.05$.

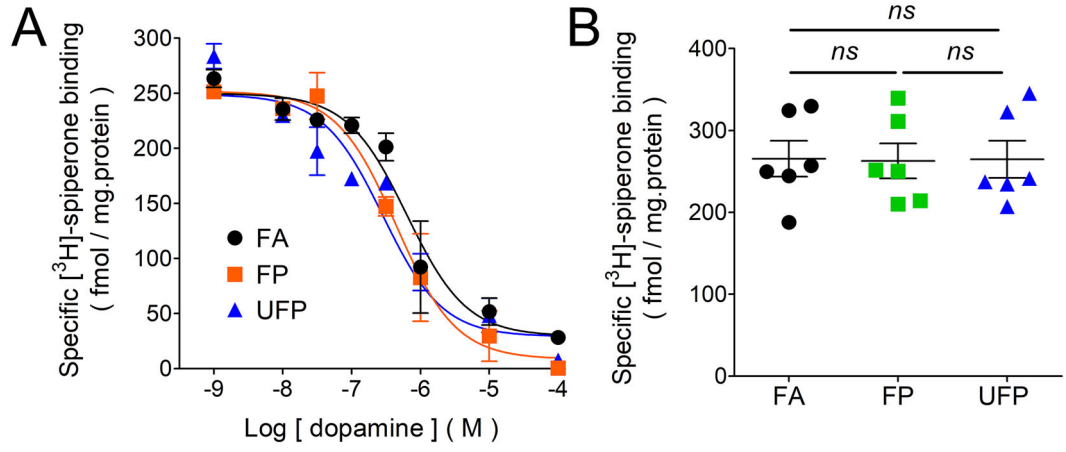

Figure 4. Effect of subchronic exposure to FP and UFP on the density and affinity of striatal D_2Rs. (A) Representative experiment of the displacement by dopamine of specific [^{3}H]-spiperone binding to striatal membranes, calculated by subtracting nonspecific [100 nM (±)-butaclamol] from total binding. Values are expressed as the percentage of control specific binding and are means of ± SEM from triplicate determinations (one membranal preparation per animal, $n = 6$). The line drawn is the best fit for a logistic equation for a one-site model. Values for maximum inhibition (I_{max}) and inhibition constants (Ki and Log_{10} Ki) calculated from the best-fit IC_{50} values are given in Table 3. (B) D_2R density in striatal membranes. Specific [^{3}H]-spiperone binding was calculated from the experiments illustrated in A and values are means ± SEM from the determinations for each animal (three replicates). *ns*: non-significant difference; one-way ANOVA and Tukey's test.

determinant of PM toxicity; our data show significant effects at lower concentrations of UFP than those required for FP-induced effects (Figures 1–3). UFP possesses higher redox activity than FP (Andrade-Oliva et al. 2020), which may be related to differences in the contents of metals, polycyclic aromatic hydrocarbons (PAHs), and heavy chain organic compounds. This, in turn, is inversely related to particle size because the surface area determines the absorption capacity

Table 3. Analysis of the effect of the subchronic exposure to ambient fine and ultrafine particles on D_2R affinity for dopamine.

	FA	FP	UFP
*p*Ki	6.76 ± 0.05	6.83 ± 0.05^{ns}	6.89 ± 0.07^{ns}
*K*i (nM)	174	148	129
I_{max} (%)	90 ± 2	88 ± 4^{ns}	91 ± 3^{ns}

I_{max}: maximal inhibition; *K*i: inhibition constant; *p*Ki: Log_{10} of *K*i. *Ns*: non-significant difference among groups; one-way ANOVA and Tukey's test.
Values are means ± SEM from individual determinations (five animals per group).

(Diociaiuti et al. 2001; Kelly and Fussell 2012). Furthermore, a smaller UFP size (aerodynamic diameter $\leq$0.1 µm) facilitates tissue penetration.

The chemical compositions of FP and UFP evaluated in this study are consistent with previous reports (De Vizcaya-Ruiz et al. 2006; Aztatzi-Aguilar et al. 2015; Andrade-Oliva et al. 2018, 2020); showing a high content (ng/m^3) of inorganic elements and relevant concentrations of organic elements, from which phthalates, alkanes, and PAHs were identified (Table 2). Both inorganic and organic compounds present in PM may be involved in the inflammatory and oxidative responses, but the isolation of individual neurotoxic factors from the complex mixture is beyond the scope of this study. Moreover, our previous work (Andrade-Oliva et al. 2020) showed that *in vitro* exposure of striatal synaptosomes to UFP and FP reduces dopamine uptake and enhances neurotransmitter release, as observed *ex vivo* in this study, suggesting that PM directly affects brain tissues.

