Chronic mild stress–induced changes of risk assessment behaviors in mice are prevented by chronic treatment with fluoxetine but not diazepam

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1. Introduction

Psychosocial stress, an important factor related to the interactions between psychological development and social-environment, has pivotal impacts on emotion and therefore in turn on related behavioral outputs (Esch et al., 2002; Duman and Monteggia, 2006; Srivareerat et al., 2009).

As an important part of risk-related defensive behavior, risk assessment in rodents is sensitive to both acute and chronic psychosocial stress. For acute stress, previous research have revealed that various acute stressors, such as one-hour restraint, aggressive attack or foot shock, can disrupt risk assessment behaviors as reflected by short entry latencies to emerge from a safe compartment into a large, well-lit open field and reduced head poke responses (Quartermain et al., 1999). In addition, the stress-influenced risk assessment may impact subsequent action selection and behavioral output. Using a mouse decision making task, previous studies have suggested that four to eight weeks of chronic mild stress (CMS) improves decision making behaviors possibly through faster processing of environmental information and more efficient risk assessment (Pardon et al., 2000; Froger et al., 2004).

Stress-related cognitive impairments, emotional disorders and behavioral abnormalities may involve alterations of the neuroendocrine systems, such as hypothalamo–pituitary–adrenocortical (HPA) axis, or etc.
neurotransmitter systems involving serotonin, adrenergic or GABA (Gold and Chrousos, 2002; Sapolsky, 2000; Graeff et al., 1996; Tsigos and Chrousos, 2002; Froger et al., 2004; Stone et al., 1996; Blanchard et al., 1990; Rodgers et al., 1999; Mikics et al., 2005). However, for chronic stress, although previous studies using EPM or decision making task have speculated the links between chronic stress and risk assessment behavior, there is still no direct experimental evidence for this correlation nor is there any explanation for the underlying neuroendocrinal and neurochemical mechanisms.

Moreover, most of the previous research results were obtained from conventional spatiotemporal measures, while specific ethological measures, including stretched attend postures (SAPs) and head dippings (HDs), were rarely used or yielded inconsistent behavioral outputs. For example, 21 days of repeated predatory stress decreased frequency of unprotected HDs but failed to change the frequency of SAPs in the EPM (Calvo-Torrent et al., 1999). Therefore, the present study was conducted to clarify the possible correlations between risk assessment and decision making behavior using a battery of mouse-specific tests, including EPM task, light/dark transition (LDT) task and open field (OF) task. In addition, we wished to clarify the possible neuroendocrinal and neurochemical basis of CMS-altered risk assessment behavior. Plasma corticosterone concentrations together with the levels of serotonin (5-hydroxytryptamine, 5-HT) and GABA in frontal cortex and hippocampus were evaluated after CMS. Pharmacological interventions with fluoxetine (one selective serotonin reuptake inhibitor) or diazepam (one GABA-A receptor modulator) were also performed.

2. Materials and methods

2.1. Animals and treatment

Adult male Swiss albino mice (20 ± 2 g body weight, 8–10 weeks old) were obtained from the Experimental Animal Center of Dalian Medical University. The mice were housed in groups under standard conditions (12 h light/dark cycle; lights on from 20:00 to 08:00 h; 22 ± 2 °C ambient temperature; 55 ± 10% relative humidity), fed with standard diet and water ad libitum and were allowed to acclimate seven days before testing. The mice were randomized into eight groups with 12 mice per group and housed 2 per cage during the paired housing stress session and 6 per cage during the remaining stress sessions. Fluoxetine (4–20 mg/kg), diazepam (0.25–4 mg/kg) or vehicle were intraperitoneally (i.p.) administered in a volume of 10 ml/kg twice daily for 4 weeks, from day 15 to day 42. The doses of fluoxetine and diazepam in this study were selected as referred to previous studies (David et al., 2009; Rygula et al., 2006; Mongeau et al., 2007; Li et al., 2006a, b). The experiment procedures involving animals and their care were conducted in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Experimental schedule

The experimental design and procedures are summarized in Fig. 1. The experiment consisted of three phases and altogether lasted for 45 days. The acclimating phase, during which the animals were adapted to laboratory and housing conditions, lasted 1 week. The animals were then subjected to CMS during the stress phase (weeks 2–6, days 8–42), together with twice daily injections of fluoxetine, diazepam or vehicle for four weeks (from week 3 to week 6). Control animals were normally housed in their cages without CMS disturbance throughout the entire experiment. In the final test phase, a battery of mouse-specific tests, consisting of EPM, LDT and OF, was subjected to animals of all groups. An additional control group of mice were normally housed in their cages without CMS disturbance throughout the entire experiment.

