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Research report

Sex differences in the effects of acute stress on cerebral glucose metabolism: A microPET study

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HIGHLIGHTS

- Acute restraint stress decreases whole brain glucose metabolism in mice.
- Effects of restraint stress in the brain ^{18}F-FDG uptake are time and sex-dependent.
- Restraint stress alters glucose uptake in the blood, liver and right adrenal of females.

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ABSTRACT

Stress has been considered as a risk factor for the development and aggravation of several diseases. The hypothalamic-pituitary-adrenal axis (HPA) is one of the main actors for the stress response and homeostasis maintenance. Positron emission tomography (PET) has been used to evaluate neuronal activity and to study brain regions that may be related to the HPA axis response. Since neuroimaging is an important tool in detecting neuroendocrine-related changes, we used fluorodeoxyglucose-18 (^{18}F-FDG) and positron emission microtomography (microPET) to evaluate sexual differences in the glucose brain metabolism after 10, 30 and 40 min of acute stress in Balb/c mice. We also investigated the effects of restraint stress in blood, liver and adrenal gland ^{18}F-FDG biodistribution using a gamma counter. A decreased glucose uptake in the whole brain in both females and males was found. Additionally, there were time and sex-dependent alterations in the ^{18}F-FDG uptake after restraint stress in specific brain regions, indicating that males could be more vulnerable to the short-term effects of acute stress. According to the gamma counter biodistribution, only females showed a significant decreased glucose uptake in the blood, liver and right adrenal after restraint stress. In addition, in comparisons between the sexes, males showed a decreased glucose uptake in the whole brain and in several brain regions compared to females. In conclusion, exposure to acute restraint stress resulted in significant decreased glucose metabolism in the brain, with particular effects in different regions and organs in a sex-specific manner.

1. Introduction

Psychiatric diseases are a major current health problem responding for approximately 13% of the total burden of disease globally (Collins et al., 2011). Stress has also been associated as a risk factor for the development of several diseases, such as cardiovascular (Carda et al., 2015), obesity (Aschbacher et al., 2014), autoimmune (Gerrard et al., 2017) and neurological disorders (Menard et al., 2017), among others. Stressful stimuli promote the homeostatic imbalance of the individual triggering a series of physiological and behavioral responses in order to destabilize the potential stressor agent. Several factors may influence this complex response, including the stressor intensity, frequency and period, as well as the individual vulnerability and resilience (Chrousos and Gold, 1992).

Abbreviations: ^{18}F-FDG, fluorodeoxyglucose-18; ACTH, adrenocorticotropic hormone; CeA, central nucleus of the amygdala; CRF, corticotropin-releasing factor; HPA, hypothalamic-pituitaryadrenal axis; microPET, positron emission microtomography; PET, positron emission tomography; PTSD, post-traumatic stress disorder; PVN, paraventricular nucleus; SUVs, standardized uptake values

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The hypothalamic-pituitary-adrenal (HPA), an important neuroendocrine system, is one of the main actors for the stress response and homeostasis maintenance. However, when a maladaptation to this response occurs, stress plays a key role in the development of neurological diseases, such as anxiety and depression (McEwen, 2005; McEwen et al., 2012). In this context, restraint stress has been used as one of the main animal models to study neurobiological mechanisms related to the activation of the HPA axis. Previous studies in rodents have shown that immobilization stress, besides increasing corticosterone secretion, is also able to affect neurogenesis, memory and cognition (Hillerer et al., 2013; Huang et al., 2015). Furthermore, several studies have reported that stress can affect brain responses in a sex-dependent manner. It is already known that women have an increased risk for the development of stress-related diseases, such as post-traumatic stress disorder (PTSD), anxiety and depression (Bangasser and Valentino, 2014). Similarly, in rodents, females have an increased vulnerability to developing neurological diseases, exhibiting increased secretion of corticosterone and adrenocorticotropic hormone (ACTH) in response to stress when compared to males. In addition, higher levels of corticosterone are secreted in the proestrus phase, when the concentration of estrogen and progesterone are increased (Iwasaki-Sekino et al., 2009). The expression of estrogen receptors has been detected in regions involved in the regulation of the HPA axis, such as the paraventricular nucleus (PVN) of hypothalamus, pituitary and adrenal gland (Isgor et al., 2003; Mitchner et al., 1998; Trejter et al., 2015).

Neuroimaging is an important tool in detecting changes related to neuroendocrine disease *in vivo*. Positron emission tomography (PET) has been used to evaluate neuronal activity and to study brain regions that may be related to the HPA axis response (Hu et al., 2010; Wei et al., 2018). The most widely used radiotracer for PET is fluorodeoxyglucose-18 (^{18}F-FDG), a glucose analogue that allows the evaluation of tissues with high metabolic activity (Mirrione et al., 2006). In humans, psychosocial stress promotes changes in the rate of cerebral glucose consumption in the prefrontal cortex and decreases glucose metabolism in limbic regions (Gianaros et al., 2008; Kern et al., 2008). In addition, exposure to acute restraint stress for 24 h promotes long-term effects, leading to a glucose uptake decrease in cortico-limbic circuitry of mice (Chu et al., 2016). Taken together, the studies show that stress might significantly alter distinct brain regions and promote CNS related diseases (Chu et al., 2016; Wei et al., 2018). Nevertheless, possible sexual differences in neuronal activation in response to acute stress are not well established. These sexual differences may be key in understanding vulnerability and increasing risk of disease development across lifespan.

Therefore, considering the importance of mental disease development, further understanding of the mechanisms related to the stress response in the brain and the singularity of this response between males and females is a relevant research problem. Thus, in this study, we used ^{18}F-FDG and positron emission microtomography (microPET) to evaluate sexual differences in the brain activity after acute stress, at distinct periods of exposure to a short-term restraint stress (10, 30 and 40 min). In addition, the response to stress was evaluated by measuring the ^{18}F-FDG biodistribution in the blood, liver and adrenal gland using a gamma counter.

