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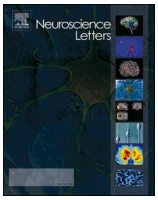

Sex differences in risk behavior parameters in adolescent mice: Relationship with brain-derived neurotrophic factor in the medial prefrontal cortex

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ABSTRACT

Adolescence is as a period of development characterized by impulsive and risk-seeking behaviors. Risk behaviors (RB) involves exposure to dangerous or negative consequences to achieve goal-directed behaviors, such as reward-seeking. On the other hand, risk aversion/assessment behaviors allow the individual to gather information or avoid potentially threatening situations. Evidence has suggested that both behavioral processes, RB and risk assessment (RA), may have sex-differences. However, sex-specific behavioral patterns implicated in RB and RA are not fully understood. To address that, we investigated sex differences in risk-behavioral parameters in a decision-making task developed for rodents. In addition, we investigated the potential role of sex-dependent differences in gene expression of brain-derived neurotrophic factor (*BDNF*) exon IV in the medial prefrontal cortex (mPFC), which has been implicated to mediate PFC-related behavioral dysfunctions. Male and female C57BL/6J adolescent mice were evaluated in the elevated plus-maze (EPM) to assess anxiety-like behaviors and in the predator-odor risk taking (PORT) task. The PORT task is a decision-making paradigm in which a conflict between the motivation towards reward pursuit and the threat elicited by predatory olfactory cues (coyote urine) is explored. After behavioral testing, animals were euthanized and *BDNF* exon IV gene expression was measured by RT-qPCR. Comparative and correlational analyses for behavioral and molecular parameters were performed for both sexes. We observed that female mice spent more time exploring the middle chamber of the PORT apparatus in the aversive condition, which is an indicative of avoidance behavior. Female mice also had a higher latency to collect the reward than male mice and presented less time exploring the open arms of the EPM. *BDNF* exon IV gene expression was higher among females, and there was a positive correlation between the *BDNF* and PORT behavioral parameters. Our findings suggest sex-dependent effects in the PORT task. Females presented higher RA and avoidance behavior profile and expressed higher levels of *BDNF* exon IV in the mPFC. Moreover, higher *BDNF* expression was correlated with RA behaviors, which suggests that adolescent females tend to evaluate the risks more than adolescent males and that *BDNF* gene expression may be mediating decision-making processes.

1. Introduction

Exposure to risk behaviors (RBs) is characterized by an impulsive response without adequate information collection and/or judgment of the outcomes and potential consequences in a given situation [42,56]. One of the periods of greatest vulnerability during development that coincides with increased RBs is adolescence. Impulsive and immediate reward and gratification seeking behaviors, as well as emotional dysregulation are common problems identified during adolescence [61]. Previous studies have shown that this period is associated with the onset for substance use (e.g., alcohol, marijuana and tobacco), involvement in risky sexual behaviors, and violent and self-injurious behaviors

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[1,11,45,52,59,62,66]. In contrast, risk aversion/assessment behaviors can be described as defensive behaviors, which can facilitate information collection and decision-making. These behaviors allow the individual to seek for information or stay away from potentially dangerous events [5,12,30]. Some cognitive tasks have been used to evaluate risk exposure behaviors, including experimental paradigms with animal models. These models are important to explore potential neurobiological mechanisms responsible for mediating the cognitive processes underlying these tasks [3,27,34,53,55,64].

The predator Odor Risk-Taking (PORT) task was proposed by Dent *et al.* [17] to evaluate risk assessment/exposure behaviors in rodents. This test reproduces the natural tendency of these animals to seek reward, but to reach for the reward they need to pass through an environment with predator odor. Shorter latency to collect the reward is associated with impulsive and risk-exposure response, while a longer time may indicate aversion or risk assessment behaviors. In this study, Dent *et al.* used two strains of male mice – C57BL/6J and in-house breeding F_2 of C57BL/6*CBA/CA – to investigate and compare behavioral responses in the PORT task. Odors from different natural mice predators (e.g., wood shavings containing rat, fox or cat odor) were tested as aversive stimuli, and a sweet condensed milk-based solution was used as reward. More responsive results were observed for the in-house breeding C57BL/6*CBA/CA, while C57BL/6J mice showed no different behavioral response between aversive vs control conditions. The task was able to naturally reproduce a decision-making paradigm that evaluated the benefits and costs of the risk exposure behavior for reward collection, so it was a starting point and an incentive for further research [17]. Since its publication, few studies were done to replicate and extend this data, which could give further insights regarding risk assessment behaviors [13,18,25,58,67]. There is one study [25] addressing sex-dependent effects, since male C57BL/6J were not responsive in the previous study. Viola *et al.* [67] also used the task to evaluate the effects of impoverished housing conditions during early development on risk behaviors during adolescence, which is considered a window of vulnerability to RBs [15,32,52].

