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Stress during adolescence increases novelty seeking and risk-taking behavior in male and female rats

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Adolescence is a period of major physical, hormonal, and psychological change. It is also characterized by a significant increase in the incidence of psychopathologies and this increase is gender-specific. Likewise, stress during adolescence is associated with the development of psychiatric disorders later in life. Previously, using a rat model of psychogenic stress (exposure to predator odor followed by placement on an elevated platform) during the pre-pubertal period (postnatal days 28–30), we reported sex-specific effects on auditory and contextual fear conditioning. Here, we study the short-term impact of psychogenic stress before and during puberty (postnatal days 28–42) on behavior (novelty seeking, risk taking, anxiety, and depression) and hypothalamus–pituitary–adrenocortical (HPA) axis activation during late adolescence (postnatal days 45–51). Peri-pubertal stress decreased anxiety-like behavior and increased risk taking and novelty seeking behaviors during late adolescence (measured with the elevated plus maze, open field and exposure to novel object tests and intake of chocopop pellets before or immediate after stress). Finally neither depressive-like behavior (measured at the forced-swim test) nor HPA response to stress (blood corticosterone and glucose) were affected by peri-pubertal stress. Nevertheless, when controlling for the basal anxiety of the mothers, animals exposed to peri-pubertal stress showed a significant decrease in corticosterone levels immediate after an acute stressor. The results from this study suggest that exposure to mild stressors during the peri-pubertal period induces a broad spectrum of behavioral changes in late adolescence, which may exacerbate the independence-building behaviors naturally happening during this transitional period (increase in curiosity, sensation-seeking, and risk-taking behaviors).

Keywords: adolescence, gender differences, stress, anxiety, risk taking, novelty seeking, resilience, vulnerability

INTRODUCTION

Adolescence is a critical developmental period characterized by change. Despite its gradual onset and offset there are major physical, hormonal, and behavioral differences between adolescence and childhood or adulthood. Adolescence is characterized by major developmental changes in the brain and the hypothalamic–pituitary–adrenal axis (HPA axis). The HPA axis matures before the brain pathways that regulate emotion, cognition, and learning (such as the prefrontal cortex, hippocampus, amygdala, and ventral striatum; Giedd et al., 1999; Sowell et al., 1999). Dynamic changes in stress-responsive regions are theorized to contribute to the unique behavior of adolescents (Cunningham et al., 2002; Casey et al., 2010).

This major biological transition is hypothesized to render the adolescent more vulnerable to stress and the development of psychopathologies. Stressful experiences during childhood and adolescence have been associated with the development of psychiatric disorders later in life (Kessler and Magee, 1993; Penza et al., 2003; Heim et al., 2004). Yet, only a percentage of the adolescents exposed to stress develop psychopathologies (Fergus and Zimmerman, 2005).

In the rat, adolescence is considered to last from either postnatal days 21–59 (Tirelli et al., 2003) or from postnatal days 28 till 42 (Spear and Brake, 1983; Spear, 2000). Recently, there has been an increase in the number of rat models developed to study the short and long-term effects of stress during adolescence. These animal models use various stressors ranging from purely social stress to exclusively physical stress (**Table 1**). While the majority of these models focus on the rat, a few have recently used mice (Ito et al., 2010; Schmidt et al., 2010a,b; Sterlemann et al., 2010; Peleg-Raibstein and Feldon, 2011). Stressors in these models are applied either before puberty, around puberty, after puberty, or during the entire adolescence (**Table 1**). Importantly, despite the original focus on males, inclusion of male and females in studies is progressively increasing investigation into the effects of peri-pubertal stress are sex specific (**Table 1**).

Until now most studies focused on the long-term effects of peri-adolescent stress. They investigated the impact on adult behavior, endocrine responses, gene expression, neuronal, and brain morphology, cell proliferation in the dentate gyrus and drug sensitization (**Table 1**). While some recent studies have investigated the short-term effects of stress during adolescence (**Table 1**), there is no sufficient information about the short-term effects of stress on risk taking and novelty seeking behaviors, and whether there are gender differences on those behaviors. Previously, using a rat model of psychogenic stress during the pre-pubertal period, we reported sex-specific effects on auditory and contextual fear conditioning (Toledo-Rodriguez and Sandi,

Table 1 | Studies investigating the effects of stress during adolescence in rodents.