**Fig. 1.** Experimental design and procedures. Animals were divided into eight groups of 12 animals each. The experiment consisted of three consecutive sessions and altogether lasted for 44 days. The acclimating session lasted for one week during which the animals were allowed to acclimate to laboratory and housing condition. During the stress session (weeks 2–6, days 8–42), the animals of CMS group, CMS + fluoxetine group and CMS + diazepam group were submitted to CMS procedure. Vehicle or drugs of various concentrations were administered twice daily for 4 weeks (weeks 3–6) while the animals remained in the daily CMS situation. Non-stressed control animals were normally housed in their cages without disturbance throughout the entire experiment.
2.3. Chronic mild stress procedure

The CMS procedure was performed as described in our previous studies (Li et al., 2006a,b, 2007, 2008). This procedure consisted of a variety of unpredictable mild environmental and social stressors, including 2 h of paired caging, 3 h of tilted cage (45°), 18 h of food and water deprivation, 1 h of restricted access to food, 1 h of exposure to an empty bottle, 21 h of housing in a wet cage (100 ml water in 100 g sawdust bedding) and 36 h of continuous light. These stressors were randomly scheduled over a one-week period and repeated throughout the 5-week stress session. In contrast to other previous procedures using rats (Pardon et al., 2000), noxious stressors were excluded.

2.4. Determination of plasma corticosterone and brain 5-HT, 5-HIAA and GABA concentrations

Trunk blood was quickly collected after sacrifice by decapitation, centrifuged and plasma samples were stored in aliquots at −80 °C. Plasma corticosterone levels from individual mice were determined by using a commercially available corticosterone assay ELISA kit (Abcam, Cambridge, UK). The corticosterone concentrations were calculated from a standard curve and expressed in ng/ml. Immediately after sacrifice, brains were quickly removed and frontal cortex and hippocampus were dissected on ice and weighed. The preparation of homogenates and neurochemical determinations were performed using HPLC with electrochemical detection as described previously (Naudon et al., 1995; Grønli et al., 1997).

2.5. Elevated plus maze task (EPM)

EPM consisted of two open arms (30 × 5 cm) and two closed arms (30 × 5 cm, surrounded by 15 cm-high walls), with the two pairs of identical arms emerging from a central platform (5 × 5 cm) positioned opposite each other. The apparatus was elevated 45 cm above the floor and was lit by four red-light lamps (60 W) placed above each arm. The animals were routinely tested during the dark phase of the light/dark cycle. The test was initiated by placing the mouse on the central platform of the maze, facing one of the open arms, and letting it explore the maze freely for 5 min. Mouse behavior was continuously videotaped by a video camera placed above the apparatus. Videotapes were analyzed offline by an observer who was unaware of the prior treatment of the animals. The maze was carefully cleaned with alcohol and rinsed with water after each test.

For EPM, behavioral parameters comprised both conventional spatiotemporal measures and ethological measures for risk-assessing. Conventional measures were the frequencies of total arms entries (arm entry = all four paws into an arm), ratio of open entries [(open / total) × 100], total duration and ratio of time spent in open arms [(time in open arms / session duration) × 100]. Ethological measures for risk-assessment comprised the frequency scores for HDs (exploratory movement in which the animal’s head / shoulder is protruding over the edge of the close arm, open arm or central area and down towards the floor) and SAPs (exploratory posture in which the body is stretched forward then retracted to the original position without any forward locomotion) (Rodgers et al., 1999; Mikics et al., 2005; Carola et al., 2002).

2.6. Light/dark transition task (LDT)

The light/dark box (45 × 27 × 27 cm) consisted of two chambers connected by an opening (7.5 × 7.5 cm) located at floor level in the center of the dividing wall. The floor was divided into 9 cm × 9 cm squares and was covered with Plexiglas. The smaller chamber (18 × 27 cm) was painted black and had the top covered with thick transparent Plexiglas. The larger chamber (27 × 27 cm) was painted white and was open-topped. Bright illumination of the white chamber was provided by a 60-W table lamp which was located 40 cm above the center of the chamber. Mice were placed in the center of the white chamber facing the opening and were allowed to explore the apparatus for 5 min. The following behaviors were scored: line crossings, number of transitions between the light and dark chambers, SAPs, and the time spent in each section of the apparatus.