One of the reasons why adolescence is considered a period of vulnerability is due to the delayed maturation process of the prefrontal cortex (PFC) [15,16,57]. Brain-derived neurotrophic favor (BDNF), a neurotrophic factor recognized by its regulatory role in neuronal development, is highly expressed in the PFC, and is suggested as a potential mediator of executive functions, especially those related to decision-making and risk assessment/exposure processes [8,54]. There are different transcription sites of the *BDNF* gene (*BDNF* gene promoters-I to -IX). The *BDNF* exon IV has been receiving special attention since its expression facilitates the neuronal activity during development [57,72]. Reduction of BDNF expression could affect neuronal activity, impairing GABAergic transmission and the inhibitory postsynaptic currents in PFC region, which might result in PFC-mediated behavioral dysfunctions [57]. On the other hand, enhanced BDNF expression has been suggested as an adaptative mechanism to promote protective effects for reward-seeking behaviors [9,26,54,68].

During adolescence, sex-specific characteristics naturally emerge, which may contribute to distinct cognitive and behavioral phenotypes between sexes [16]. For this reason, we aimed to investigate potential sex differences regarding decision-making capabilities in the PORT task during adolescence. Moreover, due to the importance of *BDNF* gene expression and its relationship with PFC mediating processes, we explored the *BDNF* exon IV gene expression in the medial prefrontal cortex (mPFC) and its correlation with the behavioral responses observed in the PORT task.

2. Methods

2.1. Animals

This study was conducted with male and female C57BL/6J mice obtained from the Center for Experimental Biological Models of Pontifical Catholic University of Rio Grande do Sul (PUCRS). All experiments were conducted according to the animal care guidelines of the National Institutes of Health (NIH Publications No. 8023, revised 1978) and approved by the PUCRS Ethics Committee on Animal Use. The mating procedures were performed in house, with two adult females allocated with one adult male for 72-h. A total of $n = 14$ litters were used for this experiment, with $n = 3$ to 4 pups, totaling $n = 52$ animals. After birth, all litters were kept in standard housing boxes with wood-shavings and 4 g of cotton for nest building. The litters were not handled until weaning on postnatal day (PND) 21, when they were separated by sex ($n = 28$ females and 24 males; $n = 3$ per cage, minimum of 2 when necessary to balance the cages). All animals were kept under laboratory conditions and controlled handling, under a 12-h light–dark cycle (lights on from 6 a.m. to 6p.m.) in Plexiglas housing boxes, automatically ventilated and with controlled temperature at $21 \pm 1\ ^{\circ}C$. Food and water were provided *ad libitum*, except when the animals were subjected to water restriction to perform the PORT behavioral protocol. The experimental design is illustrated in Fig. 1.

All animals were tested on the same day between 8 am and 11:30 am. Isopropyl alcohol was used for cleaning the experimental apparatus between each animal test and bedding was replaced between animals on PORT task to avoid additional olfactory cues. The animals were conducted to the testing room before the beginning of the task and left undisturbed for 15 min for acclimation. The illumination level was set around 120–150 lx. Estrus cycle was evaluated at the ending of PORT test period (at PND46 and PND47) using visual observation of genitalia [14]. All female mice were in diestrus stage of the estrous cycle at this timepoint.

2.2. Predator odor Risk-taking task (PORT)

All animals were exposed to the PORT task, which is a relatively new behavioral task that seeks to evaluate risk exposure vs aversion behaviors through the decision-making paradigm and reward-seeking. It consists of an acrylic apparatus composed of three chambers (30 cm wide × 30 cm long × 30 cm high each) separated by acrylic walls with openings (5 cm wide × 5 cm high). The first chamber is called the start chamber, which the animal is placed to start the task. The middle (main) chamber, is where the experimental conditions (aversive predator odor) and the control condition (standard wood shavings, neutral odor) are manipulated. The final chamber is where the reward was placed (for an illustration of the task, see Fig. 2A).

The parameters used follow those proposed by Dent *et al.* [17]: (a) time spent in the three chambers, (b) latency to collect the reward, and (c) time spent in the final chamber before collecting the reward. To induce conditioning and motivated reward-seeking behaviors, water restriction was induced for 15-h per day in all animals before the task. Water restriction was performed starting at PND30 until the last day of testing (PND47). The water bottles were removed at the beginning of the dark cycle, and access was granted in the morning of the next day (beginning of the light cycle, during the period of habituation to water restriction) or after the behavioral tasks. The tasks were recorded by a video camera and analyzed by two independent research assistants who were blinded to the sex of the animals and experimental conditions.