Type of stressor	
Social	McCormick et al. (2004, 2005, 2006, 2008, 2011), McCormick and Ibrahim (2007), Mathews et al. (2008a,b), Morrissey et al. (2011), Schmidt et al. (2010a,b), Sterlemann et al. (2010), Jacobson-Pick et al. (2011)
Physical	Tsoory and Richter-Levin (2005), Tsoory et al. (2008), Ilin and Richter-Levin (2009), Jacobson-Pick and Richter-Levin (2010), Avital and Richter-Levin (2005), Avital et al. (2006), Jacobson-Pick et al. (2008), Ito et al. (2010), Barha et al. (2011)
DEVELOPMENTAL STAGE WHEN THE STRESSOR WAS APPLIED	
Pre-puberty	Avital and Richter-Levin (2005), Avital et al. (2006), Tsoory et al. (2006), Tsoory and Richter-Levin (2005), Maslova et al. (2002), Ito et al. (2010), Jacobson-Pick et al. (2008), Jacobson-Pick and Richter-Levin (2010), Ilin and Richter-Levin (2009), Bazak et al. (2009), Peleg-Raibstein and Feldon (2011), Toledo-Rodriguez and Sandi (2007)
Puberty	McCormick et al. (2004, 2005, 2006, 2008, 2011), McCormick and Ibrahim (2007), Mathews et al. (2008a,b), Morrissey et al. (2011)
Post-puberty	Laroche et al. (2009)
Entire adolescence	Kabbaj et al. (2002), Isgor et al. (2004), Sala-Catala et al. (2005), Barha et al. (2011), Schmidt et al. (2010a,b), Sterlemann et al. (2010)
IMPACT ON ADULT	
Behavior	Avital and Richter-Levin (2005), Avital et al. (2006), Tsoory and Richter-Levin (2005), Tsoory et al. (2006), Barha et al. (2011), Schmidt et al. (2010a,b), Sterlemann et al. (2010)
Endocrine response	Barha et al. (2011), Maslova et al. (2002), McCormick et al. (2004, 2005)
Gene expression	Tsoory et al. (2008, 2010), Jacobson-Pick et al. (2008), Bazak et al. (2009), Schmidt et al. (2010a,b), Sterlemann et al. (2010)
Neuronal and brain morphology	Isgor et al. (2004), Sala-Catala et al. (2005)
Cell proliferation in the dentate gyrus	Barha et al. (2011), McCormick et al. (2011)
Drug sensitization	McCormick et al. (2004, 2005), Kabbaj et al. (2002)
IMPACT DURING ADOLESCENCE	
	Ito et al. (2010), Toledo-Rodriguez and Sandi (2007), Mathews et al. (2008b), McCormick et al. (2008, 2011), Morrissey et al. (2011), Peleg-Raibstein and Feldon (2011), Doremus-Fitzwater et al. (2009), Varlinskaya et al. (2010)
STUDIES INCLUDING FEMALES	
	Toledo-Rodriguez and Sandi (2007), McCormick et al. (2004, 2005, 2006, 2008, 2011), McCormick and Ibrahim (2007), Mathews et al. (2008a,b), Barha et al. (2011), Doremus-Fitzwater et al. (2009), Varlinskaya et al. (2010), Schmidt et al. (2010a)

2007). In the current study, we aimed to investigate the impact of psychogenic stress experienced during the peri-pubertal period, on the behavior (novelty seeking, risk taking, anxiety, and depression) and HPA axis activation displayed by animals during late adolescence. Additionally, we explored whether the anxiety trait of the parents had any influence on the offspring's reactivity to stress. For this purpose, we modified our rat model of psychogenic stress prolonging the stress period to last the entire male and female peri-pubertal phase (from 28 to 42 days of age). The stress days were chosen to cover pre-puberty (28–30 days old), and puberty (34 and 36 days old for the female, Lewis et al., 2002; and 40 and 42 days old for the male, Lewis et al., 2002; Fernandez-Fernandez et al., 2005). After 2 days recovery from the peri-pubertal stress protocol, control, and stressed animals were submitted to a battery of behavioral tests and hormonal analyses. We focused on risk taking, basal anxiety, novelty seeking, and depressive-like behaviors. Hormonal assessments included plasma corticosterone and glucose levels immediately after exposure to a mild acute stressor.

MATERIALS AND METHODS

SUBJECTS

Subjects were the offspring of rats purchased from Charles River Laboratories, France. Fifteen male and 18 female Wistar Han rats from different liters were weaned at postnatal day 21. To avoid litter effects rats from different litters were mixed, placing equivalent numbers of animals from each litter in the stress and control groups. Rats were housed in same sex cages (three or four per cage) in standard plastic cages on a 12-h light-dark cycle (light on at 7:00 AM). Food and water were available *ad libitum*. All the procedures described were conducted in conformity with the Swiss National Institutional Guidelines on Animal Experimentation, and approved by the Swiss Cantonal Veterinary Office Committee for Animal Experimentation.

PERI-PUBERTAL STRESS PROCEDURE

The stress protocol used was a longer version of the one previously developed in our laboratory (Toledo-Rodriguez and Sandi, 2007). Animals underwent stress during postnatal days 28–30

(three consecutive days pre-puberty), 34, 36 (female puberty), 40, and 42 (male puberty). During the days of stress animals were exposed to one or two consecutive stressors (see **Figure 1**). The stressors were: (a) placement for 5 min on a novel location (rectangular box) for 5 min (only the first day of stress); (b) exposure to trimethylthiazoline (TMT) odor (Phero Tech Inc., Delta, BC, Canada) during 25 min in a closed box (38 cm × 27.5 cm × 31 cm dimensions) under bright light (200–250 lx) containing a cloth impregnated with 10 mg TMT or (c) placement on an elevated platform (of 12 cm × 12 cm located 94.5 cm from the ground) during 25 min under direct bright light (500–550 lx). After stress animals were placed for 15 min in a clean cage without sawdust fitted with perforated perpex partitions. In this way animals could see hear and smell, but not touch each other. The timing of the stress was random (unpredictable for the animal) but always during the light period. See **Figure 1** for a schematic representation of the stress protocol and behavioral tests' schedule. During the days of stress, control rats were manipulated for 2 min by the experimenter.