2. Results

2.1. Acute stress decreases ^{18}F-FDG uptake in the whole brain

^{18}F-FDG SUV for the whole brain was calculated from microPET images to evaluate the effects of different restraint stress durations in the whole brain of males and females (Fig. 2). There was a significant decrease in the female brain metabolism when comparing RS40 ($p = .014$) to the CON group. In males, a significant decrease in the glucose metabolism was demonstrated for RS30 ($p = .006$) and RS40 ($p = .0001$) when compared to the CON group.

2.2. Acute stress alters ^{18}F-FDG uptake in specific brain regions in a sex dependent-manner

Acute restraint stress significantly decreased glucose metabolism in several brain regions when different durations of stress were compared to the CON group. In females, 10 min acute stress (RS10) decreased ($p = .046$) the right amygdala ^{18}F-FDG uptake (Fig. 3B). In males, acute stress (RS30) increased the ^{18}F-FDG uptake in the left ($p = .017$) and right ($p = .001$) amygdala and in the hypothalamus ($p = .017$) (Fig. 3A, B and E). On the other hand, RS10 ($p = .010$), RS30 ($p = .001$) and RS40 ($p = .0001$) males showed a significant decrease in the left hippocampus (Fig. 3C). Changes in the ^{18}F-FDG uptake were also demonstrated for both left (Fig. 4A) and right midbrain (Fig. 4B). In the olfactory areas, stress for 10 ($p = .0001$) and 30 min ($p = .016$) induced a glucose uptake decrease in males (Fig. 4C).

Several differences were also found in non-limbic regions when experimental groups were compared to CON. Males from RS10 ($p = .035$) and RS30 ($p = .010$) showed a decrease in the superior colliculi (Fig. 5E). Although 40 min stress increased ($p = .023$) ^{18}F-FDG uptake in the female cortex (Fig. 5F), 30 min stress induced a decrease ($p = .002$) in the cortex of males (Fig. 5F). As to basal forebrain/septum, an increase ($p = .021$) was seen after 30 min (Fig. 6A), whereas in the cerebellum and increase ($p = .028$) was demonstrated for 40 min stress (Fig. 6C), both in males.

In the comparisons between genders, males showed a significant ($p < .05$) decrease in the ^{18}F-FDG uptake in important limbic regions, such as amygdala and hippocampus (Fig. 3). On the other hand, also in males, stress increased ($p < .05$) the glucose metabolism in non-limbic structures, such as the cortex (Fig. 5). For the complete list of structures and sexual differences, please see Figs. 3–6. In addition, representative brain scans of the experimental groups normalized into an ^{18}F-FDG template in coronal (left), sagittal (middle) and transverse (right) views are shown in Fig. 7.

2.3. Acute stress decreases organ biodistribution of ^{18}F-FDG in a sex and tissue dependent-manner

Fig. 8 shows the ^{18}F-FDG biodistribution by tissues after intraperitoneal injection when different restraint stress durations were compared to the CON group. In the evaluation of blood biodistribution, a significant decrease of ^{18}F-FDG was found for RS30 ($p = .024$) females (Fig. 8A). Likewise, acute stress promoted a decrease in the hepatic metabolism of ^{18}F-FDG in RS30 ($p = .0001$) and RS40 ($p = .015$) females (Fig. 8B). Right adrenal gland analyses showed a significant ^{18}F-FDG uptake decrease in RS10 ($p = .001$) and RS30 ($p = .028$) females (Fig. 8D). As to sexual differences, males showed decreased blood (CON: $p = .004$; RS10: $p = .0001$ and RS40: $p = .004$) (Fig. 8A) and liver (CON: $p = .001$; RS10: $p = .004$ and RS40: $p = .015$) (Fig. 8B) ^{18}F-FDG biodistribution when compared to CON, RS10 and RS40 females. In addition, both left and right adrenal from CON males ($p = .003$ and $p = .003$, respectively) showed a decrease when compared to CON females (Fig. 8C and D).

3. Discussion

Physiological responses triggered by a single stressor event, such as restraint stress, may generate not only short, but also long-term effects, leading to functional and structural consequences. These effects may be involved in the cause of prevalent neuropsychiatric diseases, such as anxiety and depression. Nevertheless, the involvement of sexual differences in the brain response to an acute stress is not yet fully understood. To the best of our knowledge, this is the first study to show that male mice have decreased glucose uptake compared to females and that exposure to different times of acute stress leads to alterations in ^{18}F-FDG uptake in brain regions in a sex-dependent manner.