In this study, the open-field test showed reduced motor activity (decreased ambulatory distance, episodes, and time), although the lack of an effect on average velocity indicates that motor deficits were not present and that the observed motor hypoactivity could instead be related to an imbalance between the striatal direct and indirect pathways that regulate the execution of motor programs or to the response to stress, as discussed below.

An open arena is considered an averse novel environment for rodents, and it presents stressors inherent to open spaces; this test requires allowing rats to explore their environment (Carter and Shieh 2015). Our results showed decreased exploratory activity in rats exposed to FP or UFP, and this effect was observed within the first 5 min; these results correlate with emotional reactivity as evidenced by thigmotaxis (wall touching) and avoidance of the central area (Figures 1(A) and 2(F)), which was consistent with the notion that mice prefer being near a protective wall rather than exposed to danger in an open space. Reduced activity in the center of the arena was also reported in mice exposed to $PM_{2.5}$ for 10 months (Fonken et al. 2011), and this behavior could be related to anxiety because animals in an anxious stress-like state spend less time in central areas and move closer to walls (Gogas et al. 2007). Microinfusion of the general D_2R antagonist sulpiride in the striatum, which mimics the previously reported reduction in striatal D_2R signaling (Andrade-Oliva et al. 2018), induces anxiety in rats (Nguyen et al. 2019). Further research addressing more specific anxiety-like behaviors with tests such as the elevated plus maze, fear conditioning, forced swimming, marble burying, and tail suspension tests are needed.

Effect of PM exposure on dopaminergic transmission ex vivo

Exposure to FP or UFP resulted in diminished [^{3}H]-dopamine uptake by striatal synaptosomes, which could underlie the trend of increasing depolarization-evoked [^{3}H]-dopamine release reported in this study. Together, these effects could lead to increased dopamine levels in the extracellular space and, thus, to enhanced stimulation of dopamine receptors.

As mentioned above, *in vitro* exposure of striatal synaptosomes to FP or UFP also reduced [^{3}H]-dopamine uptake and release in striatal synaptosomes (Andrade-Oliva et al. 2020), suggesting a direct effect of PM and/or its components on dopaminergic nerve terminals, although peripheral effects cannot be excluded. Whole-body exposure (Chu et al. 2019) or nasal infusion of $PM_{2.5}$ (Li et al. 2015) increases the content of several metals (Li, Be, Al, Cr, Co, Ni, Se, Cd, Ba, Ti, Mn, and Pb) in the rat prefrontal cortex, and both the FP and UFP used in this study contain significant amounts of divalent cations (Fe^{2+}, Ca^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}, and Mn^{2+}; Table 2). Zn^{2+} and Cu^{2+} inhibit [^{3}H]-dopamine uptake and stimulate, albeit modestly, [^{3}H]-dopamine release in rat striatal synaptosomes (Bondy et al. 1979; Komulainen and Tuomisto 1981a, 1981b; Minnema et al. 1986; Richfield 1993; Yasui and Verity 1996), supporting the notion that the divalent cations present in UFP and FP can alter extracellular dopamine levels.

Researchers studying the effect of air pollutants toxicity on brain dopaminergic systems have most often measured the levels of dopamine and metabolites in different brain structures (Allen et al. 2014a, 2014b; Chu et al. 2019), focusing on exposure to particles in diesel exhaust (Gerlofs-Nijland et al. 2010; Levesque et al. 2011). However, these particles represent only a portion of the PM that is produced, and this could explain the lack of an effect of exposure (5 − 7 days) to nanosized particles in diesel exhaust on [^{3}H]-dopamine uptake in rat ventral mesencephalic neuron-glia cultures that was reported by Levesque et al. (2011); however, this treatment enhanced both the inhibition of [^{3}H]-dopamine transport and the stimulation of tumor necrosis factor α (TNFα) and nitrite production induced by lipopolysaccharide, indicating that diesel exhaust particles increase the sensitivity of brain tissues to proinflammatory stimuli.