ACTIVITY BOX (OPEN FIELD) AND REACTIVITY TO NOVELTY (NOVEL OBJECT)

After the stress period all rats (stressed and control) were handled during 2 min daily for 2 days before testing their spontaneous locomotor activity and reactivity to novelty in an activity box (at 45 days of age). The activity box consisted in a rectangular arena (of 37 cm × 57 cm) divided in nine zones of identical size. The test was started by placing the animals in the center of the arena and consisted in two blocks of 10 and 5 min. During the first 10 min the animal's reactivity to a novel location was measured. Afterward a novel object (dark glass bottle) was placed at the center of the arena and the reactivity of the animal toward the object was measured. The locomotor activity was monitored by a video camera, mounted on the ceiling and a computerized tracking system (Ethovision 1.90, Noldus IT, Wageningen, The Netherlands) recorded the total distance moved, speed, percentage of time spent in each zone, and latency to enter the center. An observer (blind to the animal's experimental condition) scored the animal's sniffing and rearing onto the object. The floor of the arena and the object were washed after each test with 0.1% acetic acid solution to remove odors left by previous subjects. The test was performed simultaneously to all the animals in the cage by using three or four adjacent arenas.

ELEVATED PLUS MAZE

Twenty-four hours after the activity box and reactivity to novelty tests, anxiety-like behavior of stressed and control rats was evaluated using the elevated plus maze (EPM) test. The EPM consisted of two opposing open arms (45 cm × 10 cm) and two closed arms (45 cm × 10 cm × 50 cm) that extended from a central platform (10 cm × 10 cm) elevated 65 cm above the floor. Rats were placed individually on the central platform facing a closed arm and were allowed to freely explore the maze for 5 min. The behavior of each rat was monitored using a video camera and the movement of the rats automatically registered and analyzed with a computerized tracking system (Ethovision 1.90, Noldus IT, The Netherlands). Entry into an arm was defined as entry of all four paws into the arm. Total distance moved, speed, time spent in the open and closed arms, number of times the animal entered each type of arm, latency before entering an open arm and number of defecations were measured. An observer (blind to the animal's experimental condition) scored the animal's grooming, stretching, and rearing behaviors as well as the exploration outside the maze (head-dipping). The floor of the EPM was washed after each testing with 0.1% acetic acid solution to remove odors left by previous subjects.

The parents of the experimental animals used in this study were also assessed for their anxiety-like behavior in the EPM test before breeding took place.

CHOCOPOP PELLET INTAKE

On postnatal days 45–49, animals were habituated to eat Chocopop flakes (Kellogg's, Switzerland) by feeding four flakes per animal in their homecages daily. Afterward two test of food intake were performed:

Chocopop intake in basal conditions. On the afternoon of postnatal day 50 (and at least 3 h before the forced-swim test) animals were placed in a clean cage without sawdust fitted with perforated perpex partitions. On the floor of each partition there were 10 Chocopop flakes. Animals were allowed to explore freely the partition and eat the flakes for 2 min.

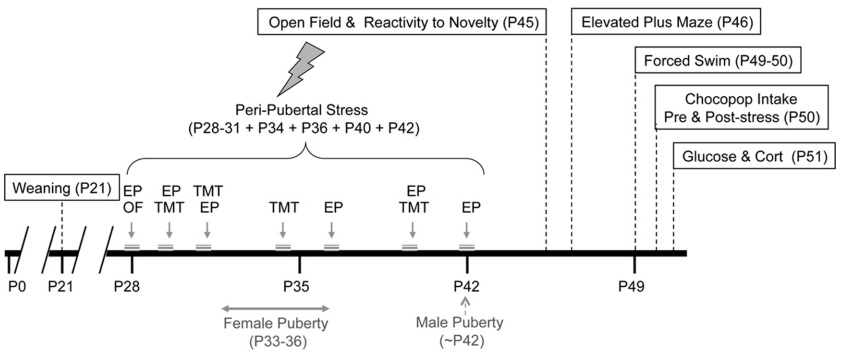

FIGURE 1 | Stress and behavioral test procedures employed in this study. P, indicates the postnatal day; EP, Elevated Platform; OF, Open Field, TMT, exposure to trimethylthiazoline odor.

Chocopop intake immediate after Forced-Swim stress. Immediate after forced-swim test (postnatal day 50, see below) animals were placed in a clean cage without sawdust fitted with perforated perpex partitions with 10 Chocopop flakes on the floor. Animals were allowed to eat the flakes for 2 min.

In all cases the number of flakes eaten by the animal were measured. The floor and walls of the arena were washed after each testing with 0.1% EtOH solution to remove odors left by previous subjects. The test was performed simultaneously to all the animals in the cage by using three or four adjacent arenas.

FORCED SWIM

To evaluate depression-like behavior animals underwent forced-swim test as previously described (Toledo-Rodriguez and Sandi, 2007). In brief, animals were individually placed in plastic cylinders (25 cm diameter, 46 cm deep) filled with 30 cm of water (25 ± 1°C). The session's duration was 15 min for the training and 5 min for the test. The test session was performed 24 h after training. Behavior was recorded with a video camera and the time spent immobile (making only those movements necessary to keep the snout above the water), swimming, climbing, or diving was quantified manually by an observer blind to the animal's experimental condition. Tests were performed simultaneously to all the animals in the cage by using three or four adjacent cylinders.

PLASMA CORTICOSTERONE AND GLUCOSE MEASUREMENT

On postnatal day 51 rats were placed individually in cylinders (25 cm diameter) for 30 min. Immediately after, rats were decapitated and their trunk blood collected in heparinized tubes. Plasma corticosterone and glucose levels were measured using an enzyme immunoassay kit (Correlate-EIA from Assay Designs Inc., USA) and (RTU BioMerieux ref *61269*, BioMerieux (Suisse SA) respectively.