Habituation to the reward: habituation to the reward solution was performed on PND33. The reward stimulus used was a solution of condensed milk (10%) mixed with water (90%) according to the original PORT task. A container holding the sweet solution (2 mL) was placed inside the cages for a period of 48 h. Between PND36 and PND39, the animals were tested daily regarding their preference for consumption of sweet solution compared to water. These 15-min sessions were performed in boxes similar to open field arenas made of Plexiglas (30 cm × 30 cm × 30 cm) containing the sweet solution (2 mL) and water (2 mL). To avoid side preference, the containers were alternated between sessions. The condensed milk solution and water were measured by

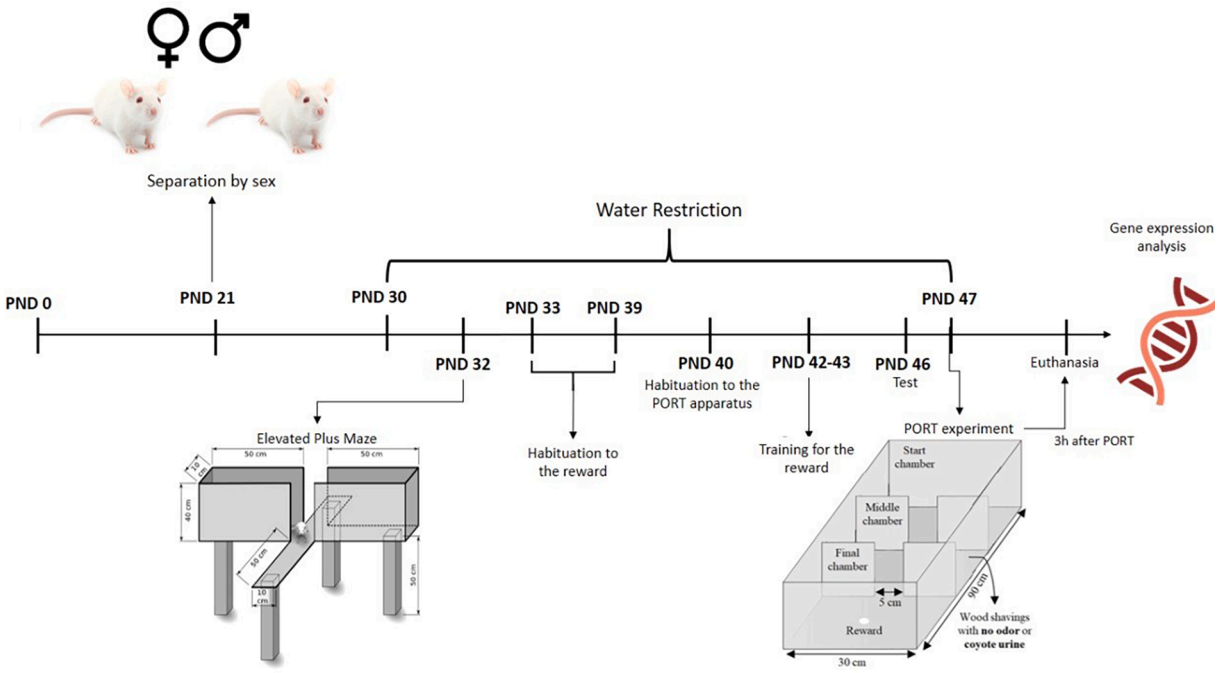

Fig. 1. Experimental design and timeline.

weighing the containers before and after the test. The preference for the reward was calculated as [consumed volume of sweet solution / (consumed volume of sweet solution + consumed volume of water)]. Animals that did not show at least 50% reward preference in the last test session were excluded from the analysis. Of the 54 animals used for behavioral training, 32% (n = 17) did not show a minimum of 50% reward preference (Fig. 2B). There were no differences between sexes regarding reward preference (chi-square = 0.107; p = 0.743). The final sample used in the PORT task was n = 37 (males, n = 17 and females, n = 20).

Habituation to the apparatus: habituation to the PORT apparatus was performed on PND40. Each animal was individually placed in the starting chamber and was allowed to explore the apparatus for 15 min. Throughout all habituation sessions, the middle chamber was covered with wood shavings.

Training for the reward: From PND42 to PND43, the animals underwent training sessions to seek and collect the reward in the PORT apparatus. Reward collection was considered when the animals started to consume the sweet liquid. Each animal performed four trials per day. In each attempt, the animal was placed in the starting chamber, and exploration of the three chambers was allowed. The reward was placed in the center of the final chamber. To collect the reward, the animals had to cross the three chambers. After collecting the reward, the animals were returned to their cages to wait for the next attempt (5 min interval between attempts). The time spent collecting the reward was recorded for each attempt.

