Evidence That Methylphenidate Treatment Evokes Anxiety‑Like Behavior Through Glucose Hypometabolism and Disruption of the Orbitofrontal Cortex Metabolic Networks

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Abstract
Methylphenidate (MPH) has been widely misused by children and adolescents who do not meet all diagnostic criteria for attention-deficit/hyperactivity disorder without a consensus about the consequences. Here, we evaluate the effect of MPH treatment on glucose metabolism and metabolic network in the rat brain, as well as on performance in behavioral tests. Wistar male rats received intraperitoneal injections of MPH (2.0 mg/kg) or an equivalent volume of 0.9% saline solution (controls), once a day, from the 15th to the 44th postnatal day. Fluorodeoxyglucose-18 was used to investigate cerebral metabolism, and a cross-correlation matrix was used to examine the brain metabolic network in MPH-treated rats using micro-positron emission tomography imaging. Performance in the light–dark transition box, eating-related depression, and sucrose preference tests was also evaluated. While MPH provoked glucose hypermetabolism in the auditory, parietal, retrosplenial, somatosensory, and visual cortices, hypometabolism was identified in the left orbitofrontal cortex. MPH-treated rats show a brain metabolic network more efficient and connected, but careful analyses reveal that the MPH interrupts the communication of the orbitofrontal cortex with other brain areas. Anxiety-like behavior was also observed in MPH-treated rats. This study shows that glucose metabolism evaluated by micro-positron emission tomography in the brain can be affected by MPH in different ways according to the region of the brain studied. It may be related, at least in part, to a rewiring in the brain the metabolic network and behavioral changes observed, representing an important step in exploring the mechanisms and consequences of MPH treatment.

Keywords Psychostimulant · Attention-deficit/hyperactivity disorder · Molecular imaging · Brain activity · Orbitofrontal cortex · Anxiety-like behavior

Abbreviations

| Term         | Abbreviation |
|--------------|--------------|
| ADHD         | Attention-deficit/hyperactivity disorder |
| CNS          | Central nervous system |
| DA           | Dopamine |
| DAT          | Dopamine active transporter |
| ¹⁸F-FDG      | Fluorodeoxyglucose-18 |
| FDR          | False discovery rate |
| MBN          | Metabolic brain network |
| MPH          | Methylphenidate |
| microPET     | Micro positron emission tomography |

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Introduction

Researchers around the world have been very concerned about the enormous incidence of the misuse of methylphenidate (MPH), a central nervous system (CNS) stimulant indicated for the treatment of attention-deficit/hyperactivity disorder (ADHD), by healthy individuals (American Psychiatric Association 2013; Perou et al. 2013; Polanczyk et al. 2014; National Institute for Health and Care and Excellence 2018). This overconsumption is mainly observed among adolescents; for several reasons, among them is an alternative to boost cognitive performance and/or for recreational use due to the effects of euphoria (Jensen et al. 2015; Clemow 2017; Kim et al. 2020a). Inappropriate use of MPH has also been observed in preschool children aged 2 to 4 years (Zito et al. 2000; Rowland et al. 2002). Although the mechanisms of MPH are not yet fully understood, it has been shown that this psychostimulant has a neuropharmacological profile similar to that of amphetamine and cocaine, increasing the levels of dopamine (DA) and norepinephrine (NE) in the synaptic cleft by binding and blocking its transporters (Kuczenski and Segal 2001; Volkow et al. 2001; Grünblatt et al. 2013; Schmeichel and Berridge 2013; Gumustas et al. 2017; Schmitz 2018; Chaaya and El Khoury 2019).

Dopaminergic neurotransmission is involved in a series of essential physiological functions, such as the regulation of brain activity and brain metabolism (Mitelman et al. 2020; Tamriage et al. 2020). There already seems to be a consensus that changes in brain metabolism are implicated in the mechanism of MPH (Fagundes et al. 2007; Scaini et al. 2008; Réus et al. 2013; Comim et al. 2014; Zhang et al. 2016, 2018). The ability to increase the transcription of several genes, as well as their corresponding proteins, suggests that MPH is capable of up-regulating neuronal activity (Yano and Steiner 2007; Banerjee et al. 2009; Benjamin et al. 2010), which probably depends on an adequate supply of ATP. In this context, we showed recently that rats subjected to chronic exposure to MPH had reduced levels of ATP in the hippocampus (Schmitz et al. 2016a). Yet, as far as we know, the effects of early chronic administration with MPH on glucose metabolism in different brain regions of rats remain largely unknown. However, since glucose is the main energy source for the brain, the effects of MPH on energy metabolism may be modulated by changes in glucose consumption in the brain.