2.7. Open field task (OF)

Twenty-four hours after the LDT task, the open field test was performed. The open field consisted of a base (100 × 100 cm) divided into 25 (5 × 5) identical sectors (20 × 20 cm) by white stripes. The squares were subdivided into peripheral and central sector, where the central sector included the 9 central squares (3 × 3) and the peripheral sector contained the squares close to the surrounded wall (20 cm high). The arena was lit by two red-light lamps (60 W) placed over its center. The animals were placed in the central sector and their activity was video-recorded for 10 min for further analysis. No stressor was applied to the animals for 24 h before the test. Motility was scored when an animal crossed a sector border with both its hind-limbs. The following behavioral parameters were scored: the total frequency of squares crossed, ratio of central squares crossed frequency [(central / total) × 100], ratio of central area duration [(central / total) × 100], and frequencies of SAPs. The open field arena was thoroughly cleaned between each test.

2.8. Statistical analysis

For statistical analysis, SPSS 16.0 (SPSS Inc., Chicago) was used. All data were expressed as mean ± S.E.M. and were analyzed by ANOVA. Post hoc comparisons were realized with Tukey’s HSD tests. Comparisons between treatment groups and control were carried out using Dunnett’s t-test.

3. Results

3.1. Plasma corticosterone

After a 5-week CMS exposure, plasma corticosterone levels of mice were significantly higher in the CMS group than in the non-stressed control group [F(1,22) = 20.89, P = 0.000, Fig. 2]. The corticosterone response to CMS exposure was ameliorated by 4 weeks treatment with fluoxetine [F(3,44) = 5.009, P = 0.004]. Meanwhile, chronic treatment with diazepam had no effects on CMS-induced corticosterone response [F(3,44) = 0.21, P = 0.889, Fig. 2].

![Fig. 2. Impacts of CMS exposure on plasma corticosterone concentration. Plasma corticosterone levels from individual mice were determined by ELISA and expressed in ng/ml. *P < 0.05, compared with non-stressed control; #P < 0.05, compared with CMS group.](image-url)
3.2. Neurochemical analysis for 5-HT, 5-HIAA and GABA

In the present study, we measured the levels of 5-HT, 5-HIAA and GABA in frontal cortex and hippocampus, two major brain regions related to emotion and cognition. As shown in Table 1, compared with non-stressed control animals, the CMS-treated mice showed significant decreases in 5-HT, 5-HIAA and GABA levels in frontal cortex and hippocampus. In addition, long-term therapy with fluoxetine reversed the CMS-induced neurochemical changes of 5-HT and GABA, whereas chronic treatment with diazepam only reversed the CMS-induced alteration of GABA without impacting 5-HT level (Table 1). Neither fluoxetine nor diazepam altered 5-HIAA levels.

3.3. Behavioral analysis of EPM task

3.3.1. CMS-induced alterations of conventional spatiotemporal measures

As shown in Figs. 3A and C, CMS exposure had no significant effects on closed arms entries and durations when compare to control group, which indicated no significant alteration in locomotor activity after CMS exposure. Meanwhile, consistent with previous research reports, the CMS-treated mice showed an elevated anxiety level induced by decreased frequency of open arms entries (F(1,22) = 7.95, p = 0.01, Fig. 3B) and decreased durations of open arms exploring (F(1,22) = 21.43, p = 0.000, Fig. 3D). These stress-induced alterations of conventional spatiotemporal measures were ameliorated by chronic treatment with fluoxetine and diazepam, in a dose-dependent manner (F(3,44) = 7.73, p = 0.000 for fluoxetine; F(3,44) = 10.29, p = 0.000 for diazepam, Fig. 3D).

3.3.2. CMS-induced changes of risk-assessing behaviors

As shown in Figs. 4 and 5, CMS exposure produced a marked elevation in risk assessment behaviors. Saline-treated CMS mice had significantly higher behavioral scores of SAPs in closed arms (F(1,22) = 21.80, p = 0.000) and HDs (F(1,22) = 36.80, p = 0.000 in open arms; F(1,22) = 8.163, p = 0.009 in closed arms) than control unstressed animals. Moreover, while fluoxetine ameliorated the effects of CMS on SAPs and HDs, diazepam had no impacts on these CMS-affected risk assessment behaviors.