Neither striatal D_2R density nor receptor affinity for dopamine was affected by the *in vivo* exposure to FP or UFP (Figure 4 and Table 3). The lack of effect on D_2R density is consistent with our previous report (Andrade-Oliva et al. 2018), although that study also showed that subchronic exposure to FP reduced dopamine-stimulated [^{35}S]-GTPγS binding to membranes by 43%, indicating decreased D_2R signaling. In contrast, direct exposure to both FP and UFP enhanced the D_2R affinity for dopamine in striatal membranes and increased the ability of the selective D_2R agonist quinpirole to inhibit cAMP production in striatal slices (Andrade-Oliva et al. 2020). The reduction in receptor signaling after subchronic exposure to PM (Andrade-Oliva et al. 2018) could therefore involve adaptative changes in

the responses to the acute effects, most likely the desensitization of D_2Rs induced by prolonged exposure to PM.

Pathophysiological implications

In the striatum, D_2Rs are mainly expressed by a subpopulation of GABAergic medium-sized spiny neurons (MSNs) that represent over 90% of striatal neuronal cells. MSNs are divided into two cell types, which preferentially express D_1Rs or D_2Rs (Fuxe et al. 2007) and originate the direct (D_1-MSNs) and indirect (D_2-MSNs) synaptic pathways of the basal ganglia. According to the disinhibition model of basal ganglia function (Bolam et al. 2000; Obeso et al. 2008; Obeso and Lanciego 2011), increased striatal dopamine levels resulting from the decrease in dopamine uptake and the increase in release reported in this study, along with the reduced D_2R signaling reported previously (Andrade-Oliva et al. 2018), would lead to an imbalance between the direct and indirect pathways, favoring the former and facilitating the execution of motor programs. However, recent studies have shown that motor activity requires the coordinated firing of D_1-MSNs and D_2-MSNs (Freeze et al. 2013; Nguyen et al. 2019), and alterations in striatal dopaminergic transmission and D_2R signaling induced by PM exposure could therefore modify the balance in the activity of the basal ganglia's direct and indirect pathways and thus reduce motor activity, as shown here.

The loss of striatal dopaminergic innervation underlies most of the motor manifestations of Parkinson's disease. While some epidemiological evidence links $PM_{2.5}$ to increased risk for the disorder, other studies do not support such an association (reviewed by Palacios 2017 and Wang et al. 2021). However, the diagnosis of Parkinson's disease is preceded by a long pre-clinical latent period, hampering the study of air pollution as a risk factor for the disorder. We have shown that exposure of rats to PM alters *ex vivo* striatal D_2R density and function (Andrade-Oliva et al. 2018) and dopamine uptake and release (this study) and that direct exposure to PM affects dopamine uptake and release and D_2R affinity and signaling in striatal tissues (Andrade-Oliva et al. 2020). Furthermore, exposure to $PM_{2.5}$ reduces the number of dopaminergic neurons in the culture (Block et al. 2004). This information adds to the proposed link between PM pollution and alterations in dopaminergic transmission, which could contribute to the etiopathology of Parkinson's disease. Further studies on the potential interactions between pollutant exposure with other factors (e. gr. gender, smoking, use of non-steroidal anti-inflammatory drugs) and on the gene-environment interaction with air pollution are needed to enhance the understanding of the relationship between air pollution and Parkinson's disease.

Functional deficits related to dopaminergic neurotransmission are generally associated with aging, and most people diagnosed with Parkinson's disease are 60 years or older when 60–80% of the dopaminergic neurons of the substantia nigra pars compacta are lost. However, the prodromal phase spans 20 or more years before symptom onset, typically motor alterations (Goldman and Postuma 2014). Furthermore, parkinsonism can also be induced by the exposure to pesticides (rotenone and paraquat), metals (manganese), or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MTPT), and can thus appear at any stage of life. Noteworthy, there is evidence that the loss of dopaminergic terminals precedes the loss of neuronal bodies in the substantia nigra in both Parkinson's disease patients and rodent parkinsonism models (Wong et al. 2019). We found that *in vivo* (this study) and *in vitro* (Andrade-Oliva et al. 2020) PM exposure alters dopamine uptake and release by striatal synaptosomes, indicating functional alterations in dopaminergic terminals. As for pesticides, solvents, and metals, exposure to air pollutants has been associated with an increase in Parkinson's disease risk, but the evidence is still inconclusive (Periñán et al. 2022). The findings of our studies support that exposure to PM alters dopaminergic transmission and adds to the evidence that links air pollutants to increased risk for Parkinson's disease.