Glucose metabolism changes have been correlated with anxiety-like and depression-like behaviors (Hu et al. 2010). In addition, excessive levels of DA and NE are positively related to the state of anxiety in organisms (Goddard et al. 2010; Bailer et al. 2012; Yorgason et al. 2013; Kaçprzak et al. 2017). Corroborating this, many anxiety-like and depression-like behaviors are often induced by drugs that disturb neurotransmitter systems (Leret et al. 2003; Zhou et al. 2010; Matsuda et al. 2012; Baculis et al. 2015), including the dopaminergic/norepinephrinergic system (Liu et al. 2020). However, the effects on plus-maze observed following chronic MPH treatment have yielded mixed results: increases (Bolaños et al. 2003), decreases (Gray et al. 2007), and no change in anxious behaviors (LeBlanc-Duchin and Taurkulis 2007). Vendruscolo et al. (2008) showed that chronic treatment with MPH (2 mg/kg intraperitoneally; twice daily for 16 days) elicited anxious-like behavior in the open field but not in the elevated plus-maze.

The huge increase in the incidence of the misuse of MPH, especially for children and adolescents, whose CNS is in full development, raises concerns about the long-term consequences (Klein-Schwartz 2003; Scaini et al. 2008; Evans et al. 2010; Bruchmüller et al. 2012; Simchon-Tenenbaum et al. 2015). Taking all this into account, we investigated brain glucose metabolism and the metabolic network in chronically MPH-treated rats, using $^{18}$F-FDG and micro-positron emission tomography (microPET) as a noninvasive imaging tool. MPH effects on light–dark transition box, eating-related depression, and sucrose preference tests were also evaluated. We hypothesized that changes in brain glucose metabolism, metabolic network architecture, and depressive-and anxiety-like behaviors may be involved in the MPH effects since their regulating may be modulated by dopaminergic neurotransmission, MPH’s main target.

Methods

Ethics Approval

All animal experimental procedures developed in the present study were approved by the Institutional Ethics Committee on Animal Use (No. 37027) and were following the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 80–23, revised 1996), the guide of the Federation of Brazilian Societies for Experimental Biology and the Arouca Law (no. 11.794/2008).
Animals and Reagents

Ten female Wistar rats at the beginning of the second half of pregnancy were obtained from the Central Animal House of the Department of Biochemistry of the University Federal of Rio Grande do Sul and maintained in a controlled room temperature (22 ± 1 °C) on a 12:12-h light/dark cycle, with food and water available ad libitum. The litters were standardized on 8 puppies on postnatal day (PD) 3 (day of parturition = PD 0), resulting in eighty puppies (the surplus was decapitated, keeping preferably the largest number of male puppies). On the 15th PD, randomly, half of the puppies from each litter received saline and the other half received MPH, resulting in two experimental groups: control (treated with saline) and MPH (treated with the drug). Animals were maintained with their dams until PD 21 when they were weaned and housed 3–4 per cage (Plexiglas cages). The dams were decapitated. All pups continued receiving treatment until the 44th PD. Sample sizes were determined as 10 and 17 animals per group for 18F-FDG microPET study and behavioral testing, respectively, based on standard deviation values of previous studies, with power estimation of 0.80 and alpha = 0.05. Taking into account a 15% loss rate throughout the experiment, forty male Wistar rats (n = 20 for each group) were used. The forty surplus animals (remaining males and all females) were used in studies that are being carried out in parallel.

All chemicals were obtained from Sigma Chemical Co., St. Louis, MO, USA.

Chronic Early Treatment with Methylphenidate

Beginning on PD 15th, rats were weighed and injected daily intraperitoneally with saline solution (0.9%) or MPH (2.0 mg/kg), for thirty consecutive days, as previously described in reports of our research group (Scherer et al. 2010; Schmitz et al. 2012a, b, 2015, 2016a, b, 2018). Briefly, MPH was dissolved in 0.9% saline solution and injected at a volume of 1 mL/100 g of body weight. The control group received an equivalent volume of saline solution. This dose and route of administration were selected because they mimic the therapeutic doses in terms of the magnitude of neurochemical and behavioral effects (Gerasimov et al. 2000). MPH administrations started at 15 days of life because this period is characterized by intense synaptogenesis, myelination, and gliogenesis, comparable to early childhood in humans (Rice and Barone 2000). Treatment lasted for 30 days because it has been shown that this period mimics chronic use in humans (Andreazza et al. 2007). Twenty-four hours after the last administration of MPH, the rats were subjected to the MicroPET scanning of 18F-FDG uptake in the brain. The next day, we started the functional behavioral assessments. Then, the animals were decapitated. We did not observe any significant difference in weight gain between the groups during the treatment (p > 0.05), as shown in Supplementary Fig. 1. The study design is outlined in Fig. 1.