3.3.3. Correlations between risk assessment and action choice

For non-stressed control animals, the Pearson correlation analysis further indicated a close correlation between SAPs and frequency of arm entries (negative for open arm entries and positive for close arm entries, Figs. 6A and 7A). For CMS stressed animals, however, 5 weeks of CMS exposure not only increased risk assessment behaviors, but also abolished the correlations between risk assessment and action selection (Figs. 6B and 7B). The CMS-disturbed correlations between risk assessment and subsequent decision making were restored by four-weeks of chronic treatment with fluoxetine (Figs. 6C and 7C) but not diazepam (Figs. 6D and 7D).

3.4. Behavioral analysis of LDT task

3.4.1. CMS-induced alterations of conventional measures

Compared with non-stressed control mice, CMS-stressed mice spent less time in the light box (Fig. 8D) and had an increase in the number of transitions between the light and dark chambers (Fig. 8B), which indicated a higher anxiety level. In addition, CMS exposure had no effects on locomotion as indicated by the number of line crossings (Fig. 8A). These anxiety-related behaviors were ameliorated under repeated fluoxetine and diazepam treatment.

3.4.2. CMS-induced changes of risk-assessing behaviors

Similar to behavioral performance in EPM, CMS increased risk assessment behavior in LDT task. The saline-treated CMS-stressed mice had significantly higher counts of SAPs (forward elongation of head and shoulders towards the opening door, followed by retraction to original position) than that of control animals (F(1,22) = 5.92, p = 0.024; Fig. 8C). While fluoxetine ameliorated the effects of CMS on SAPs, diazepam had no significant impact.

3.4.3. Correlations between risk assessment and decision making

In LDT task, the statistical analysis indicated that SAPs in non-stressed control mice were positively correlated with number of transition between the two boxes (Fig. 9A) and negatively correlated with time spent in the light chamber (Fig. 10A). For CMS treated animals, the counts of SAPs were increased, and the correlations between risk assessment and action selection were abolished (Figs. 9B and 10B). Four-weeks of chronic treatment with the selective serotonin reuptake inhibitor fluoxetine not only reversed the CMS-affected risk-assessment behaviors, but also restored the CMS-impaired correlations between risk assessment and action selection (Figs. 9C and 10C).

3.5. Behavioral analysis of OF task

3.5.1. CMS-induced alterations of conventional behavior measures

While a 5-week period of CMS did not alter total locomotor activity (Fig. 11A), CMS elicited as significant effect on anxiety-like behavior as determined by the OF task. Mice treated with 5-week CMS exhibited significantly lower ratios of central squares crossed and central area duration than non-stressed controls (P < 0.05, Fig. 11B and C). In addition, repeated treatment with fluoxetine and diazepam showed potent anxiolytic-like effects. Mice treated with fluoxetine (10–20 mg/kg) or diazepam (1–4 mg/kg) made more central area entries and showed a trend for longer duration in central areas than CMS-treated control animals (P < 0.05), while total locomotor activity remained unaffected by all doses of drugs.

3.5.2. CMS-induced changes of risk-assessing behavior

Similar to EPM and LDT task, CMS-stressed mice showed a significant elevation of risk assessment behavior in comparison to control animals, as indicated by increased SAP counts in both central and peripheral areas of open field (P < 0.05). In addition, 4-weeks repeated treatment with fluoxetine dose-dependently ameliorated the CMS-elicited increased SAPs behavior, while diazepam had no such effect (Fig. 12).