^{18}F-FDG is a glucose analog that competes with glucose for its

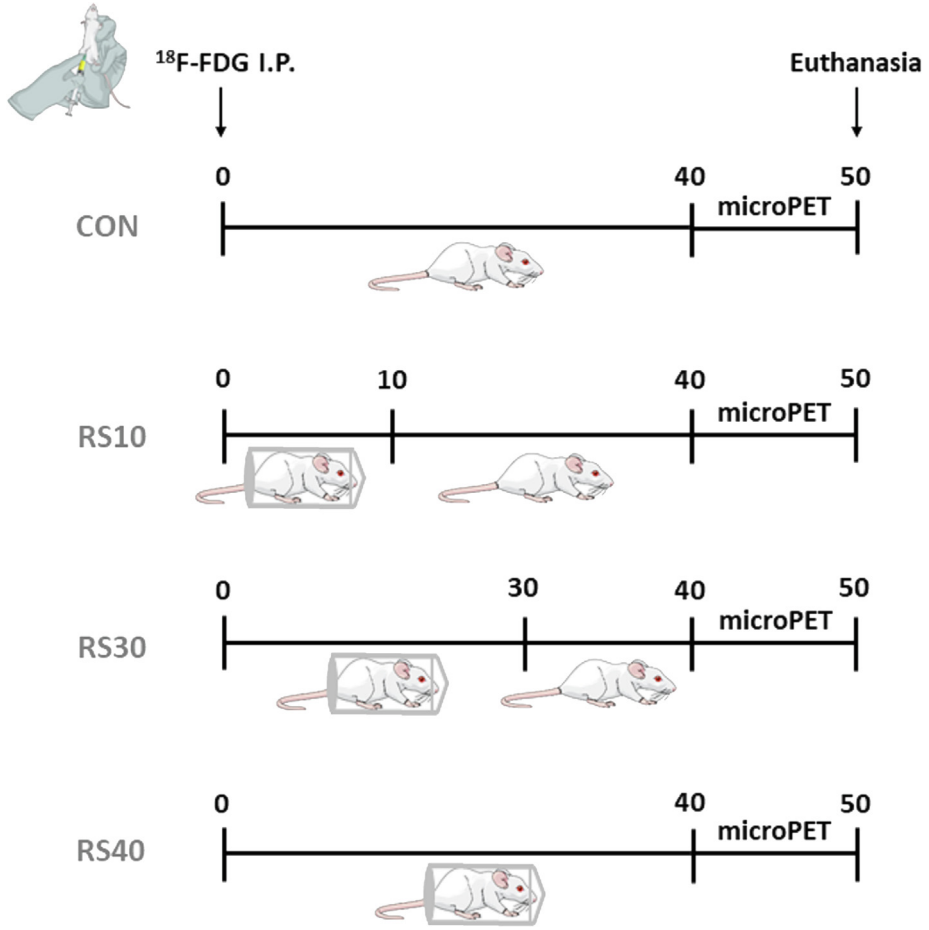

Fig. 1. Experimental design of the study. The timeline is expressed in minutes. CON: control; RS10: 10-min restraint stress; RS30: 30-min restraint stress; RS40: 40-min restraint stress.

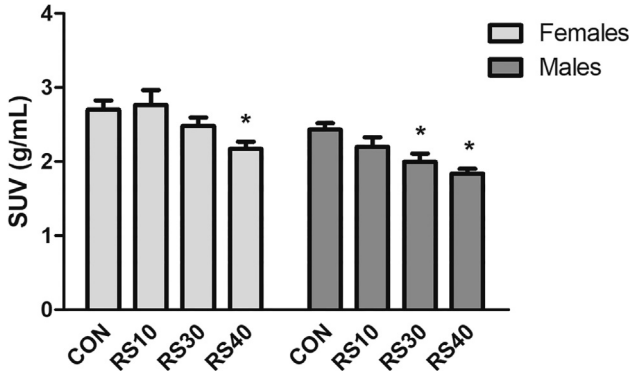

Fig. 2. Effects of restraint stress on glucose metabolism in the whole brain. The ^{18}F-FDG uptake was measured in females and males and expressed as standardized uptake value (SUV). Data are shown as mean and standard error of the mean and were analyzed using one-way ANOVA. *$p < 0.05$ indicate significant differences compared to CON group in the same sex. $n = 6–7$ mice for each group.

intracellular transporter and phosphorylation (Khan et al., 2011). Since glucose is the main source of energy for the brain functioning, alterations in the absorption of the PET tracer can be observed in several diseases. It is well established that activation of the HPA axis mobilizes energy store and culminates in increased glucose blood levels and inhibition of glucose uptake in peripheral tissues (Marik and Bellomo, 2013). Decreased brain glucose uptake has been found in diseases involving alterations in cognition and mood, such as PTSD, in both studies with humans and animals (Chu et al., 2016; Molina et al., 2010). In the present study, female and male Balb/c mice were exposed to restraint stress for 10, 30 and 40 min. When glucose metabolism in the whole brain was evaluated, we have demonstrated that stressed females exhibit decreased brain absorption of ^{18}F-FDG only after 40 min of stress, whereas males showed this response after 30 and 40 min of exposure. In addition, in several regions, males showed a significant decrease in the ^{18}F-FDG uptake in comparison to females. This decrease in brain glucose metabolism in males has already been reported in a study with healthy humans (Hu et al., 2013), although the comparison between genders in response to a short-term stress event has not yet been studied. In contrast with several studies, our data indicate that males could be more vulnerable to the short-term effects of acute stress. The results also suggest that acute restraint stress is able to generate brain alterations that may impair the control of stress response in a sex and time-dependent manner. In male rats, Sung et al. showed that restraint stress for 1 or 2 h, or 1-h stress followed by 1-h recovery decreased ^{18}F-FDG uptake in several brain regions (Sung et al., 2009). Another study observed that immobilization for 24 h produces distinct short (increased) and long-term (decreased) glucose brain uptake response in male mice (Chu et al., 2016). No animal data showing this response in females is available to date. Moreover, corroborating our findings, a study demonstrated that females could be less susceptible to the effects of stress due to the protective effect of gonadal hormones (Karisetty et al., 2017), although this association remain controversial. The administration of estrogen has been shown to attenuate the activation of neurons in response to stress, besides decreasing depression and anxiety-like behavior in rodents (Ueyama et al., 2006; Rachman et al., 1998; Walf and Frye, 2005). Although our findings are consistent with