18F-FDG Micro-PET Brain Scan

Twenty-four hours after the last saline and MPH injection, the animals were individually anesthetized using a mixture of isoflurane and medical oxygen (3–4% induction dose) and injected with 0.4 mL 18F-FDG (sham = 38.05 ± 1.06 MBq and MPH = 37.56 ± 1.08 MBq) in the tail vein, after overnight fasting. Then, each rat was returned to its home cage for a 40-min period of conscious (awake) in vivo metabolism of 18F-FDG. After the uptake period, ten rats per group were placed in a head-first prone position and scanned with the Triumph™ micro-PET (LabPET-4, TriFoil Imaging, Northridge, CA, USA) under inhalational anesthesia (2–3% maintenance dose). Throughout these procedures, the animals were kept on a pad heated at 37 °C. For radiotracer readings, 10-min list mode static acquisitions were acquired with the field of view (FOV; 3.75 cm) centered on each rat’s head (Baptista et al. 2015; Zanirati et al. 2018). All data were reconstructed using the maximum likelihood estimation method (MLEM-3D) algorithm with 20 iterations. Each microPET image was reconstructed with a voxel size of 0.2 × 0.2 × 0.2 mm and spatially normalized into an 18F-FDG template using brain normalization in PMOD v3.8 and the Fuse It Tool (PFUSEIT) (PMOD Technologies, Zurich, Switzerland). An MRI rat brain volume of interest (VOI) template was used to overlay the normalized images previously
coregistered to the microPET image database. Activity values were normalized for the injected dose and the animal body weight and were therefore expressed in standard uptake values (SUVs). The SUV was calculated for the whole brain and each region. To correct for weight variations, we calculated the SUV ratio (SUVr) of each brain region by dividing the SUV value of the region by the whole brain SUV (Silva et al. 2018; Zanirati et al. 2018; Bellaver et al. 2019). Mean SUVs of 14 brain regions were extracted using a predefined VOI template. For analysis at the voxel level, MINC tools (www.bic.mni.mcgill.ca/ServicesSoftware) were used for image processing and analysis.

**Metabolic Networks**

Metabolic brain networks (MBNs), derived from 18F-FDG SUV regional data, were constructed by computing Pearson’s correlation coefficient based on 1000 bootstrap samples. The correlation maps were built using the VOIs. The graph-theoretical measures, global efficiency, density, assortativity coefficient, and average clustering coefficient were computed using the 10 MBNs most similar to the mean MBN of each group. Networks computed this way were corrected for multiple comparisons using false discovery rate (FDR) at $p < 0.001$ (Benjamini and Hochberg 1995). In short, global efficiency is a measure of how effectively the network interchange information between its nodes; density quantifies the overall number of edges of a network; assortativity coefficient measures the network resilience, and the clustering coefficient corresponds to the portion of the node’s neighbors that are also neighbors of each other (Rubinov and Sporns 2010). Likewise, the local graph measures degree, strength, local efficiency, and betweenness centrality were computed to evaluate specific VOIs. The degree of a node corresponds to the total number of nodes that are connected to it; the strength is the sum of all weights of edges (e.g. correlation coefficients) connected to a given node; local efficiency measures how effectively a node interchange information with its neighbors; nodes with high betweenness centrality are often hub nodes and may be responsible for linking two or more interconnected parts of a network (Rubinov and Sporns 2010).

**Behavioral Assessment**

Behavioral evaluation was carried out 24 h after the microPET assessment of 18F-FDG uptake in the brain, and the tests were performed on the 7 following days. The behavioral performance of the animals was evaluated on the following tests: light–dark transition, eating-related depression, and sucrose preference.

**Light–Dark Transition Test**

A light–dark transition test was conducted as previously described by Takao and Miyakawa (Takao and Miyakawa 2006). The apparatus used for the light–dark transition test comprised a cage (21 × 42 × 25 cm) divided into two sections of equal size by a partition with a door (Ohara & Co., Tokyo). One chamber was brightly illuminated (390 lx), whereas the other chamber was dark (2 lx). Rats were placed into the dark side and allowed to move freely between the two chambers with the door open for 10 min. The total number of transitions and time spent in each chamber was evaluated.

**Eating-Related Depression Test**

This test examines the levels of depression and motivation to eat after food deprivation (Upadhya et al. 2016). In this test, all food was removed from the animal cages for 24 h. After, a few food pellets were placed on a piece of filter paper positioned at one of the 4 corners of the home cage, and each animal was placed in a corner opposite to the food, free to explore for 5 min. To exclude odor-related issues, the food pellets were changed for each rat. The latency of the first bite was measured.