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Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Brain regions</th>
<th>Neurochemicals (ng/mg)</th>
<th>5-HT</th>
<th>5-HIAA</th>
<th>GABA</th>
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<tr>
<td>Control</td>
<td>FC</td>
<td>620.8 ± 22.3</td>
<td>270.1 ± 16.9</td>
<td>30.1 ± 1.8</td>
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<tr>
<td>CMS</td>
<td>FC</td>
<td>500.2 ± 31.7</td>
<td>194.6 ± 11.6</td>
<td>17.8 ± 1.4</td>
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<tr>
<td></td>
<td>Hip</td>
<td>256 ± 15.4</td>
<td>301.3 ± 16.2</td>
<td>1.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>CMS + Flu 5.0 mg/kg</td>
<td>Hip</td>
<td>418.7 ± 17.2</td>
<td>201.6 ± 12.1</td>
<td>18.8 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>CMS + Flu 10.0 mg/kg</td>
<td>FC</td>
<td>264.5 ± 18.9</td>
<td>309.3 ± 15.7</td>
<td>1.8 ± 0.3</td>
<td></td>
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<tr>
<td></td>
<td>FC</td>
<td>467.9 ± 26.3</td>
<td>221.4 ± 19.6</td>
<td>22.9 ± 0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hip</td>
<td>357.6 ± 22.1</td>
<td>425.9 ± 21.7</td>
<td>2.1 ± 0.2</td>
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<tr>
<td>CMS + Dia 0.25 mg/kg</td>
<td>FC</td>
<td>523.8 ± 26.2</td>
<td>254.9 ± 12.5</td>
<td>25.6 ± 1.2</td>
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<tr>
<td>CMS + Dia 1.0 mg/kg</td>
<td>Hip</td>
<td>435.5 ± 19.6</td>
<td>539.7 ± 15.8</td>
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<td>CMS + Dia 4.0 mg/kg</td>
<td>FC</td>
<td>417.2 ± 16.4</td>
<td>200.8 ± 15.1</td>
<td>2.5 ± 1.0</td>
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</tbody>
</table>

* p < 0.05, compared with non-stressed control.

** p < 0.05, compared with CMS-stressed group.
3.5.3. Correlations between risk assessment and decision making

In contrast to EPM and LDT, behavioral data of OFT presented no significant correlations between risk assessment and behavioral output. In unstressed control and CMS treated mice, SAPs and enter quarters or durations in both central (Fig. 13A, B) and peripheral areas were not significantly correlated (Fig. 13E, F). Peripheral SAPs and central area duration were also not significantly correlated, even after determining average SAP counts per min (Fig. 13I–L).

![Fig. 3. Conventional spatiotemporal measures of mice behaviors in elevated plus maze. A: frequencies of closed arms entries; B: ratio of open arm entries; C: duration in closed arms; D: ratio of time spent in open arms. *P < 0.05, compared with non-stressed control; #P < 0.05, compared with CMS group.]

![Fig. 4. Ethological measures for risk-assessment behaviors of mice in elevated plus maze. A: scores of stretched attend postures in open arms; B: scores of stretched attend postures in closed arms. *P < 0.05, compared with non-stressed control; #P < 0.05, compared with CMS group.]
4. Discussion

4.1. CMS-induced changes of conventional measures and risk assessment behaviors

Risk assessment has been proved to faithfully represent the approach/avoidance conflict and decision making behaviors under aversive or stressed situations. Detailed ethological measurements have been applied to evaluate risk assessment in standard animal models. For instance, some studies have demonstrated the usefulness of risk assessment behaviors such as SAPs and HDs in the EPM task (Rodgers et al., 1999; Mikics et al., 2005; Griebel et al., 2000; Carola et al., 2002), SAPs and return behaviors in the OF task (Mikics et al., 2005; Carola et al., 2002; Choleris et al., 2001) or SAPs, freezing and avoidance behaviors using rat-exposure test (Blanchard et al., 2003b; Yang et al., 2004). These specific ethological measures for risk assessment may provide some preliminary evidence for possible links between psychosocial stress, emotion reactivity and risk assessing behaviors.

In agreement with previous research reports, in the present study, behavioral analysis of conventional spatiotemporal parameters recorded in EPM, LDT or OF led to the conclusion that CMS-stressed animals displayed an elevated anxiety level compared with non-stressed controls. For the EPM task, the CMS-stressed animals avoided open

![Figure 5](image)

**Fig. 5.** Ethological measures for risk-assessment behaviors in elevated plus maze. A: scores of head dipping in open arms; B: scores of head dipping in closed arms. *P < 0.05, compared with non-stressed control; #P < 0.05, compared with CMS group.

![Figure 6](image)

**Fig. 6.** Correlation analysis for SAPs and frequency of open arm entries in elevated plus maze. A: non-stressed control group; B: CMS-stressed group; C: fluoxetine treatment (20 mg/kg); D: diazepam treatment (4 mg/kg).
arms exploration and spent more time in closed arms. Similarly, the stressed mice had a decreased time spent in the light box and an increase in the number of transitions in LDT task, as well as a lower ratio of central area entries and preferred to stay in the periphery in OF task.