STATISTICS

All data from the adolescent animals was analyzed using two-way ANOVA. When the sex × treatment interaction was significant, Student's *t*-tests were performed as *post hoc* analyses. ANCOVA analysis was employed to study the impact of the covariate "parental basal anxiety" (measured as time spent in the open arms of the EPM by either the mothers or the fathers before breeding took place) on the levels of corticosterone of their offspring during testing. PASW Statistics 18.0 statistical package was used for the analyses. Data represent the mean ± SEM. Significance of results was accepted at $p < 0.05$.

RESULTS

SPONTANEOUS LOCOMOTOR ACTIVITY (OPEN FIELD TEST)

First, we studied whether peri-pubertal stress affected the spontaneous locomotor activity of adolescent animals. Two days after the end of the stress period (i.e., 45 days of age) the basal locomotor activity and exploratory behavior of stressed and control animals were measured. Stressed animals showed significantly longer latency to reach the center of the arena [$F(1,29) = 6.553$, $p = 0.016$; **Figure 2A**] but did not differ from control animals in the time spent at the center of the arena [$F(1,29) = 1.049$, $p = 0.314$]. Additionally, two-way ANOVA revealed that, while there was not a treatment or gender effect, there was a significant treatment × sex interaction in the distance moved at the corners of the arena [$F(1,29) = 6.123$, $p = 0.019$]. *Post hoc* comparisons indicate that in the control group females walked longer distances [$t(13) = -2.89$, $p = 0.013$ (**Figure 2B**). No sex differences were observed in this test.

REACTIVITY TO NOVELTY (NOVEL OBJECT)

Next we examined whether peri-pubertal stress influenced the reactivity to explore a novel object. Stress during adolescence had a significant effect on the exploratory behavior of the animals. Stressed animals showed significant lower latency to approach the object [$F(1,29) = 5.75$, $p = 0.023$; **Figure 3A**), spent more time sniffing the object [$F(1,29) = 8.701$, $p = 0.006$; **Figure 3B**) and sniffed the object more times [$F(1,29) = 4.961$, $p = 0.034$; **Figure 3C**) than controls. No sex or treatment differences were found in any for these parameters. However, a significant treatment × sex interaction was observed in the number of times the animals reared on the object [$F(1,29) = 10.46$, $p = 0.003$]. *Post hoc* comparisons indicated that within the control group males reared more times on the object [$t(13) = 3.80$, $p = 0.002$] while with females peri-pubertal stress lead to a significant increase in the number of rearings on the object [$t(16) = -3.15$ (two-tailed), $p = 0.006$; **Figure 3D**). No sex differences were observed in this test.

ANXIETY-LIKE BEHAVIOR (EMP)

On postnatal day 46, animals underwent the EMP test to measure their anxiety-like levels. Two-way ANOVA revealed that stress during adolescence had a significant effect on anxiety-like behavior in the adolescent rat. Stressed animals: (a) spent more time in the open arms [$F(1,29) = 5.73$, $p = 0.023$; **Figure 4A**]; (b) entered more times the open arms [$F(1,29) = 6.81$, $p = 0.014$; **Figure 4B**] and center [$F(1,29) = 6.00$, $p = 0.021$]; (c) walked longer distances at the EPM [$F(1,29) = 7.69$, $p = 0.010$; **Figure 4C**]; and (d) had a faster mean velocity [$F(1,29) = 7.51$, $p = 0.010$; **Figure 4D**]. Moreover, stress during adolescence had also a significant effect on the behaviors displayed by the adolescent rats at the EPM. Stressed animals: (a) reared more times at the EPM [$F(1,29) = 13.53$, $p < 0.001$; **Figure 4F**] and (b) spent more time head-dipping at the EPM [$F(1,29) = 5.03$, $p = 0.033$; **Figure 4G**]. A two-way ANOVA also revealed a significant sex effect. Female rats: (a) entered more times into the open arms [$F(1,29) = 5.56$, $p = 0.025$; **Figure 4B**] and center [$F(1,29) = 4.89$, $p = 0.035$]; (b) spent more time rearing [$F(1,29) = 9.39$, $p = 0.005$; **Figure 4E**]; (c) reared more times at the EPM [$F(1,29) = 19.33$, $p < 0.000$; **Figure 4F**]; (d) spent more time head-dipping at the EPM [$F(1,29) = 6.80$, $p = 0.014$; **Figure 4G**]; and (e) performed head-dipping more frequently [$F(1,29) = 4.81$, $p = 0.036$; **Figure 4H**]. No treatment × sex interactions were found.

DEPRESSIVE-LIKE BEHAVIOR (FORCED-SWIM TEST)

On postnatal day 50 peri-pubertal stressed and control animals underwent forced-swim test (training took place 24 h before). Exposure to peri-pubertal stress did not affect swimming behavior as revealed by two-way ANOVA performed on the percentage of time spent floating [treatment effect, $F(1,29) = 2.14$, $p = 0.154$; treatment × sex interaction $F(1,29) = 0.445$, $p = 0.510$; **Figure 5A**]. There was a significant sex effect on the number