**Sucrose Preference Test**

The sucrose preference test was performed as described by Upadhya et al. (Upadhya et al. 2016). Briefly, on the first day, the animals were trained to adapt to sucrose solution for 24 h. Animals were housed in individual cages with free access to 2 identical bottles, each containing 100 mL of 1% sucrose and food provided ad libitum. On the second day, the animals had free access to 2 bottles, one containing 100 mL of 1% sucrose and the other containing 100 mL of water (food was provided ad libitum). On the third day, the animals were deprived of water and food for 22 h. On the last day, the animals had free access to 2 bottles, one containing 100 mL of 1% sucrose and the other containing 100 mL of water. After 2 h of testing, water and sucrose consumption were measured.

**Statistical Analyses**

The analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, in a PC-compatible computer. The Student’s $t$ test was used to evaluate all different parameters after checking data normal distribution with Shapiro–Wilk test. Results are expressed as means ± standard deviation or standard error mean, and differences were considered statistically significant when $p < 0.05$. Networks were corrected for multiple comparisons using FDR at
Results

Brain Glucose Metabolism Homeostasis

Initially, we evaluated the effect of chronic MPH treatment on brain glucose metabolism. As we can see in Fig. 2A, MPH did not alter the whole-brain $^{18}$F-FDG uptake ($p > 0.05$) when compared to the control group. Figure 2B shows the representative microPET images of cerebral $^{18}$F-FDG uptake for control and MPH groups: axial (top), sagittal (middle), and coronal (bottom) sections.

To closely observe the effect of MPH treatment on glucose metabolism, we evaluated the $^{18}$F-FDG uptake in each brain region. MPH provoked glucose hypermetabolism in the auditory ($p < 0.01$), parietal ($p < 0.05$), retrosplenial ($p < 0.05$), somatosensory ($p < 0.05$), and visual ($p < 0.01$) cortices. $^{18}$F-FDG uptake was not affected by MPH in the orbitofrontal, frontal association, medial prefrontal, and motor cortices ($p > 0.05$) (Table 1).

$^{18}$F-FDG uptake in the left and right sides of those brain regions was also evaluated. MPH provoked glucose hypermetabolism in the auditory cortex (right ($p < 0.05$) and left ($p < 0.01$)), parietal cortex (left ($p < 0.01$)), retrosplenial cortex (left ($p < 0.05$)), somatosensory cortex (left ($p < 0.01$)), and visual cortex (right ($p < 0.05$) and left ($p < 0.01$)), as showed in Fig. 3A–E, respectively. On the other hand, glucose hypometabolism was identified in the left orbitofrontal cortex ($p < 0.05$), as we can see in Fig. 3F. Figure 3G–I show that $^{18}$F-FDG uptake was not affected by MPH treatment in the frontal association, medial prefrontal, and motor ($p > 0.05$) cortices, when compared to the control group.

Table 1 Effect of chronic treatment with MPH on glucose metabolism in the brain $^{18}$F-FDG SUVR) from juvenile rats

| Brain region                  | Control                  | MPH                       |
|-------------------------------|--------------------------|---------------------------|
| Auditory cortex               | 0.0292 ± 0.00054         | 0.0304 ± 0.00083**        |
| Parietal cortex               | 0.0312 ± 0.00128         | 0.0325 ± 0.00080*         |
| Retrosplenial cortex          | 0.0319 ± 0.00121         | 0.0331 ± 0.00127*         |
| Somatosensory cortex          | 0.0337 ± 0.00097         | 0.0346 ± 0.00055*         |
| Visual cortex                 | 0.0300 ± 0.00097         | 0.0316 ± 0.00094**        |
| Orbitofrontal cortex          | 0.0363 ± 0.00130         | 0.0351 ± 0.00169          |
| Frontal association cortex    | 0.0283 ± 0.00197         | 0.0276 ± 0.00192          |
| Medial prefrontal cortex      | 0.0410 ± 0.00132         | 0.0407 ± 0.00085          |
| Motor cortex                  | 0.0334 ± 0.00159         | 0.0340 ± 0.00088          |
| Hippocampus                   | 0.0628 ± 0.00132         | 0.0621 ± 0.00095          |
| Anterodorsal hippocampus      | 0.0340 ± 0.00086         | 0.0340 ± 0.00059          |
| Posterior hippocampus         | 0.0288 ± 0.00086         | 0.0282 ± 0.00051          |

Results are expressed as mean ± standard deviation for 10 animals in each group. Different from control, *$p<0.05$ and **$p<0.01$ (Student’s t test)

MPH methylphenidate, $^{18}$F-FDG fluorodeoxyglucose-18, SUVR standard-ized uptake value ratio
We also evaluated the $^{18}$F-FDG uptake in the hippocampus, an important brain region associated with MPH effects. $^{18}$F-FDG uptake in the total hippocampus, anterodorsal, and posterior hippocampus were not affected by MPH treatment ($p > 0.05$) (also showed in Table 1). We also did not observe any lateralized effects in the hippocampus ($p > 0.05$) (Fig. 4). Although it is not statically significant, the left hippocampus posterior of MPH-treated rats presented a tendency to show a decrease in glucose metabolism ($p = 0.054$) (Fig. 4C).