Test: The animals were initially placed in the starting chamber and released to explore the apparatus to collect the reward. The tests were performed with two wood shavings conditions. The neutral condition (on PND46) had the middle chamber covered with neutral scent shavings. On PND47 the experimental condition (wood shavings with predator odor) was performed. To induce unconditioned predator stimuli, we used 2 mL of coyote urine odor mixed on wood shaving in the middle chamber of the PORT apparatus. Specifically, coyote urine has been suggested as the more reliable threat stimulus to promote anxiety-like defensive behaviors in comparison to other commonly used predatory cues, such as synthetic odor 2-phenylethylamine (PEA) or trimethylthiazoline (TMT) [23,38].

2.3. Elevated plus-maze

The elevated plus-maze (EPM) is a classic task used to assess anxiety-like behavior based on the tendency that rodents prefer dark and enclosed environments compared to open areas [70]. For this purpose, a black acrylic apparatus containing two open arms (30 cm long × 5 cm wide) and two closed arms (30 cm long × 5 cm wide, surrounded by 15-cm high walls), raised at a height of 40-cm from the floor was utilized. The animals were placed individually on the central platform facing one of the open arms and were allowed to explore the apparatus for 5 min. All animals were video-recorded and the recordings were analyzed following the same model used in the PORT task. Considering that the PORT protocol started on PND33, the EPM was conducted on PND32.

The parameters analyzed were: total time spent in the open arms, total time spent in the closed arms, and total time in the center of the apparatus.

2.4. Gene expression analysis

Three hours after behavioral testing the animals were euthanized. This period was chosen because evidence suggests that changes in gene expression involved in glutamatergic and neurotrophic signaling takes approximately 3 h, especially after exposure to olfactory stressors in rodents [35]. The mPFC tissue was manually dissected using a scalpel and stored at −80 °C until further molecular analysis. Total RNA was isolated from n = 6–7 randomly selected animals per group following the QIAzol (Qiagen; Hilden, Germany) and chloroform standard protocols. The RNA concentration was measured using a NanoDrop spectrophotometer. A total of 500 ng of RNA from each sample was used for cDNA synthesis following the instructions of the miScript II RT kit (Qiagen). The following primers were designed and tested for *BDNF exon IV*: forward, 5′-GCAGCTGCCTTGATGTTTAC-3′; reverse, 5′-CCGTGGACGTTTACTTCTTTC-3′; and *Pgk*: forward, 5′-TGCACGCTTCAAAAGCGCACG-3′; reverse, 5′-AAGTCCACCCTCATCACGACCC-3′ [4]. To check the primer specifications, melting curve and agarose gel analyses were performed. Each PCR was performed in duplicate for each sample using SYBR green in a Rotor-Gene real-time PCR machine (Qiagen). Relative expression was calculated using the ΔΔCt method