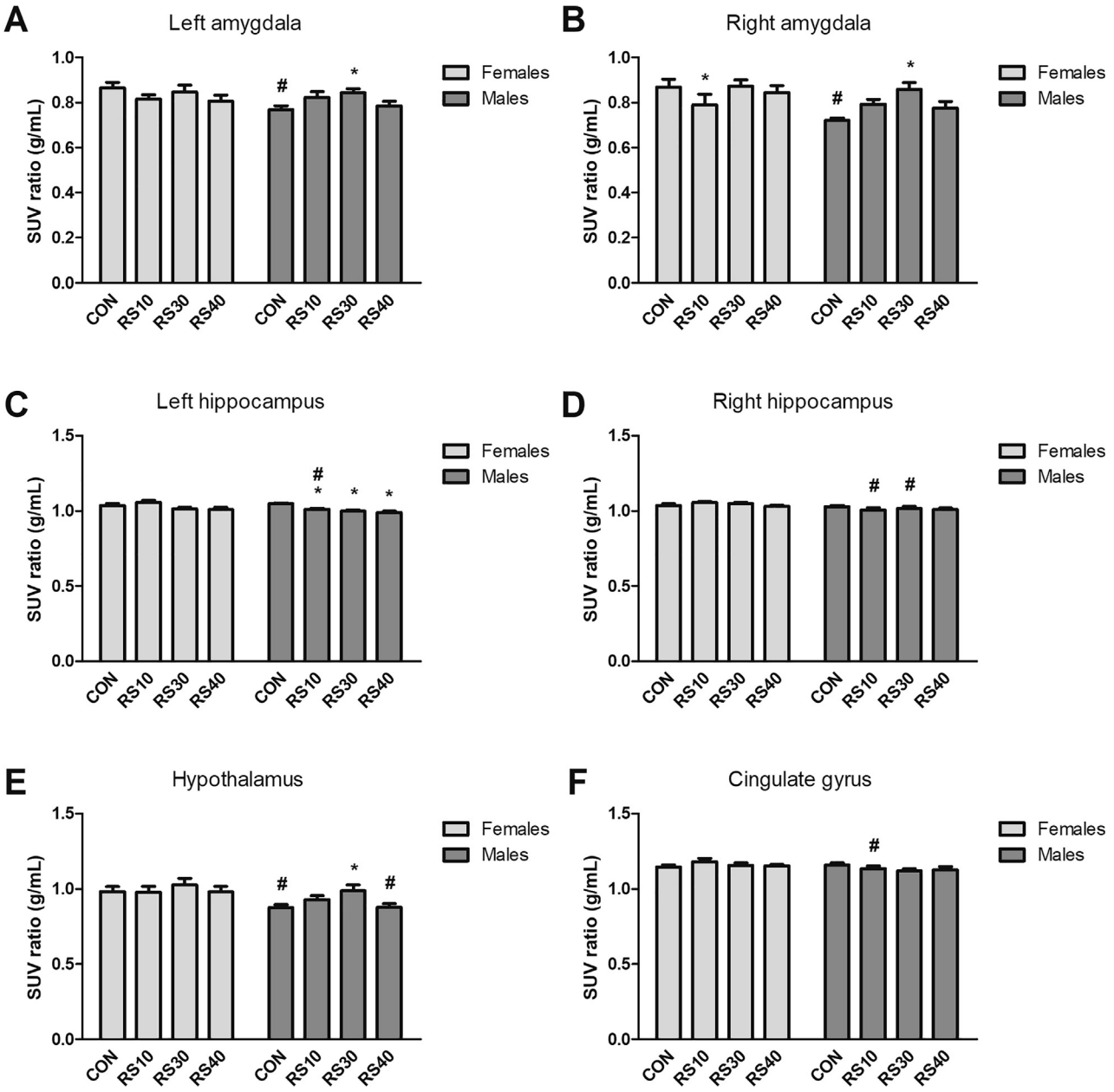

Fig. 3. Effects of restraint stress on glucose uptake in the amygdala (left and right), hippocampus (left and right), hypothalamus and cingulate gyrus. The ^{18}F-FDG uptake was measured in females and males and expressed as standardized uptake value (SUV) ratio. Data are shown as mean and standard error of the mean and were analyzed using two-way ANOVA. *$p < 0.05$ indicate significant differences compared to CON group in the same sex; and $^{\#}p < 0.05$ indicate significant sex differences within the same group. $n = 6–7$ mice for each group.

studies showing that stress promotes a decreased glucose metabolism in the brain, different protocols of exposure and sex hormones may play an important role.

Several brain regions are involved in the development of neurological diseases related to HPA axis regulation and stress response. Our microPET results show that males and females have significant changes in the glucose metabolism in some limbic regions. The hippocampus is a key region responsible for the glucocorticoid feedback, and dysfunctions in this region are usually related to altered cognitive functions, such as learning and memory (Kim et al., 2015). Although short duration (10, 30 or 40 min) single-restraint stress effects in cerebral glucose metabolism have been poorly studied, previous data using longer protocols have shown that acute stress promotes a decrease in synaptic plasticity (Foy et al., 1987) and neurogenesis (Thomas et al., 2007) in the hippocampus, which may impair future cognitive abilities. Stressor events seem to trigger specific dysfunctions for each hippocampus hemisphere. Studies with PTSD have already shown a reduction of the left hippocampus in humans (O'Doherty et al., 2015; Smith, 2005). Similarly, in males, a decrease in the left hippocampal volume was observed in a PTSD model and in chronic stress using immobilization (Rahman et al., 2016; Golub et al., 2011). Our data demonstrates that exposure to acute restraint stress decreased ^{18}F-FDG uptake in the left hippocampus only in males. Studies with humans have demonstrated a strong association with decreased hippocampal volume in patients with PTSD and depression (Davidson et al., 2002; Gilbertson et al., 2002). These findings are consistent with a study that reported a greater cognitive impairment in stressed males (Conrad et al., 2004).

In addition to the hippocampus, the hypothalamus is a key structure involved in the HPA axis control and in the neuropathology of psychiatric diseases, regulating glucocorticoid secretion. The primary