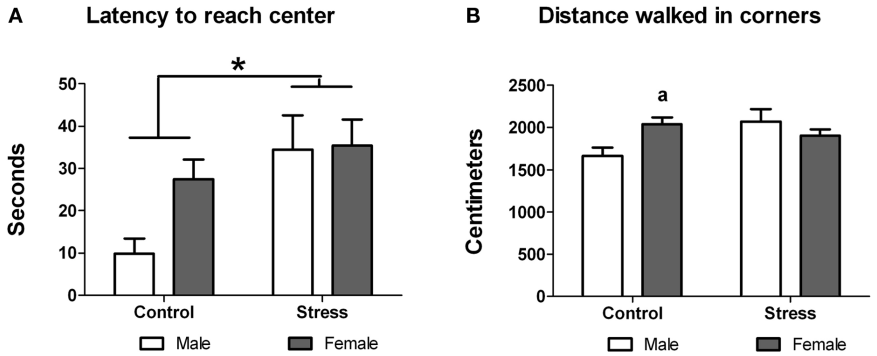

FIGURE 2 | Effect of peri-pubertal stress on behavior at the open field test. Mean (SEM) behavioral measures in the open field test for male and female rats that underwent either peri-pubertal stress (Stress) or no stress (Control). **(A)** Latency to reach center and **(B)** distance walked in corners. *$p < 0.05$ treatment effect; a $p < 0.05$ *post hoc* comparison between control male and females.

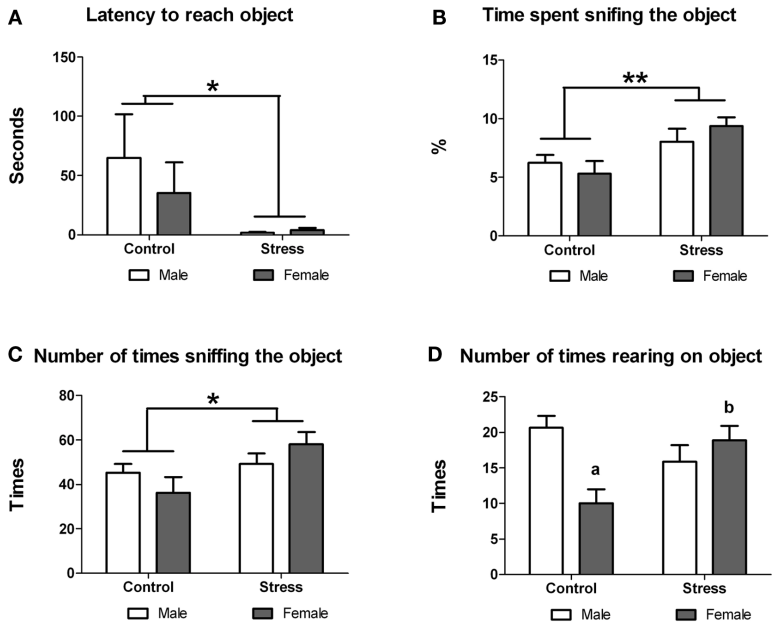

FIGURE 3 | Effect of peri-pubertal stress on behavior at the novel object test. Mean (SEM) behavioral measures of reactivity to novelty (novel object test) for male and female rats that underwent either peri-pubertal stress (Stress) or no stress (Control). **(A)** Latency to reach object, **(B)** time spent sniffing the object, **(C)** number of times sniffing the object, and **(D)** number of times rearing on the object. *$p < 0.05$ treatment effect; **$p < 0.01$ treatment effect; a $p < 0.01$ *post hoc* comparison between control male and females; b $p < 0.01$ *post hoc* comparison between stressed and control females.

of times the animals floated during the test [F (1,29) = 9.774, p = 0.004; **Figure 5B**], with females showing more floating episodes than males, though there was neither a treatment effect, nor a treatment × sex interaction.

LOW NOVELTY REACTIVITY UNDER BASAL AND STRESSFUL CONDITIONS (CHOCOPOP INTAKE)

In this test, performed on postnatal day 50, each rat was allowed 2 min to eat 10 chocopop pellets. Statistical analysis showed significant treatment effect with stressed animals eating significantly more chocopop pellets [F (1,29) = 7.57, p = 0.010; **Figure 6A**]. Next, we studied whether an additional acute stress (forced swim) affected chocopop intake during 2 min. For this purpose we measured the number of chocopops eaten by each animal immediately after the forced-swim test. Two-way ANOVA revealed that despite the acute stress, animals than underwent peri-pubertal stress ate significantly more chocopop pellets [F (1,29) = 7.95, p = 0.009; **Figure 6B**]. No sex differences or treatment × sex interaction were observed in both tests.

PLASMA GLUCOSE AND CORTICOSTERONE LEVELS

On postnatal day 51, animals were exposed to a novel environment for 30 min and immediate afterward blood samples were taken to assess plasma corticosterone and glucose levels. Two-way ANOVA analysis revealed that while peri-pubertal stress did not have any effect on glucose levels [F (1,29) = 0.29, p = 0.5935; **Figure 7A**], it induced a mild but not significant decrease in corticosterone levels [F (1,28) = 3.31, p = 0.080; **Figure 7B**]. No sex differences or treatment × sex interaction were observed in both tests.