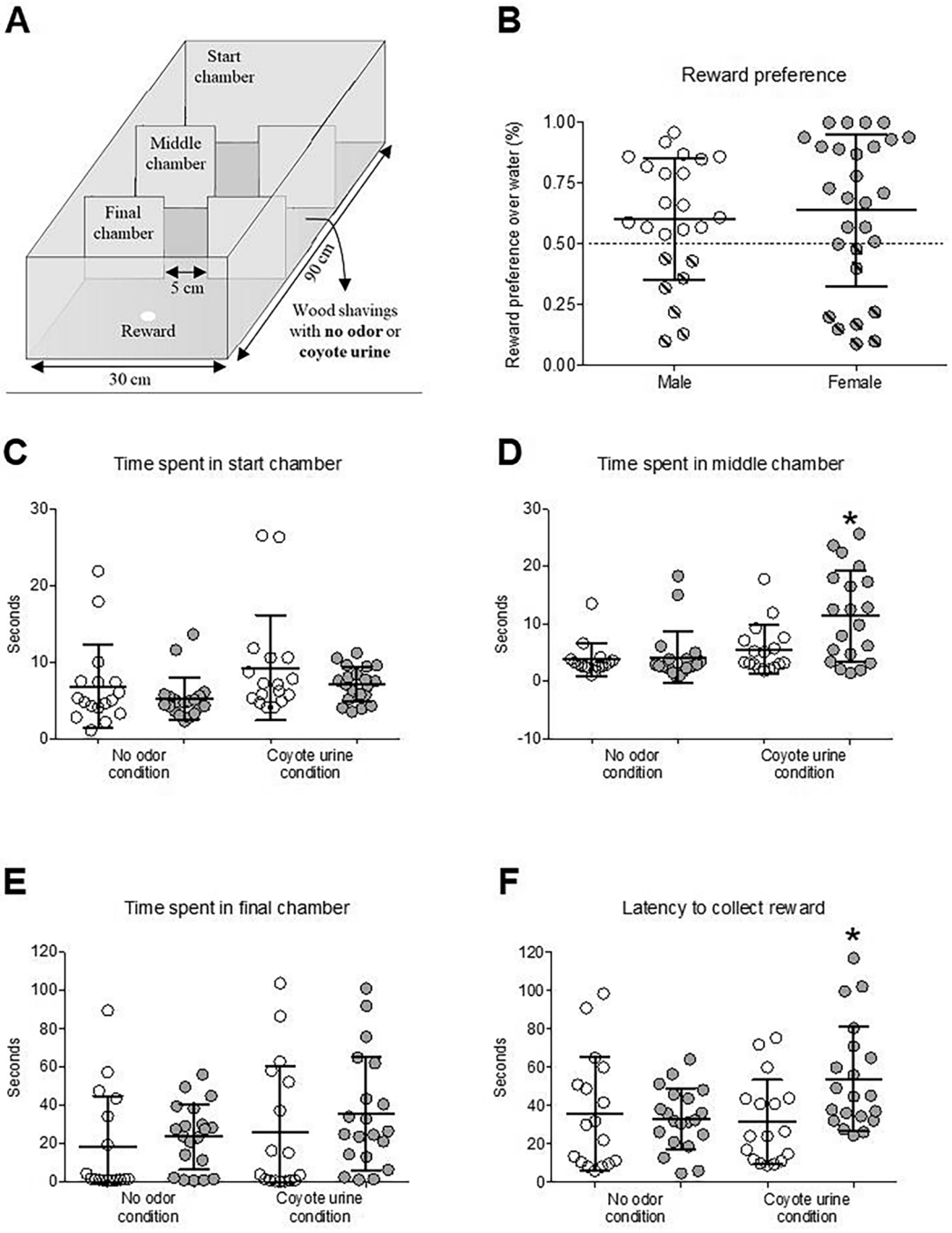

Fig. 2. Predator odor risk-taking task; (A) Representation of the PORT apparatus composed of three chambers, with the middle chamber used for manipulation of the experimental vs. neutral condition. Task used to evaluate risk assessment and risk exposure behaviors; (B) Percent preference for the reward, cutoff point of 50% (n = 24 males and n = 28 females; before the preference test); (C) Time in seconds spent in the start chamber (n = 17 males and n = 20 females); (D) Time spent in seconds in the middle chamber in the experimental vs. neutral condition (n = 17 males and n = 20 females); (E) Time in seconds spent in the final chamber (n = 17 males and n = 20 females); (F) Latency in seconds to collect the reward (n = 17 males and n = 20 females); * sex × experimental condition interaction effect with $p < 0.05$. For all graphs: males = white, females = gray.

[36] with the group of male animals as a reference. *Pgk* ct values were utilized as endogenous controls.

2.5. Statistical analysis

The PORT task data were analyzed using general linear models with two factors (experimental condition: no odor × coyote urine; sex: male × female). Post hoc comparisons were run using Bonferroni correction. The EPM and BDNF expression data were analyzed using *t*-test for independent samples. Pearson correlation was performed to analyze the association of BDNF levels with behavioral parameters. Statistical significance was defined as $p < 0.05$. Values are expressed as the mean ± SEM. All results were analyzed using SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA). Graphs were generated using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA, USA).

3. Results

3.1. Port

The results of the PORT task revealed the existence of an experimental condition effect (neutral × predator odor; $F = 9.42$; $df = 3,70$; $p = 0.004$) and sex × experimental condition interaction ($F = 4.34$; $df = 3,70$; $p = 0.045$; Fig. 2D) on the time spent in the middle chamber of the PORT apparatus. An increase in the time spent in the middle chamber among females was observed during the aversive condition (coyote urine) in comparison with females in neutral condition ($p < 0.001$) and males in both aversive ($p = 0.009$) and neutral ($p < 0.001$) conditions. Regarding the total latency to collect the reward, a sex × experimental condition interaction was observed ($F = 5.70$; $df = 3,70$; $p = 0.022$; Fig. 2F). The post hoc analysis showed that the females took longer to collect the reward during the aversive condition compared to males ($p = 0.037$) and females in neutral condition ($p = 0.048$). There were no significant differences in the analyses of the time spent in the start and final chambers.

3.2. Epm

In the EPM, less time was spent by females in the open arms of the apparatus compared to males ($t = 2.16$; $df = 52$; $p = 0.035$; Fig. 3A). No significant differences were observed regarding the time spent in the center ($p = 0.691$) and the time spent in the closed arm ($p = 0.276$).