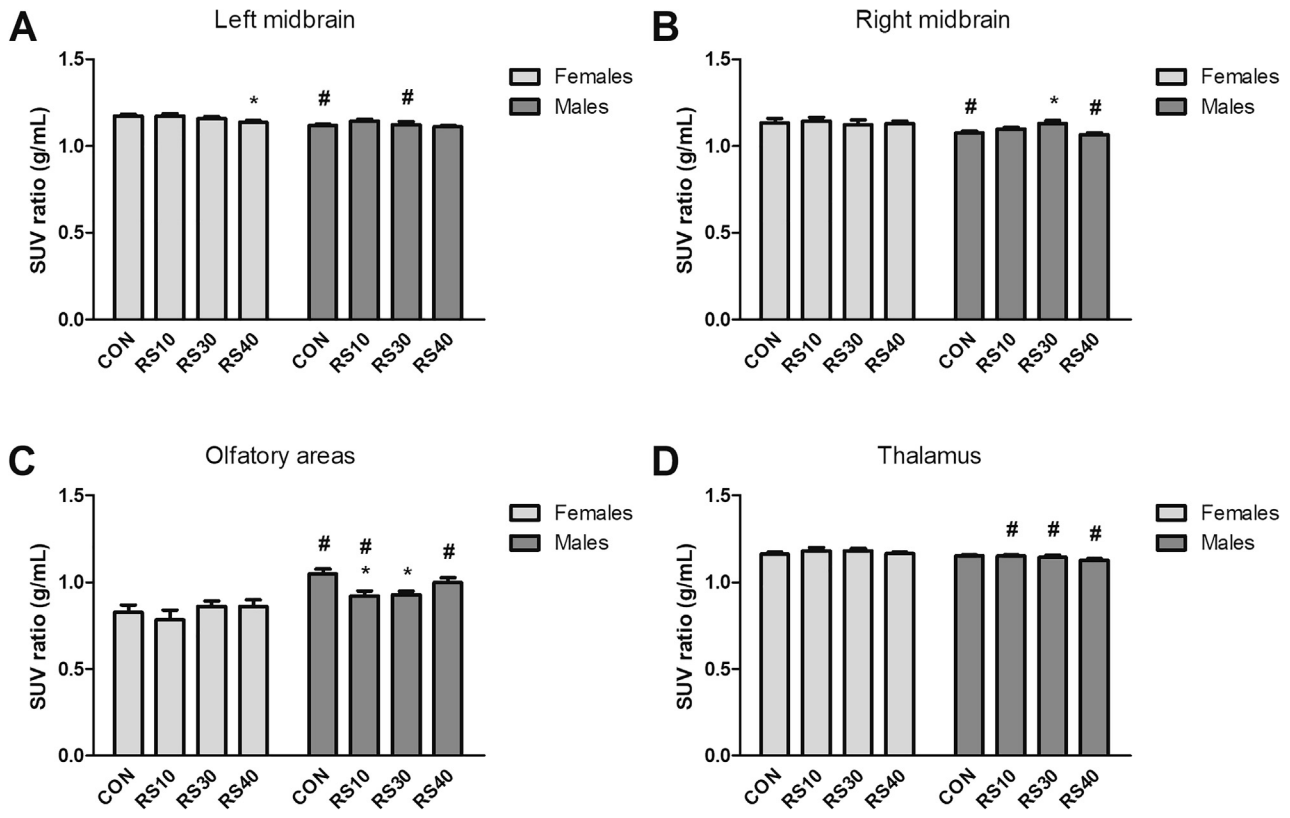

Fig. 4. Effects of restraint stress on glucose uptake in the midbrain (left and right), olfatory areas and thalamus. The ^{18}F-FDG uptake was measured in females and males and expressed as standardized uptake value (SUV) ratio. Data are shown as mean and standard error of the mean and were analyzed using two-way ANOVA. *$p < 0.05$ indicate significant differences compared to CON group in the same sex; and $^{\#}p < 0.05$ indicate significant sex differences within the same group. n = 6–7 mice for each group.

neuroendocrine response to stress involves, mainly, the activation of corticotropin-releasing factor (CRF) neurons in the PVN, generating the systemic release of ACTH (Herman et al., 2016). Our data shows that only males have an increased glucose uptake in the hypothalamus after 30 min of stress. The involvement of hypothalamus and possible sexual differences on the stress response seems to be controversial. Although the PVN of males and females resemble each other morphologically, it has been reported that females have more neurons in this region than males (Ishunina and Swaab, 1999). Zavala et al. demonstrated that a single 30 min restraint decreases the recruitment of PVN neurons in response to stress only in females (Zavala et al., 2011), suggesting a lower sensitivity to a single stress exposure in the hypothalamus, which is in agreement with our results. Similarly to our data, the study conducted by Chu et al. demonstrated an increased glucose uptake after acute stress in the hypothalamus of male mice (Chu et al., 2016).

A decreased glucose metabolism in the olfactory area after 10 and 30 min of stress was observed in males, which may lead to changes in the sensory system in an effect that is sex-dependent. In contrast to our results from other limbic regions, the effects of stress on the amygdala and midbrain appear to be opposite between females (decrease) and males (increase), depending on the time of stress exposure. The amygdala plays an important role in mediating the stress-response, including behavioral alterations and secretion of glucocorticoids Shin and Liberzon, 2010. Furthermore, the relationship of CRF neurons located in the central nucleus of the amygdala (CeA), which is involved in regulating the stress response, and the sexual differences related to this response are controversial (Iwasaki-Sekino et al., 2009). Viau et al. demonstrated that 30 min of restraint stress was not able to change corticosterone secretion, but decreased the CeA expression of CRF mRNA in males (Viau et al., 2005). On the other hand, Iwasaki-Sekino et al. showed that females have a higher CRF expression in response to an acute stress than males (Iwasaki-Sekino et al., 2009). In addition, midbrain plays an important role in the motor movement. Although its participation in the response to short-term acute stress is not elucidated, Laeken et al. have recently demonstrated that chronic administration of corticosterone in male rats elevated glucose consumption, which is similar to our findings (Van Laeken et al., 2018). Taken together, our data indicate that males may present a greater sensitivity to HPA axis activation in the limbic system after an acute stress in comparison to females.

Our findings also indicate important sexual differences in the involvement of non-limbic regions in response to acute stress. In our study, females and males exhibited alterations in brain glucose metabolism in the cortex, an important region for the control of diverse behaviors, including cognition (Bicks et al., 2015). The cortex was the only non-limbic region affected by stress in females, which showed an increase in the ^{18}F-FDG metabolism, while males exhibited a metabolic hypoactivity. On the other hand, males showed a brain hypoactivity in the superior colliculus, which acts on sensorimotor control (superior) and auditory system (inferior). In addition, males showed a decreased ^{18}F-FDG uptake in the superior colliculus after 10 and 30 min of restraint stress. Recently, Olivé et al. demonstrated, in humans, that the superior colliculus has a connection with defensive responses observed in PTSD (Olive et al., 2018). When we evaluated the basal forebrain/septum, brain stem and cerebellum, we have observed that stress was able to increase ^{18}F-FDG uptake only in males. The expression of glucocorticoid receptors in both forebrain and cerebellum is essential for the HPA axis regulation in response to stress. Several studies have reported associations between cerebellar size, behavioral changes, such as anxiety, and patients with PTSD (Furay et al., 2008; Schutter, 2012). To our knowledge, there are no studies in Balb/c mice showing the effects of a short-term restraint stress in the ^{18}F-FDG uptake in these regions