Further, $^{18}$F-FDG uptake was not affected by MPH treatment in other brain regions, namely: accumbens, amygdala, striatum, cingulate cortex, entorhinal cortex, cerebellum, insular cortex, hypothalamus, olfactory, colliculus superior, midbrain, ventral tegmental area, colliculus inferior, cortex, and thalamus, as shown in Table 2.

In Table S1, we also show the right and left side results found for each one of these structures, as well as the $^{18}$F-FDG uptake measured in the pituitary, cerebellum blood, central canal, pons, septum, and medulla.

**Brain Metabolic Network**

Metabolic networks were constructed using selected VOIs aiming at identifying brain reorganization patterns after MPH treatment. MPH administration induced metabolic brain hypersynchronicity, represented by more connections in multiple brain regions (Fig. 5A–B, symmetric matrices, and C–D, 3D brain metabolic network surface). More specifically, the following brain regions are more connected in the MPH group compared to the control group: the right entorhinal cortex, the right amygdala, left auditory cortex, left and right insular cortex, left and right medial prefrontal cortex, left cingulate cortex, left auditory cortex, left parietal cortex, left and right motor cortex, left and right somatosensory cortex, left posterior and anterodorsal hippocampus, left and right hypothalamus, left and right midbrain, left and right colliculus superior, left and right thalamus, and the cerebellum.

For directly accessing network differences between MPH and control groups, global graph theoretical measures were
computed. Hypersynchronicity was evidenced by a consistent reorganization in the brain metabolic network, revealing a higher global efficiency \((p < 0.0001; \text{Fig. 5E})\) and density \((p < 0.0001; \text{Fig. 5G})\) in the MPH group. Likewise, the assortativity coefficient \((p < 0.0001; \text{Fig. 5F})\) and average clustering coefficient \((p = 0.0089; \text{Fig. 5H})\) were found to be significantly higher in MPH-treated rats when compared to controls. For additional information about graph measures specifically for the in auditory, visual, parietal, retrosplenial, and somatosensory cortices, see Supplementary Fig. 3. Additional information regarding network interpretation is provided in the “Discussion” section.

In contrast, MPH reduced the number of metabolic connection patterns in the orbitofrontal cortex. Local graph measures demonstrated consistent segregation of the orbitofrontal cortex region indexed by a lower degree \((p < 0.0001, \text{for left and right sides})\) and strength \((p = 0.0007 \text{ and } p < 0.0001 \text{ to the left and right sides, respectively})\). A lower local efficiency in the right orbitofrontal cortex \((p < 0.0001)\) and betweenness centrality in the left orbitofrontal cortex \((p = 0.0009)\) were also triggered by the MPH treatment.

### Behavioral Results

In the light–dark transition test, a tool to analyze anxiety-like behavior, we found that the MPH group spends less time in the light box \((p < 0.05)\), when compared to the control group (Fig. 6A). Total light–dark transitions were not affected by MPH treatment (Fig. 6B). Less time spent on the light side suggests that animals undergoing early chronic treatment with MPH exhibit anxious behavior when compared to the group that received saline solution.

In the eating-related depression test, a tool to examine the levels of depression and motivation to eat after food deprivation, we did not identify differences between MPH and control \((p > 0.05)\) (Figure S2).

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**Table 2** Effect of chronic treatment with MPH on glucose metabolism in the brain \((^{18}\text{F-FDG SUVr})\) from juvenile rats

| Brain region     | Control       | MPH            |
|------------------|---------------|----------------|
| Accumbens        | 0.0345 ± 0.00075 | 0.0339 ± 0.00114 |
| Amygdala         | 0.0243 ± 0.00069 | 0.0240 ± 0.00089 |
| Striatum         | 0.0384 ± 0.00074 | 0.0387 ± 0.00076 |
| Cingulate cortex | 0.0402 ± 0.00118 | 0.0401 ± 0.00116 |
| Entorhinal cortex| 0.0236 ± 0.00102 | 0.0233 ± 0.00108 |
| Cerebellum       | 0.0551 ± 0.00240 | 0.0541 ± 0.00186 |
| Insular cortex   | 0.0301 ± 0.00090 | 0.0304 ± 0.00096 |
| Hypothalamus     | 0.0266 ± 0.00083 | 0.0264 ± 0.00086 |
| Olfactory        | 0.0296 ± 0.00167 | 0.0287 ± 0.00172 |
| Colliculus superior | 0.0367 ± 0.00117 | 0.0369 ± 0.00129 |
| Midbrain         | 0.0328 ± 0.00070 | 0.0331 ± 0.00078 |
| Ventral tegmental area | 0.0282 ± 0.00066 | 0.0280 ± 0.00103 |
| Colliculus inferior | 0.0372 ± 0.00118 | 0.0373 ± 0.00137 |
| Thalamus         | 0.0359 ± 0.00130 | 0.0362 ± 0.00053 |
| Cortex           | 0.3890 ± 0.00802 | 0.3933 ± 0.00682 |