3.3. Bdnf

We observed an increased expression of *BDNF exon IV* mRNA levels in the mPFC of females compared to males ($t = 4.64$; $p = 0.001$; Fig. 4A). In addition, correlation analyses were performed with pooled data between *BDNF* expression and the different parameters evaluated in the PORT task. There was a significant positive correlation between the time spent in the middle chamber (aversive condition) and *BDNF exon IV* gene expression ($r = 0.62$; $p = 0.022$; Fig. 4B). The other behavioral parameters showed no significant correlation with BDNF expression.

4. Discussion

The present study investigated possible differences between sexes in a paradigm of RB assessment in rodents. In addition, biomolecular correlates of *BDNF* gene expression in the mPFC were investigated to establish associations with the behavioral parameters evaluated by the PORT task. Our results indicate significant sex differences in terms of risk assessment/exposure behaviors, through an increase in the time spent collecting the reward and total time spent in the middle chamber of the PORT apparatus (during the aversive condition with predator odor). This difference was reinforced by the shorter open arms exploration time in EPM. Both findings converge to the presence of a pattern of avoidance/aversion in the face of threatening situations among females and/or indicates a longer period evaluating potential risks in contexts associated with exposure to potentially dangerous stimuli. *BDNF* exon IV gene expression also supported the differences between sexes. Furthermore, a higher BDNF expression was positively associated with time spent in the middle chamber during the aversive condition, which suggests a relationship with risk aversion and/or assessment behaviors regardless of the sex.

Risk aversion/assessment is considered a behavioral pattern contrary to an impulsive and non-deliberate response of an individual. Such behaviors refer to the ability to assimilate the benefits and harms of an action/response through the choice of available options [5,30,31]. On the other hand, RB are characterized by an impulsive (sometimes reckless) response, without an adequate judgment of the possible consequences or the collection of information about the surrounding environmental conditions [42,56]. Although such concepts are debated by

Fig. 3. Analysis of anxiety-like behaviors in the EPM; (A) Time spent in open arms; (B) Time spent in closed arms; (C) Time spent in the center; (n = 17 males and n = 20 females) *p < 0.05.

several authors, it has not been extensively explored in animal models. The PORT task, developed and proposed by Dent *et al.* (2014), allows to estimate the time spent by the animals in each of the task chambers. A shorter time is associated with impulsive responses and risk exposure, while a longer time may indicate risk aversion or assessment (exploration) before decision-making. In our study, when investigating possible sex distinctions in adolescent animals, a pattern of risk aversion and/or higher risk assessment was observed among females. In male, however, similar to what was reported by Dent, we did not observe an aversive effect to coyote urine, which reinforces strain- and sex-specific responses facing the presence of predator odor cues [17]. C57BL/6 indeed has been suggested as a less responsive strain to stress-induced paradigms

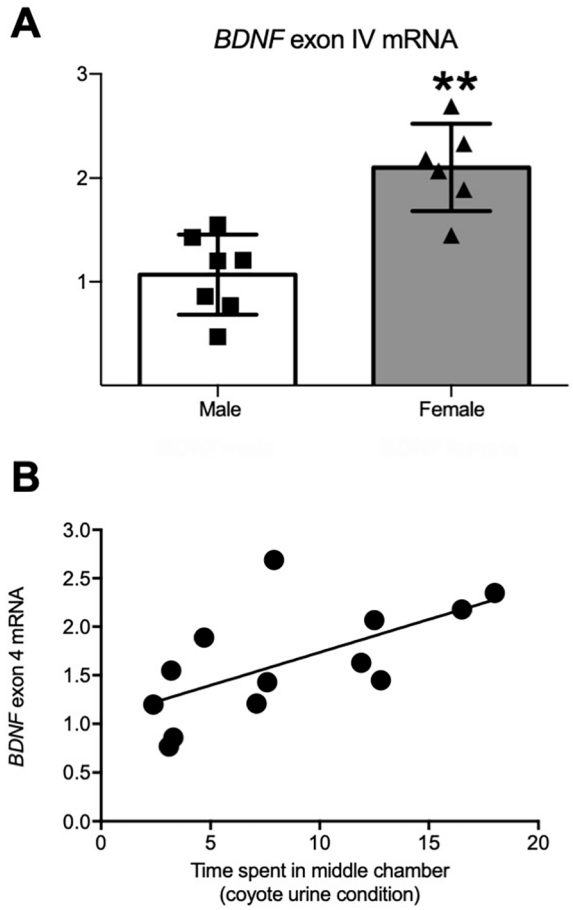

Fig. 4. Analysis of BDNF gene expression in the medial prefrontal cortex and correlation with time spent in the middle chamber; (A) Expression of BDNF exon IV mRNA in females relative to males; (B) Pooled data of relationship between BDNF exon IV mRNA expression and time spent in the middle chamber in coyote urine condition; n = 7 males and n = 6 females; **p < 0.01.