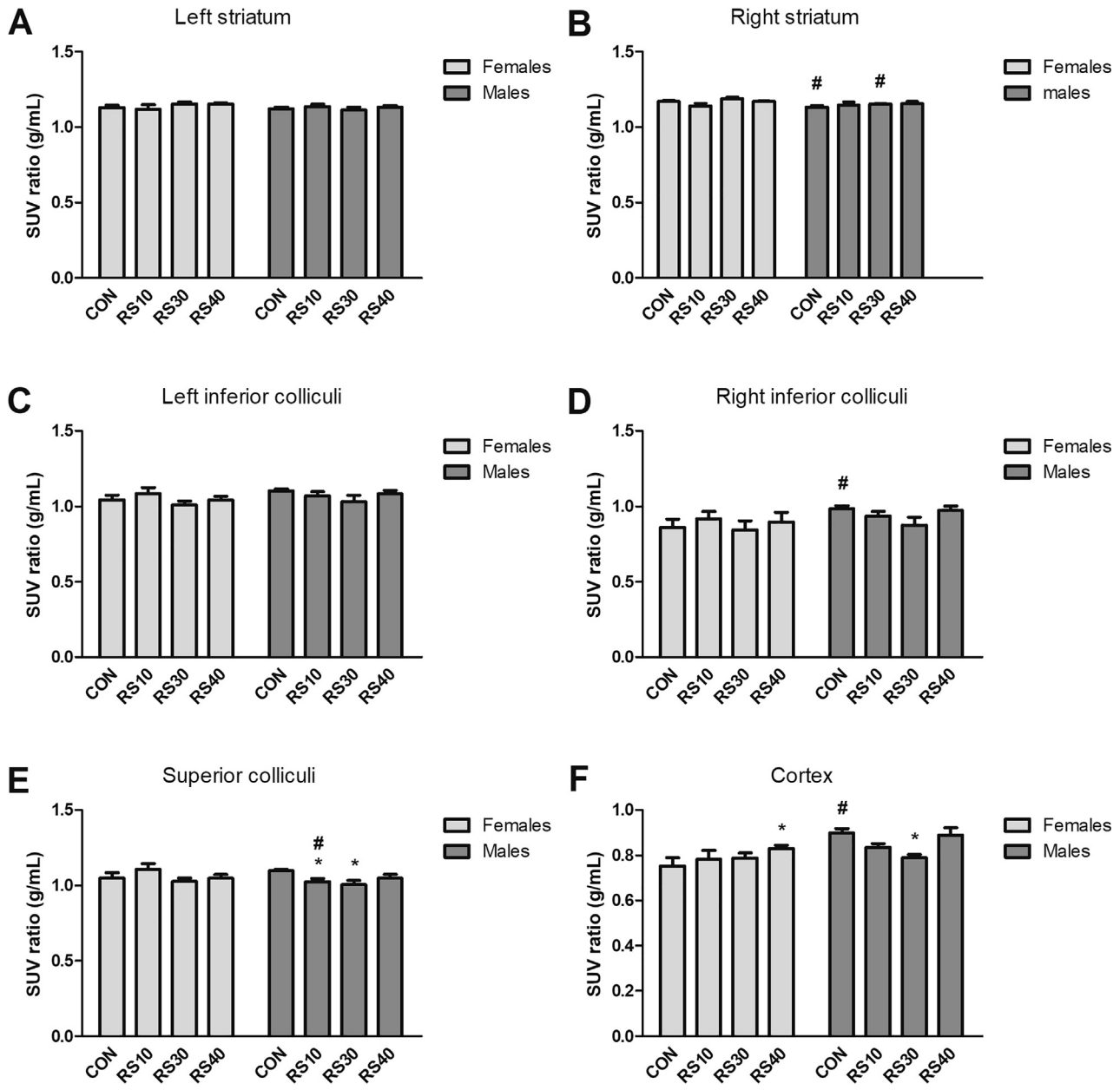

Fig. 5. Effects of restraint stress on glucose uptake in the striatum (left and right), inferior colliculi (left and right), superior colliculi and cortex. The ^{18}F-FDG uptake was measured in females and males and expressed as standardized uptake value (SUV) ratio. Data are shown as mean and standard error of the mean and were analyzed using two-way ANOVA. *$p < 0.05$ indicate significant differences compared to CON group in the same sex; and $^{\#}p < 0.05$ indicate significant sex differences within the same group. n = 6–7 mice for each group.

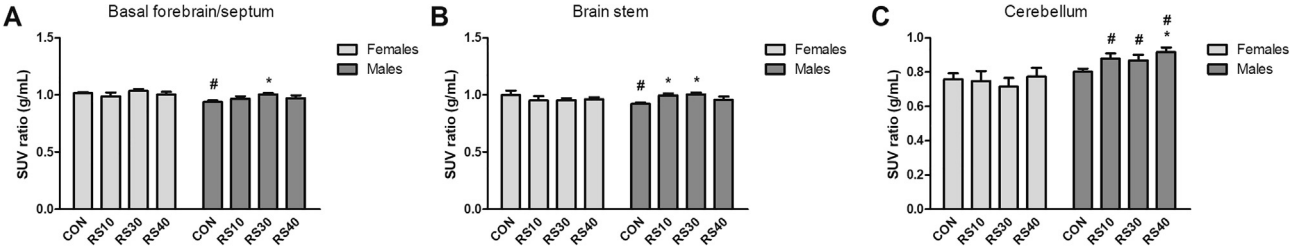

Fig. 6. Effects of restraint stress on glucose uptake in the basal forebrain/septum, brain stem and cerebellum. The ^{18}F-FDG uptake was measured in females and males and expressed as standardized uptake value (SUV) ratio. Data are shown as mean and standard error of the mean and were analyzed using two-way ANOVA. *$p < 0.05$ indicate significant differences compared to CON group in the same sex; and $^{\#}p < 0.05$ indicate significant sex differences within the same group. n = 6–7 mice for each group.