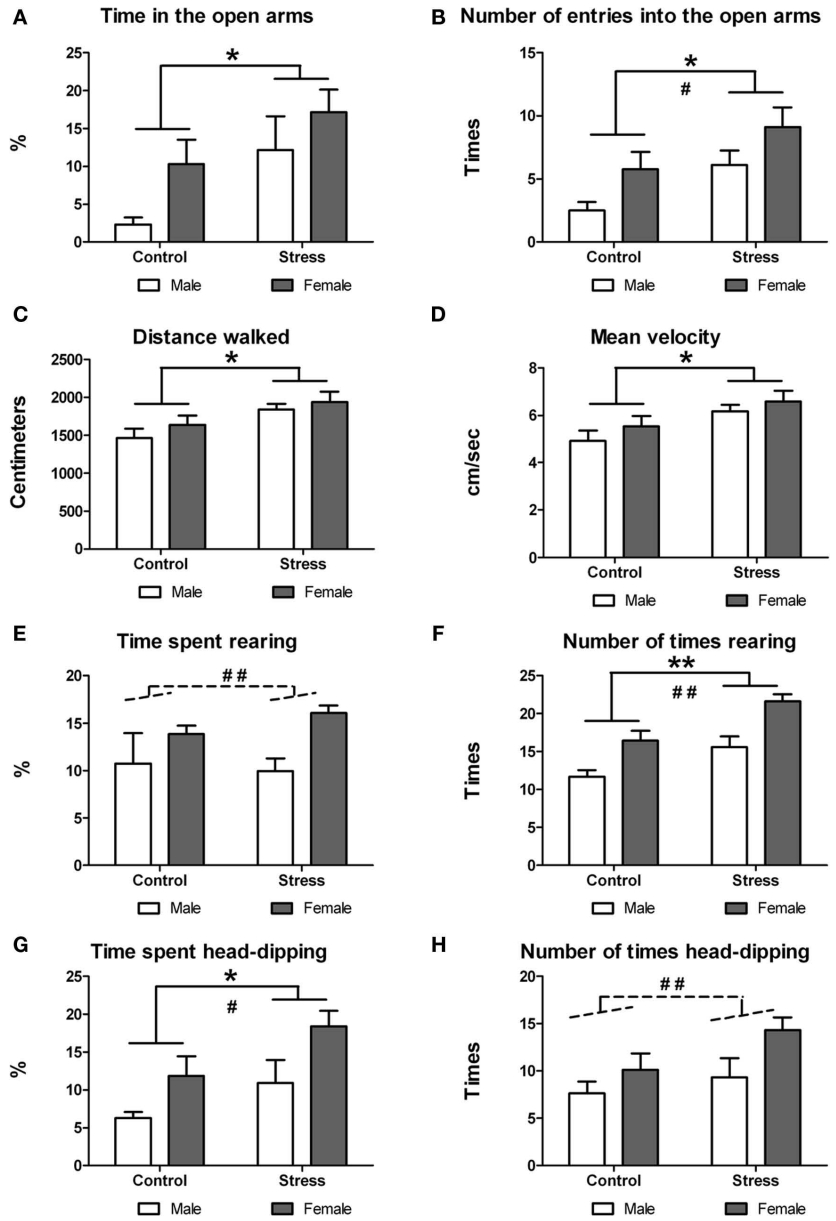

FIGURE 4 | Effect of peri-pubertal stress on behavior at the elevated plus maze (EPM) test. Mean (SEM) behavioral measures in the EPM test for male and female rats that underwent either peri-pubertal stress (Stress) or no stress (Control). **(A)** Time in the open arms, **(B)** number of entries into the open arms, **(C)** distance walked, **(D)** mean velocity, **(E)** time spent rearing, **(F)** number of times rearing, **(G)** time spent head-dipping, and **(H)** number of times head-dipping. $^{*}p < 0.05$ treatment effect; $^{**}p < 0.01$ treatment effect, $^{\#}p < 0.05$ sex effect, $^{\#\#}p < 0.01$ sex effect.

INFLUENCE OF PARENTAL ANXIETY ON ADOLESCENT PUPS BEHAVIOR AND REACTIVITY TO STRESS

Finally, we evaluated whether the behavior and reactivity to stress of the adolescent rats was influence by the parental anxiety-like traits. For this purpose, we repeated the previous analyses including the covariate "basal anxiety of either the mother of the father before breeding" (measured as the time spent in the open arms of the EPM; see Materials and Methods).

ANCOVA analysis revealed that the covariate "basal anxiety of the mothers before breeding" was significantly related only to the offspring's latency to approach the object [$F(1,28) = 4.85$, $p = 0.005$, $r = 0.38$] and blood corticosterone levels [$F(1,27) = 9.20$, $p = 0.005$, $r = 0.50$]. Interestingly, the effect of peri-pubertal stress on corticosterone levels became significant after controlling for the mothers' basal trait anxiety [$F(1,27) = 5.06$, $p = 0.033$]. Controlling for the mothers' basal trait anxiety did not change the significance of the effect of stress on latency to approach the object [$F(1,28) = 6.60$, $p = 0.016$].

The covariate "basal anxiety of the fathers before breeding" was marginally but not significantly related to the offspring's blood corticosterone levels [$F(1,27) = 3.45$, $p = 0.074$, $r = 0.37$] and significantly related to the offspring's' distance moved at the center and corners of the open field [$F(1,28) = 4.34$, $p = 0.046$, $r = 0.37$]

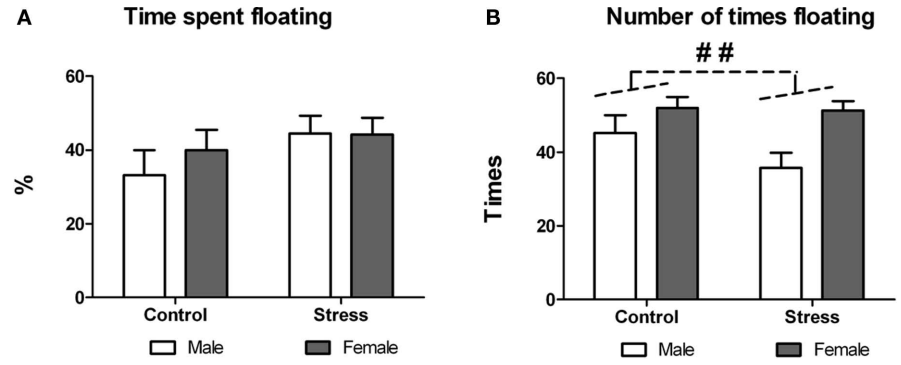

FIGURE 5 | Effect of peri-pubertal stress on behavior at the forced-swim test. Mean (SEM) behavioral measures in the forced-swim test for male and female rats that underwent either peri-pubertal stress (Stress) or no stress (Control). **(A)** Time spent floating and **(B)** number of times floating. $^{\#\#}p < 0.01$ sex effect.