and anxiety-like behavior response [46,50]. The time spent in each chamber could be interpreted as a novelty-seeking behavior induced by the task itself, but we suppose that is not the case. In the previous study by Viola et al. [67], an independent cohort of animals was tested to evaluate if the higher time spent in central chamber was due to the coyote urine odor or novelty-seeking effects. Animals were exposed to a citronella odor condition and showed a significant reduction in time compared to coyote urine condition animals. In addition, we evaluated the animals in the EPM as a complementary task, including parameters of locomotor activity, (e.g., number of entries). There are no significant differences in these parameters, suggesting that the behaviors during the PORT task did not reflect distinct patterns of locomotor activity or were induced by a novelty-seeking environment.

The sex differences observed in our study support previous evidence that suggests a defensive response among females in risky and threatening situations (e.g., predatory odor stimuli) in various experimental paradigms [6,29,31,41,67,73]. In a prior study with similar conditions, it was observed that control females displayed increased risk assessment behavior under reward-seeking condition compared to an experimental stressed group. However, this study limited its findings by analyzing males and females independently [67]. Other studies using different predatory-cue paradigms, such as Yokota [73] and Kavaliers [29,31], observed more pronounced aversion response to risk in females, which suggests that males tend to make riskier decisions. Nevertheless, these risk-seeking comparisons between males and females were not assessed during adolescence [10,31]. In this sense, our results provide additional insights about sex differences during adolescence that should be further explored, especially about the role of gonadal hormones in these behavioral distinctions.

A more recent study by Francesconi [25] using the PORT task observed a distinct pattern between males and females. Males presented higher latency to collect the reward, which was interpreted as a more risk-aversive response. Although this study identified a pattern opposite to previous evidence, it seems that this effect may have occurred because some of the male animals showed an extreme response compared to the overall behavioral pattern. In addition, the predatory odor used in the study (synthetic PEA) has not been providing reliable data when eliciting defensive behaviors underlying anxiety- and fear-like states (e.g., risk assessment, avoidance and freezing) [38], which might contribute for the conflicting results in predator odor-induced avoidance and risk assessment behaviors in PORT.

Data from clinical studies also converge to a difference between sexes in relation to risk assessment and exposure. A clinical study comparing both sexes in regard to impulse control and substance-seeking behaviors revealed that women tend to have greater control over impulsive responses, and present reduced substance-seeking behaviors [60]. This corroborates with the data that men present greater exposure to risk behaviors, such as drug use, involvement in situations of violence, or with potentially harmful outcomes [21,48]. One of the possible explanations for this pattern is related to a lower sensitivity to adversity and greater sensitivity to reward compared to females. In this sense, males tend to decide faster and show greater behavioral perseverance, while females tend to engage more fully with the environment, acquire more detailed information, and retain associations more effectively [30].

Anxiety behavior was also distinct between sexes. Our findings revealed trends of anxiogenic stimuli (open and unprotected area of the apparatus) avoidance, which could be related to a pattern of higher risk assessment. Some studies exploring sex differences in anxiety and stress-related paradigms, such as open field and EPM, revealed an increased anxiety response among adolescent and adult females [2,10,41,44,65]. Anxiety behaviors are greatly influenced by sex hormones, especially estradiol and testosterone, which markedly vary throughout different life stages. These hormones also were suggested as able to facilitate anxiogenic responses in different behavioral tasks, including auditory-cued fear conditioning and predatory avoidance tests [29,31,49]. In this sense, the anxiety-like behavior data found in this study appears to be complementary to the pattern of female risk assessment/avoidance observed in the PORT task.

One of the regions most studied and involved in the risk assessment and risk exposure responses during adolescence is the PFC. This region plays a role in regulating and mediating emotional responses, impulses, and reward-seeking behaviors. Its involvement has been discussed as especially important for deliberate behaviors, such as risk assessment and anxiety modulation. Moreover, this region is considered extremely susceptible to environmental effects and influences throughout childhood and adolescence [54,57,68]. One of the factors responsible for stimulating the neuronal development and plasticity of this region is the BDNF, which is an important neurotrophic factor with significant expression in the PFC. Due to its role in neuronal development and integrity, as well as in adequate cognitive functioning, BDNF has been associated with risk behaviors, including drug-seeking behavior, and decision-making process [8,22,69].