Results are expressed as mean ± standard deviation for 10 animals in each group. MPH methylphenidate. \(^{18}\text{F-FDG} \text{fluorodeoxyglucose-18, SUVr standardized uptake value ratio}\)
**Fig. 5** Metabolic brain networks derived from 18F-FDG SUV. Intersubject Pearson correlation matrices displaying region-to-region associations in control (A) and MPH (B) and 3D brain surfaces displaying metabolic brain networks of control (C) and MPH (D). Metabolic brain network graph measures global efficiency (E), assortativity coefficient (F), density (G), and average clustering coefficient (H). The orbitofrontal cortex local graph measures degree (I), strength (J), local efficiency (K), and betweenness centrality (L) are also demonstrated. Different from control, **\( p < 0.01 \), *** \( p < 0.001 \), and **** \( p < 0.0001 \). Correlation matrices were corrected for multiple comparisons using FDR and non-significant correlations are depicted in white. MPH methylphenidate.
**Light-dark transition test**

A

Time spent in light (s)

| Control | MPH |
|---------|-----|
| ![](image) |

B

Light-dark transitions

| Control | MPH |
|---------|-----|
| ![](image) |

**Sucrose preference test**

C

Consumption (mL)

| Control | Water | Sucrose |
|---------|-------|---------|
| ![](image) |

D

Consumption (mL)

| MPH | Water | Sucrose |
|-----|-------|---------|
| ![](image) |

E

Sucrose preference (%)

| Control | MPH |
|---------|-----|
| ![](image) |

F

Total volume consumption (mL)

| Control | MPH |
|---------|-----|
| ![](image) |

Fig. 6 Effects of chronic treatment with MPH in the light–dark box transition and sucrose preference tests. Time spent in light (A), light–dark transitions (B), water and sucrose consumption in the control group (C) and MPH group (D), sucrose preference (E), and total volume consumption (F). Data are expressed as mean ± standard error mean for 14–17 animals in each group. Different from control, *p < 0.05 and ***p < 0.001 (Student’s t test). MPH methylphenidate.
In the sucrose preference test, a tool to analyze anhedonia, a depressive-like behavior, control, and MPH-treated rats showed a bigger consumption of sucrose solution ($p < 0.05$ and $p < 0.001$, respectively) (Fig. 6C, D). Differently, than we imagined, Fig. 6E shows that MPH increased the preference for sucrose concerning the control group ($p < 0.05$). This result suggests that MPH increases the positive reinforcement caused by sucrose. We did not identify differences between groups about total volume consumption ($p > 0.05$) (Fig. 6F).

**Discussion**

The current study was proposed to examine the effects of early chronic treatment with MPH on glucose metabolism and the metabolic network patterns in juvenile rat brains using microPET. We also attempted to determine whether the MPH treatment affects the performance in the light–dark transition box, eating-related depression, and sucrose preference tests. First, we showed that MPH caused changes in glucose uptake in several regions of the brain, with more pronounced effects on the left side. In this context, the left orbitofrontal cortex was the only structure that showed a decrease in glucose metabolism. Then, we also observed that rats treated with MPH have a more efficient and connected brain metabolic network between most of the evaluated regions, but the orbitofrontal cortex showed a loss of its connectivity with other areas of the brain. Finally, the results of behavioral assessments suggest that rats treated with MPH developed behavior similar to anxiety and also showed greater sensitivity to the positive reinforcement promoted by sucrose when compared to the control group. As far as we know, the effects on glucose metabolism in different brain regions of juvenile rats submitted to early chronic treatment with a clinically relevant dose of MPH have not been extensively investigated to date. Also, new is the possible relationship among changes in brain glucose metabolism with the brain metabolic network and the behavioral changes shown here.