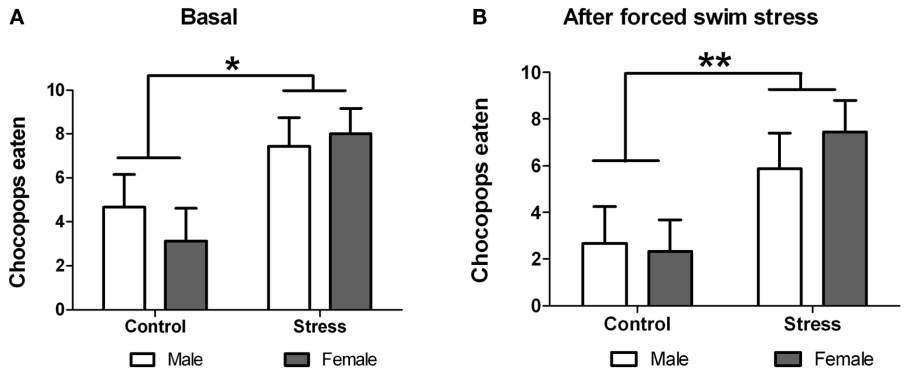

FIGURE 6 | Effect of peri-pubertal stress on behavior at the Chocopop pellet test. Mean (SEM) number of Chocopop pellets eaten by male and female rats that underwent either peri-pubertal stress (Stress) or no stress (Control) in their home cage **(A)** or immediately after stress **(B)**. $^{*}p < 0.05$ treatment effect; $^{**}p < 0.01$ treatment effect.

and [F (1,28) = 6.40, p = 0.017, r = 0.43] respectively. Controlling for the fathers' basal trait anxiety did not change the significance of stress on offspring behavior (i.e., non-significant effect for distance walked in the center [F (1,28) = 0.64, p = 0.429] and significant sex × treatment interaction of the distance walked in the corners [F (1,28) = 6.50, p = 0.017]).

DISCUSSION

The present study investigated the behavioral and hormonal effects of psychogenic stress (exposure to predator odor and placement on an elevated platform) during the peri-pubertal period in adolescent male and female rats. Our findings indicate that peri-pubertal stress reduces anxiety-like behaviors without affecting depressive-like behavior in late adolescence. Stressed animals exhibited increases in: (a) risk-taking behaviors (measured as time spent in the open arms of the EPM, time spent head-dipping, and number of entries to the EPM open arms) and (b) novelty seeking behaviors (measured as latency to reach a novel object, distance walked at the EPM, frequency, and time spent sniffing the object and number of chocopop pellets eaten under basal or stress conditions). Yet, stressed animals did not differ from controls in: (a) time spent floating during the forced-swim test or (b) blood corticosterone and glucose levels immediate after an acute stress.

At first glance our results may seem paradoxical when compared with previous studies reporting an increase of anxiety-like behaviors in rats exposed to stress during adolescence (Avital et al., 2006; Tsoory et al., 2006; Ilin and Richter-Levin, 2009). However, this contradiction can be explained by the fact that most these studies investigated behavior in adulthood. Indeed recent reports studying the effects of stress on behavior during adolescence show a decrease in anxiety-like and in risk-taking behaviors (i.e., increased time spent in the open arms of the EPM and increased locomotor activity in the open field and EPM; McCormick et al., 2008; Burke et al., 2010; Ito et al., 2010; Jacobson-Pick and Richter-Levin, 2010; Jacobson-Pick et al., 2011; Peleg-Raibstein and Feldon, 2011).

The increase in risk-taking behaviors in animals that underwent stress during adolescence seems to be restricted to the adolescent period. In fact, many of the above cited studies reported that the decrease in anxiety-like behaviors following adolescent stress disappeared when animals were tested in adulthood, both for males (Peleg-Raibstein and Feldon, 2011; Jacobson-Pick et al., 2011; and females, McCormick et al., 2008; Jacobson-Pick et al., 2011). Risk taking and novelty seeking behaviors emerge around puberty and are considered by many the hallmarks of adolescent behavior (Spear, 2000; Macri et al., 2002; Dahl, 2004; Kelley et al., 2004). Our results suggest that exposure to mild psychogenic stress