Previous studies showed that BDNF is involved in the potentiation of the synaptic strength required for decision-making and modulation of RBs [8,33,51,54]. A decrease in *BDNF* expression, for example, has been reported in addictive behaviors, being related to RBs and relapse [40]. Although there are no previous studies investigating *BDNF* expression during decision-making paradigms, such as the PORT, our findings indicated a positive association between *BDNF exon IV* gene expression in the mPFC and a pattern of risk aversion/assessment response (i.e., an increase in the time spent in the middle chamber during the task). Such association might be related to the distinct pattern of behavioral

response between sexes, since we found an increase in *BDNF exon IV* gene expression in females. For example, *BNDF exon IV* deficient mice exhibited less GABAergic transmission and pike-timing-dependent activity in the mPFC [57]. Since dysregulation of *BDNF* gene expression affects neuronal activity and inhibitory postsynaptic potential, it is conceivable that lower levels of *BDNF exon IV* expression might be involved in cognitive dysfunctions underlying PFC functioning, as suggested by risk-seeking behaviors and decision-making problems observed in addictive disorders [19,20,47,63]. An adequate balance between excitatory and inhibitory neurotransmission (E/I balance) is essential for PFC integration of multiple inputs from sensory, limbic, and neuromodulatory regions [24,39,43]. In contrast, overexpression of *BDNF* has been suggested as a potential marker for regulatory process of development and function of parvalbumin GABAergic interneurons in the PFC [57].

The neuronal activity during the PORT task was recently explored by the study of Francesconi and colleagues [25], indicating that the avoidance condition increased *c-fos* expression in females, while no difference was observed in males. The distinct pattern of activation during the task was associated with decision-making processes (benefits vs costs to ignore threat and reach the reward). Interestingly, differential *c-fos* activity in the cortex indicates that animals have higher response to aversive and threatening stimulus [28], which reinforce the sex-differences findings. A distinct pattern of neuronal activity has been directly correlated with *BDNF* gene expression and its receptors in the mPFC, as well as other sexual hormones, including estrogen, which plays an important role in the distinctions between sexes and is highly expressed among females [7,37]. It is known that estrogen is a stimulant of BDNF production [71] and therefore might influence *BDNF* expression, which could lead to alterations in BDNF-related behaviors during adolescence [74,75]. However, these conclusions should be considered exploratory since the function of *BDNF* expression is dependent on the region where it is expressed and the developmental stage [40,75]. Thus, our findings provide only evidence of a possible mediating role of *BDNF* exon IV expression that must be further explored.

Considering that our study aimed to explore sex-differences in the PORT task, some limitations should be highlighted for data interpretation. Our findings are strain- specific and time-point limited. We do not know how other strains perform in this relatively new task or how the behavioral pattern can change during adulthood. There are just a few studies using the PORT task and future studies comparing different strains and time points are important to consolidate this decision-making paradigm. We also investigated the expression of a specific *BDNF* exon (exon IV). The reason was in part because our research group has been exploring the role of this exon in PFC-mediated behavioral dysfunctions. Complementary studies measuring total BDNF levels or other exons underlying motivational behavior should provide additional insights to the present data. In the same way, we just assessed *BDNF exon IV* in the whole mPFC region, without being selective for the mPFC subregions (e.g., anterior cingulate, prelimbic, infralimbic). We also did not include a control region within the cortex to determine the specificity of our findings or whether BDNF levels could also be altered in different regions of the cortex. Finally, the correlation between time spent in middle chamber in the PORT task and the expression of *BDNF exon IV* should be interpreted with caution. Since gene expression was measured 3-h after testing, this expression could reflect the behavioral differences and not its cause per se. To assess this possibility, future studies should include a baseline cohort in their experimental design.

5. Conclusions

The sex distinctions regarding risk aversion/assessment and risk exposure behaviors suggest that females present a pattern of greater risk aversion/assessment and anxiety response to potentially threatening and anxiety-eliciting situations. Moreover, the alterations in *BDNF* exon IV expression highlights the importance of exploring and associating sex-dependent behavioral specificities and biomolecular markers. This could lead to the understanding of susceptibility patterns that culminate in sex-specific dysfunctions. In addition, periods such as adolescence may represent windows of opportunity for the investigation of sex differences, since several neurodevelopmental and hormonal alterations are occurring throughout this period, which could be related with the differences in decision making processes and stress-related responses.

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CRediT authorship contribution statement

Marco G.S. Gomes: Investigation, Methodology, Writing – original draft. **Saulo G. Tractenberg:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Rodrigo Orso:** Writing – review & editing. **Thiago W. Viola:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – review & editing. **Rodrigo Grassi-Oliveira:** Conceptualization, Project administration, Resources, Supervision.

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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