Increased enzymes in the mitochondrial respiratory chain (Fagundes et al. 2007), enzyme disorders in the Krebs cycle (Pamplona et al. 2007; Scaini et al. 2008), changes in glucose uptake (Réeus et al. 2015), and increased creatine kinase activity (Scaini et al. 2008) have been reported after treatment with MPH in the brain of rats. Based on these studies, although the MPH’s precise neurochemical mechanism of action is under debate, it is reasonable to hypothesize that cerebral glucose metabolism changes are associated with MPH treatment, since there is no doubt about MPH’s ability to affect the dopaminergic system (Kuczynski and Segal 2001; Berridge et al. 2006), which has been strongly related to the glucose supply and uptake by the brain (Brus et al. 1995). Corroborating our hypothesis, we observed a hypermetabolism of glucose in the auditory and visual cortices on both sides, with a more pronounced effect on the left side after chronic MPH administration. We also observed hypermetabolism of glucose on the left side of the parietal, retrosplenial, and somatosensory cortices, further suggesting that treatment with MPH promotes greater dopaminergic activity, especially on the left side. According to these findings, amphetamine, a psychostimulant that acts by increasing available DA, improved glucose uptake in the rat striatum, frontal cortex, and hippocampus (Nowak et al. 2007). Changes in glucose uptake in the brain have been associated with DA receptor agonists (Brus et al. 1995). In line with this, MPH administered for 6 weeks (0.3 mg/kg per day) increased cerebral glucose metabolism in patients with impaired consciousness after traumatic brain injury (Kim et al. 2009). Therefore, it is likely that the elevation of glucose metabolism in this study is related to the known blockage of DAT by MPH, which increases the synaptic levels of DA and, consequently, the stimulation of the DA receptor. However, it is not yet possible to establish the precise mechanism of MPH on this effect.

The left orbitofrontal cortex was the only brain region studied that showed a decrease in $^{18}$F-FDG-uptake after chronic MPH administration. It is a subregion of the rat prefrontal cortex (Hoover and Vertes 2007), which is responsible for evaluating the interpretation of threat and adjust the anxiety response accordingly (Calhoon and Tye 2015). Also, the rat orbitofrontal cortex includes distinct subregions: medial orbital area, ventral orbital area, lateral orbital area, and dorsolateral orbital area (Shiba et al. 2016; Izquierdo 2017; Kunishi et al. 2017) and has multiple connections with sensory systems such as the hippocampus and amygdala (Kalin et al. 2007; Wallis 2012). In this context, analysis of the $^{18}$F-FDG uptake in the hippocampus, following chronic MPH treatment revealed no significant effect; however, there was a trend towards a decreased glucose metabolism in the left posterior hippocampus ($p = 0.054$). Zhang et al. (2016) showed that the uptake of $^{18}$F-FDG in the cerebellum of young adult male rhesus monkeys was significantly decreased after a long treatment when compared to the control group. Since cerebral glucose hypometabolism has been associated with impairment in energy metabolism, as well as with the pathophysiology of neuropsychiatric diseases (Zimmer 2009; Tenney et al. 2014), our result suggests that chronic MPH treatment could impair the left orbitofrontal cortex activity, affecting its role in brain activities.

Based on classic notions of neuroenergetics, it is a consensus that cerebral $^{18}$F-FDG metabolism indicates brain energy consumption and, thus, brain activity (Kim et al. 2009; Wang et al. 2013), as well as allows the identification of interregional connections as an index of the functional metabolic architecture of the brain (Zimmer et al. 2017). To identify whether the $^{18}$F-FDG metabolism changes observed...
Many anxiety-like and depression-like behaviors were significantly correlated with glucose metabolism changes in structures with a pivotal role in depression and anxiety (Hu et al. 2010). To identify whether the glucose brain metabolism and metabolic network changes caused by chronic MPH administration in juvenile rats observed in this study coincide with anxiety-like and depression-like behavior, we evaluated the performance in the light–dark transition box, eating-related depression, and sucrose preference tests.

Similar to our previous report when we showed that juvenile rats treated with MPH explored less the open arms in the elevated plus-maze and the environment in the open field test (Schmitz et al. 2016b), we observed that MPH-treated rats spend less time in light box, suggesting that MPH treatment evoked an anxiety-like behavior. Corroborating our findings, Britton and Bethancourt (2009) show that MPH treatment at doses of 3 and 5 mg/kg elicited a significant decrement in locomotor behavior in the open field test and MPH treatment at a dose of 2 mg/kg increased anxiety in the light–dark transition test as evidenced by less time spent in the light compartment relative to controls. In addition, this result could be related to the decrease in glucose metabolism and reshaping of the metabolic network impairment observed in the orbitofrontal cortex since this brain region is involved in the psychological and neurobiological processes of anxiety and their interruption in pathological anxiety (Grupe and Nitschke 2013). Corroborating this, studies have found increased responses to fear in rodents injured in the orbitofrontal cortex (Lacroix et al. 2000; Zelinski et al. 2010) and increased anxiety displayed by injured non-human primates in the orbitofrontal cortex (Shiba et al. 2016).