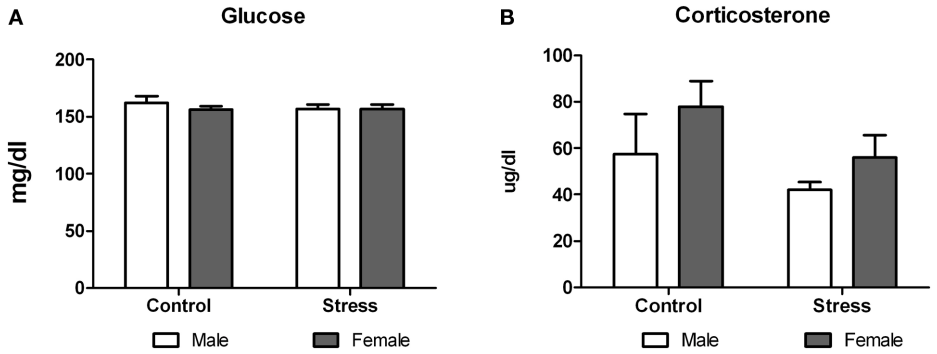

FIGURE 7 | Effect of peri-pubertal stress on blood glucose and corticosterone. Mean (SEM) plasma corticosterone **(A)** and glucose **(B)** concentrations immediately after being at a novel location (acute stress) for male and female rats that underwent either peri-pubertal stress (Stress) or no stress (Control).

before and during puberty exaggerates the independence-building behaviors naturally happening during this transition period (Macri et al., 2002).

What could be the implications of the increase in novelty seeking and risk-taking behaviors resulting from exposure to stress during adolescence? Early life stress has been hypothesized to lead to an increase in vulnerability to develop psychopathologies later in life (Kessler and Magee, 1993; Penza et al., 2003; Heim et al., 2004; Avital and Richter-Levin, 2005; Tsoory et al., 2006; Mathews et al., 2008b). Excessive novelty seeking and risk taking are characteristics of sensation-seeking behavior. Sensation-seeking behavior has been correlated with drug experimentation and recklessness (Laviola et al., 2003). Interestingly, chronic stress during adolescence was shown to enhance locomotor sensitization to amphetamine (Mathews et al., 2008a) or cocaine (Lepsch et al., 2005) in both male and female rats tested days after the stress period.

At this stage, it is not possible to indicate what is the predictive value of the stress-induced changes in behavioral reactivity for maladaptive behavior later in life. Further studies are required in order to determine how these alterations relate to behavioral responses in adulthood.

The use of males and female rats in our study enabled us to investigate whether the effects of peri-pubertal stress were sex specific. We found a significant interaction between treatment and sex factors in some parameters of the open field and novel object tests, but not in the standard variables from these tests (such as those related to visits to the center of the arena or to the exploration of the novel object). Interestingly, there was a significant sex difference in the behavior at the EPM with females showing increased risk-taking behaviors. So far there are only a handful of studies investigating the effects of stress during adolescence on behavior at the EPM in males and females. The results from these studies are not consistent. Exposure to chronic social stress during adolescence was shown to result in only females displaying higher risk-taking behaviors at the EPM, when tested in late adolescence (McCormick et al., 2008). Nevertheless, a recent study reports no sex differences in the behavior at the EPM of adolescent rats that did not undergo stress (Lynn and Brown, 2010), while other study reports only treatment effect on rats stressed and tested before puberty (Jacobson-Pick and Richter-Levin, 2010). Currently we cannot identify the source of such discrepancy on the effects of adolescent stress on males and females. Yet we can only speculate that it may be due to either the stage of sexual maturation of the animals during stress and test (Spear, 2000) or to differences in the nature of the stressors and/or stress protocols.

In addition, we found that the basal anxiety of the mothers was significantly related to the offspring's blood corticosterone levels after acute stress. Future experiments should address the molecular and/or behavioral mechanisms underlying this link between maternal anxiety and the offspring's HPA reactivity after adolescent stress.

One should be cautious when comparing the results of the present study with human adolescence, since brain maturation in rodents and humans follows different developmental time patterns. For example, while the rodent hippocampus continues to develop well into adolescence (Meyer et al., 1978), the human hippocampus is fully developed by 2 years old (Lupien et al., 2009).

It should be noted that a shortcoming in the present study is the fact that only one measure of the HPA axis and glucose responses to acute stress was performed, with basal levels not being included. Given that corticosterone (McCormick et al., 2005) and glucose (Bialik et al., 1988, 1989) show dynamic changes that can last up to 2 h post stress, a single datapoint only provides partial information on the HPA axis response.

Adolescence, and in particular the peri-pubertal period, is a critical developmental period when major changes take place in the brain (i.e., synapse pruning, fiber sprouting, and myelination; Giedd et al., 1999; Cunningham et al., 2002). It has been hypothesized that the different speeds in maturation of brain areas involved in emotional regulation and cognitive function may be partly responsible for the sharp increase in risk taking and novelty seeking observed during adolescence (Rosenberg and Lewis, 1995; Cunningham et al., 2002; Erickson and Lewis, 2002; Cruz et al., 2003). Due to incomplete maturation of the HPA negative-feedback system, adolescent rats show prolonged activation of the HPA axis when exposed to stress (Romeo et al., 2004), which might be implicated in the observed changes in curiosity and risk-taking behaviors observed in the stressed rats.

Juvenile stress has been reported to delay the maturational changes in expression of GABAa receptor subunits in the amygdala (Jacobson-Pick et al., 2008) and of the cell adhesion molecules PSA-NCAM (Tsoory et al., 2008) and L1 (Tsoory et al., 2010) in the basolateral amygdala and hippocampus. Future studies should address the cellular and molecular changes induced by the stress protocol used in our study and explore their potential impact on later life behaviors.

In conclusion, this set of experiments shows that exposure to stress during the peri-pubertal period results in an increase of novelty seeking and risk-taking behavior in adolescent male and female rats. These data suggest that peri-pubertal stress exacerbates the independence-building behavior characteristic of this transitional period. This behavioral change may reflect alterations in the plastic maturational changes undergone by brain regions regulating emotionality and stress responsiveness during this developmental stage.

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