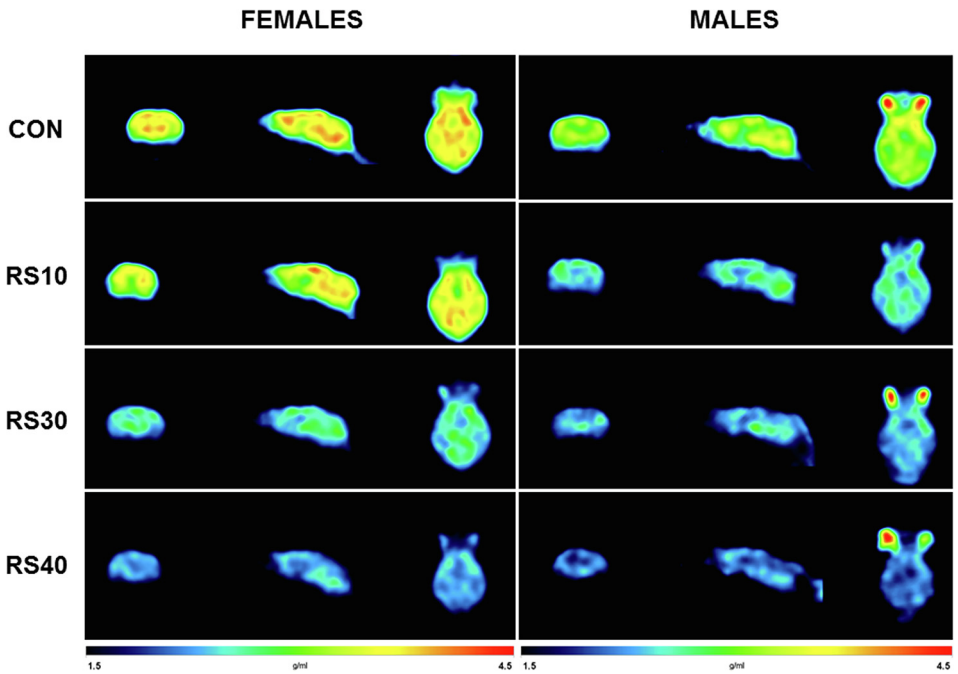

Fig. 7. Representative microPET images of cerebral ^{18}F-FDG uptake. Images for all experimental groups (control, RS10, RS30 and RS40) in both females and males show coronal (left), sagittal (middle) and transverse (right) sections.

implicating sexual differences. Taken together, our data in non-limbic regions suggests that females appear to be less affected by acute stress in regions related to cognition and learning.

Blood, liver and adrenal gland were also analyzed using a gamma counter. The decreased blood, liver and adrenal ^{18}F-FDG uptake observed in females after acute stress may be attributed to the control of glucose homeostasis. The adrenal cortex is the region responsible for the secretion of cortisol (corticosterone, in rodents), which also

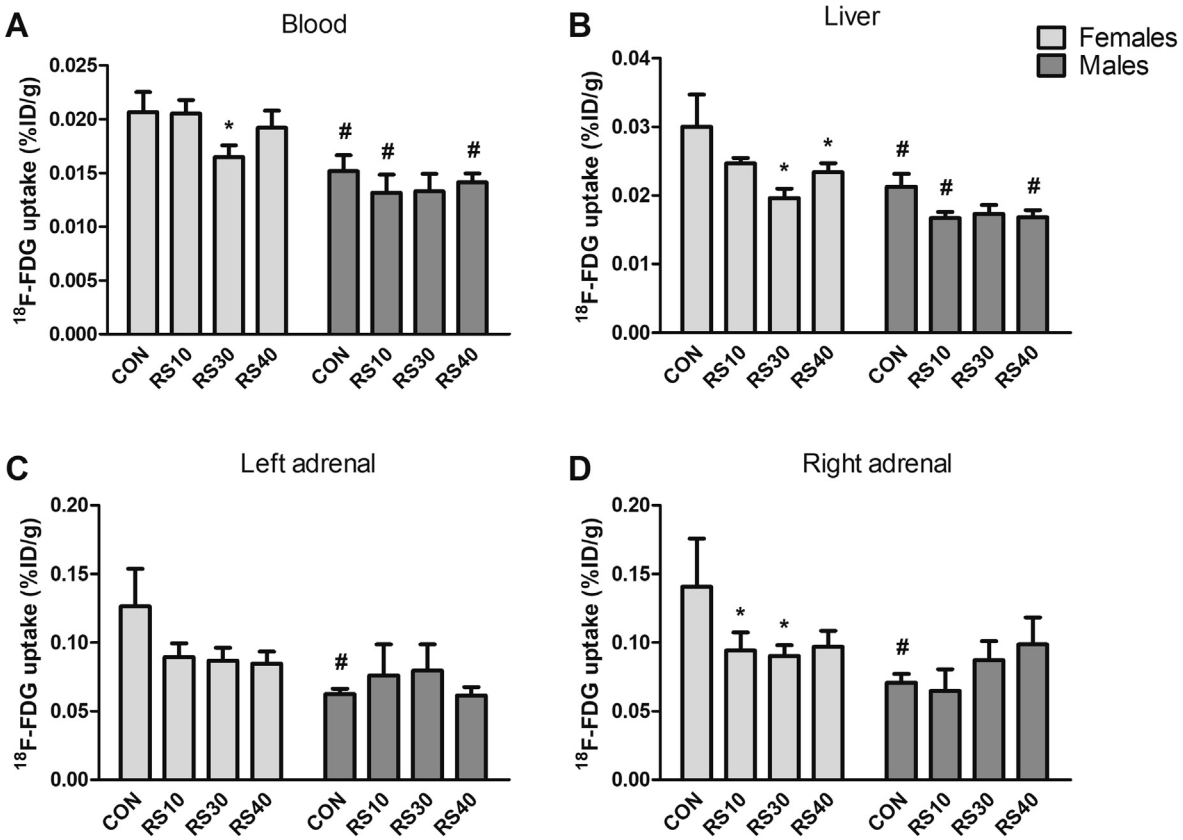

Fig. 8. Effects of restraint stress on the ^{18}F-FDG biodistribution in mice. The ^{18}F-FDG uptake was measured in females and males, and expressed as %ID/cc (% injected dose per g of tissue). Data are shown as mean and standard error of the mean and were analyzed using two-way ANOVA. *$p < 0.05$ indicate significant differences compared to CON group in the same sex; and $^{\#}p < 0.05$ indicate significant sex differences within the same group.

participates in the negative feedback control in several brain regions. The increase in energy demand needed after a stressor event is catalyzed by the liver, which hydrolyzes glucose-6 phosphate and elevates blood glucose levels (Rui, 2014; van den Berghe, 1991). In contrast to females, restraint stress did not decrease liver glucose uptake in males, suggesting that energy homeostasis in response to stressful events may also be regulated in a sex-dependent manner.