PM exposure has also been associated with autistic- or depressive-like behaviors in humans and rodents (Fonken et al. 2011; Lim et al. 2012; Allen et al. 2014a; Li et al. 2018), and reduced D_2R signaling has been related to anhedonia (Heinz et al. 1994). Additional behavioral alterations reported after *in vivo* exposure to FP or UFP include behaviors of despair in mice in the forced swim test, a depressive-like response (Fonken et al. 2011), and greater preference for immediate reward (which has been related to addiction, obesity, and attention deficit) in mice (Allen et al. 2013), as well as reduced ultrasonic vocalizations, sociability, burying behavior, and novel object exploration in rats after nasal instillation of $PM_{2.5}$ on postnatal days 8–22 (Li et al. 2018). Of note, these alterations resemble behavioral features of autism, including communication deficits, poor social interaction, and novelty avoidance. However, the autism spectrum disorder is a complex neurodevelopmental condition, and environmental and genetic factors are proposed to contribute to the disorder pathogenesis (Eve et al. 2022). Dopamine is involved in neurogenesis, neuronal migration, and synaptogenesis (Saad et al. 2022), and perturbations in dopaminergic innervation and transmission have therefore been proposed to participate in the etiology of the autism spectrum disorder. Although in this study animals were exposed to PM in their adult life, changes in dopaminergic transmission in adulthood could also mimic, at least to some extent, behavioral changes originated by altered development of the brain dopaminergic systems.

Limitations of the study

The limitations of this study are as follows: (1) The effects of FP and UFP on behavior result from their action in the whole body, and D_2Rs are also expressed at high levels in several brain structures, including the nucleus accumbens and the prefrontal cortex. The nucleus accumbens (ventral striatum) plays a key role in emotion and motivation processing, modulating reward and pleasure processing, and serves as a critical limbic-motor interface (Salgado and Kaplitt 2015). Regarding the striatal control of motor behavior, the coordinated activity of

accumbal D_1-MSNs and D_2-MSNs likely underlies goal-directed actions (Simpson et al. 2022). In turn, the pyramidal neurons and interneurons of the prefrontal cortex (Quintana and Beaulieu 2019) play critical roles in cognitive and executive processes that involve motivation, emotion, learning, and memory (Euston et al. 2012). Therefore, the effects of PM on the prefrontal cortex and nucleus accumbens are likely to participate, in conjunction with the striatum, in the effects reported here, and further studies are thus required to address the participation of both regions. (2) High expression of D_1Rs is also found in the striatum, nucleus accumbens, and prefrontal cortex (Beaulieu and Gainetdinov 2011), and thus, the second limitation of this work is the lack of analysis of the effect of PM on D_1R expression and function, which deserves investigation. (3) The enriched aerosol exposure system used in this study allows for the separation of FP and UFP, but FP includes a UFP fraction. For this reason, in some of the behavioral responses evaluated, a relevant difference between FP and UFP exposure may have been masked. (4) There is evidence for innate sex differences in the expression and function of D_2Rs in rodent and human brains, as well as for sex-specific differences in prevalence, symptom manifestation, and treatment responses of several neurological disorders that involve the dopaminergic system, such as drug addiction, depression, anxiety, schizophrenia, and attention-deficit/hyperactivity disorder (Williams et al. 2021). This study was conducted on male rats to further extend our previously reported work on the impact of the exposure to FP and UFP on striatal dopaminergic transmission (Andrade-Oliva et al. 2018, 2020) also performed using male animals. Further research on the reported effects should include both male and female animals. (5) Although we chemically identified and quantified FP and UFP components, we cannot attribute or distinguish the observed effects on dopaminergic transmission to a specific inorganic or organic component(s) contained in PM.

Conclusion

Subchronic exposure to FP or UFP induces hypoactivity in rats and decreases dopamine uptake by striatal synaptosomes obtained from the same animals. This study thus provides information that supports the notion that dopaminergic transmission can be affected by exposure to FP or UFP due to their effects on dopamine reuptake and release as well as on D_2R affinity and signaling (Andrade-Oliva et al. 2018, 2020). Alterations in dopaminergic transmission have been reported in several motor and mood disorders, and our results, therefore, add to the evidence that links environmental factors with changes in brain function that could in turn be related to neurological and psychiatric diseases.

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Author contributions

M.A.A.O., J.A.A.M., and A.D.V.R designed the study. M.A.A.O., G.E.M.F., Y.D.G., and J.E.S. performed the experiments. M.A.A.O., J.A.A.M., and A.D.V.R performed data analysis. B.F. contributed experimental resources. O.A.M. performed organic compound analysis. R.D.G. performed elemental analysis. M.A.A.O., J.A.A.M., and A.D.V.R. wrote the manuscript. M.K. participated in data analysis and reviewed and edited the manuscript. All authors revised and approved the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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