The performance of MPH-treated rats on the sucrose preference test, a tool to analyze anhedonia, a depressive-like behavior, and in the eating-related depression test, was assessed. Interestingly, contrary to what we initially expected, the animals treated with MPH showed greater sensitivity to the positive reinforcement promoted by sucrose when compared to the control group. We also did not observe any depressive-like behavior from the eating-related depression test. However, these data are in agreement with previous studies that have provided evidence for an anti-depressive effect of MPH (Lazarus et al. 1994; Homsi et al. 2002; Kerr et al. 2012; Golubchik et al. 2017). Hardy (2009) even demonstrated the effectiveness of MPH in treating symptoms similar to depression in humans. On the other hand, there are reports of a depressive effect of MPH in rodents (Bolaños et al. 2003; Carlezon et al. 2003; Vendruscolo et al. 2008; Brookshire and Jones 2012; Motaghtinejad et al. 2015a, b). The increase in positive reinforcement by sucrose triggered by MPH is quite interesting, since this suggests psychostimulant as a potential therapeutic agent in the treatment of obesity or chemical dependency, for example, replacing the positive reinforcement obtained through the cafeteria diet.
and drug abuse. On the other hand, the increase in positive reinforcement to other substances may favor the development of chemical dependence by normal individuals in cases of non-medical use of MPH. Corroborating this, Vendruscolo et al. (2008) showed that chronic treatment with MPH (2 mg/kg intraperitoneally; twice daily for 16 days) enhanced ethanol intake.

The understanding of the consequences of chronic treatment with MPH on early stages of brain development is particularly important since this psychostimulant has been used extensively in preschool-age children and young adults who do not meet full diagnostic criteria for ADHD (Zito et al. 2000; Gonçalves et al. 2014; Jensen et al. 2015; Clemow 2017; Loureiro-Vieira et al. 2017; Coelho-Santos et al. 2018, 2019). This is of great concern to neuroscientists since it has been described that the interruption of brain connectivity causes cell death in the immature brain more rapidly and with higher frequency than in the mature brain (Nyakas et al. 1996; Kudryashov et al. 2001). Also, there is evidence that early-life exposure to MPH induces long-lasting behavioral adaptations (Wiley et al. 2009). In line with this, studies show that adult rats submitted to chronic treatment with psychostimulants during childhood and adolescence present changes in neurochemical, behavioral, and molecular parameters (Andersen et al. 2001; Carlezon et al. 2003; Carlezon and Konradi 2004; Adriani et al. 2010; Hsu et al. 2019; Kim et al. 2019, 2020b). In this context, alteration in the dopaminergic system, redox status, mitochondrial function, glutamatergic/GABAergic systems, neuroinflammation, blood–brain barrier, and neurogenesis have been associated with MPH treatment (Lagace et al. 2006; Sadasivan et al. 2012; Gonçalves et al. 2014; Réus et al. 2014; Motaghinejad et al. 2017). Here, we provide some novel insights into the biochemical and behavioral effects of MPH on the adolescent brain. For this purpose, the 18F-FDG-microPET scan was employed to analyze the left and right cerebral hemispheres of rats that have not been genetically manipulated. The effects of MPH observed in the present study could be related to the mechanism of action of the drug in the adolescent brain. These findings also show evidence of the potential of early MPH exposure to modify emotional responses, as well as contribute new information that has implications for the neurobiological understanding of anxiety, and by extension, to the future development of more directed/specific pharmacological treatments for anxiety disorders.

Summing up, we showed that MPH caused changes in glucose uptake in several regions of the brain. Furthermore, there were lateralization alterations in the 18F-FDG uptake in specific brain regions, indicating that the left side could be more sensitive to the MPH effects. Also, MPH-treated rats show a brain metabolic network more efficient and connected. However, unlike most of the brain regions analyzed, the orbitofrontal cortex shows glucose hypometabolism and impaired metabolic network connectivity and efficiency, which probably may be related to the observed anxious behavior, since this region of the brain is involved in psychological and neurobiological processes of anxiety. As far as we know, this is the first study showing the relationship between changes in glucose metabolism with the brain metabolic network and the behavioral changes in juvenile rats submitted to early chronic treatment with a clinically relevant dose of MPH. We chose to study the effect of MPH only in male rats because several studies are showing that this drug is used more in boys than in girls (Bauermeister et al. 2007; Park et al. 2014; Ehrhardt et al. 2017; Bouziane et al. 2019; Efron et al. 2020). However, further work needs to be done to determine the effects that MPH may cause on females. Finally, given the marked increase in MPH consumption over the past decade, vigilance is crucial to prevent potential drug abuse and its long-term detrimental consequences.
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