Our study is not without limitations, including the lack of hormonal assessment, such as corticosterone and gonadal hormones. However, the stress model used has been widely studied and previous data has reported increased plasma corticosterone levels in rodents submitted to restraint stress (Hare et al., 2014). In addition, the impossibility to subdivide brain regions, such as the cortex, did not allow us for a more informative analysis.

In conclusion, exposure to acute restraint stress resulted in decreased glucose metabolism in the whole brain, with particular effects in different brain regions and organs, in a sex-specific manner. Although the mechanisms involved in these changes are still unclear, our data shows differences in the ^{18}F-FDG absorption in essential regions for the stress response control, which may help in future research for the understanding of sexual differences in stress-related diseases.

4. Experimental procedures

4.1. Animals

BALB/c mice were acquired from the Center of Biological and Experimental Models (CeMBE) at PUCRS. Animals were kept at a maximum of 5 individuals per cage on ventilated racks with controlled temperature and humidity. All animals were kept under a 12 h light-dark cycle, with free access to food and water. All procedures were performed according to the guidelines described in the *Guide for the Care and Use of Laboratory Animals*. The study was approved by the university ethics committee in animal use (CEUA), under registry number 15/00446.

4.2. Experimental design

Male and female mice aged 6–8 weeks old were divided into four experimental groups: control (CON); 10-minute restraint stress (RS10); 30-minute restraint stress (RS30); and 40-minute restraint stress (RS40). The animals were injected intraperitoneally with ^{18}F-FDG and subjected to a restraint stress protocol according to their experimental group, as described below. Immediately after the stress protocol, a 10 min microPET brain scan was performed and then animals were euthanized (Fig. 1). Blood, liver and adrenal glands were removed for biodistribution evaluation using a gamma counter.

4.3. Acute restraint stress

A single acute restraint stress protocol was used, as previously reported (Vargas et al., 2016). Briefly, mice were restrained in an acrylic tube (34 mm high, 42 mm wide, 100 mm circumference) with air holes (Insight, Brazil) for 10, 30 or 40 min, according to the experimental group. The restraint stress protocol was performed during the light cycle (9:00 a.m. to 1:00 p.m.) to minimize variations in basal hormone levels. Animals in the control group were not submitted to any intervention.

4.4. ^{18}F-FDG microPET brain scan

All animals were fasted overnight before the scan to increase the ^{18}F-FDG uptake in the brain. The mice were kept on a heating pad at 36 °C for 30 min before imaging and the stress protocol. After, they were removed from their cages and injected intraperitoneally with 250 μCi of ^{18}F-FDG immediately before the acute stress protocol (RS10, RS30 and RS40 experimental groups). Conscious ^{18}F-FDG tracer uptake lasted approximately 40 min. Animals from RS30 and RS10 groups were removed from the restrainer, after the acute stress protocol, and maintained for 10 and 30 min, respectively, in their housing cages for the remainder time of the radiopharmaceutical metabolism. Control animals were maintained conscious in their cages during ^{18}F-FDG uptake. After the complete uptake of the tracer, animals were immediately anesthetized with inhaled isoflurane mixed with oxygen (3–4% for anesthetic induction and 2–3% for anesthesia maintenance). The mice were placed in a headfirst prone position and scanned with the Triumph™ microPET (LabPET-4, TriFoil Imaging, Northridge, CA, USA). Throughout these procedures, animals remained at a controlled temperature of 36 °C. For radiotracer readings, 10-min list mode static acquisitions were acquired with the field of view (FOV; 3.75 cm) centered on the mouse's head. All data were reconstructed using the maximum likelihood estimation method (MLEM-3D) algorithm with 20 iterations. Each reconstructed microPET image was spatially normalized into an ^{18}F-FDG template using PMOD v3.5 and the Fusion Toolbox (PMOD Technologies, Zurich, Switzerland). An MRI mouse brain volume of interest (VOI) template was used to overlay the normalized images, previously coregistered to the microPET image database. The ^{18}F-FDG uptake in the right and left striatum, cortex, right and left hippocampus, thalamus, basal forebrain/septum, hypothalamus, right and left amygdala, olfactory areas, cingulate gyrus, superior colliculi, right and left midbrain, and right and left inferior colliculi were normalized for the injected dose and body weight. The standardized uptake value (SUV) was calculated for the whole brain and each individual region. In order to correct for weight variations, we calculated the SUV ratio (SUVr) of each individual brain region by dividing the SUV value of the region by the whole brain SUV (Silva et al., 2018; Zanirati et al., 2018).

4.5. ^{18}F-FDG biodistribution

After the microPET scan, mice were immediately decapitated and blood, liver and adrenal glands were removed and weighed. The ^{18}F-FDG radioactivity was measured by well-type gamma counter (2480 WIZARD^2 automatic gamma counter, PerkinElmer, Waltham, MA, USA). Samples were read after radioactivity decayed to optimal gamma counter detection levels. The whole tissues and 10 μL of blood were used. Radioactivity uptake in the samples was expressed as percentage of injected dose (activity) per gram of tissue (%ID/g).

4.6. Statistical analysis

All results are presented as mean ± standard error of the mean (SEM). After normality was verified, one-way analysis of variance (ANOVA), followed by the LSD post-hoc test, was used for the whole brain data analysis. For the comparisons between different experimental groups and sexes, a two-way ANOVA was performed, followed by the LSD post-hoc test. All data were analyzed using SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL) and the graphs were made using Prism GraphPad (version 5.0, GraphPad Software Inc, San Diego, California). Statistical differences were considered when $p < .05$.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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