In addition, specific ethological measures of risk-assessing revealed that CMS caused an elevation of risk assessment behaviors. The CMS-exposed mice displayed more frequencies of SAPs and/or HDs in EPM, LDT and OF tasks, which indicated that the stressed mice spent more time in assessment of the potential risks. The concurrence of elevated risk-assessment behavior and repeated risk-approach/avoidance behaviors might indicate a disrupted environment information processing and a higher hesitation level. These behavioral findings were contradictory with previous observations of McCormick et al. (2008) and Calvo-Torrent et al. (1999). The study by McCormick and colleagues has demonstrated that 15-days of adolescent social stress may cause female rats to spend less time in risk assessment/exploration (time in center of maze) than controls. Calvo-Torrent et al. have also reported that 21-days repeated predator-exposure induces a significant decrease in unprotected HDs behavior but has no impacts on protected HDs and SAPs. Our present results are also inconsistent with the observations that chronic stress facilitates “choice” behavior with an absence of “no choice” behavior, as well as facilitates a more rapid capacity to process information and with a lower level of hesitation (Pardon et al., 2000). These inconsistencies between our present and previous studies might be due to different animal genders, different stress intensities, different animal models and accordingly different ethological parameters. In the study of McCormick et al., the researchers used time in center of maze as index for risk assessment and/or exploration. Pardon’s conclusion has been drawn from the behavioral observation and analysis of choice behaviors using a decision making task, while the present study measured ethological indices of risk-assessment which could reflect directly the risk-assessing behavioral response to CMS.

4.2. CMS abolished correlations between risk assessment and decision making

Risk as feelings refers to individuals’ fast, instinctive, and intuitive reactions to danger. Risk as analysis brings logic, reason, and scientific deliberation to bear on risk management. Decision-making is closely related to risk assessment, which consists in gathering information on the environment and its potential dangers, and involving varying degrees of caution when doing so. In the present study, the EPM and LDT tasks appear to be of particular help in evaluating correlations between risk assessment and decision-making, as well as the potential impacts of CMS. For non-stressed control animals, the risk assessment behaviors were closely related with their action selection. For instance, the control mice showed a negative correlation between SAP counts and number of open arm entries in EPM task, and a negative correlation between SAP counts and number of light/dark transitions in LDT task. Although analysis is certainly important in some decision-making circumstances, reliance on affect and emotion is a quicker, easier, and more efficient way to navigate in a complex, uncertain, and sometimes dangerous situation. Many theorists and models have given affect a direct and primary role in motivating behavior (Slovic et al., 2005). Alhakami and Slovic have found that the inverse relation between perceived risk and perceived benefit of an activity was linked to the strength of positive or negative affect associated with that activity as measured by rating the activity on bipolar scales such as good/bad, nice/awful, dread/not dread, and so forth. This implies that people base their judgments of an activity not only on what they think about it but also on how they feel about it. A positive affect may lead to judging the risks as low and the benefits as high, while a negative affect may cause the opposite judgment—high risk and low benefit. Researchers have called this process the affect heuristic. In present study, for CMS-stressed animals, the negative correlations between risk assessment and decision-making were diminished.
between risk assessment behaviors and action selection could be abolished by CMS exposure. The separation of risk assessment and decision making might be due to the CMS-induced affective disorders, such as depression or anxiety (Lazarus, 2006; Duman and Monteggia, 2006).

Another possible explanation for CMS-induced hyper risk assessing tendency is a bi-directional response to repeated threatening stimuli (Blanchard et al., 2003a). Previous research reports have indicated that rodents may present different risk assessment behavioral responses to different stressors with various intensities (Blanchard et al., 2003b; Yang et al., 2004). At the latter phase of CMS, the animals were familiar to the stressors and for them the threatening intensity was decreased, threat ambiguity increased and the animals began to present a hyper-risk assessing tendency to explore the possible threatening stimuli. In addition, after a long period of exploring with hyper-risk assessment level in a genuinely nonthreatening situation, animals gradually reduce their levels of risk assessment and return to normal activities (Blanchard et al., 2003a). This kind of dynamic behavioral performance is compatible with findings that risk assessment is associated with gathering of information about the threat source as well as analyses of the role of these activities in maintaining or reducing defensiveness, in accord with the information that these activities provide concerning threat or danger (Blanchard et al., 2003a).

4.3. Neurochemical basis of CMS-altered risk-assessment

GABA is implicated in fear and anxiety, on the evidence of both pharmacological (Sanders et al., 1995; File et al., 1998) and gene-targeting (Rudolph et al., 1999; McKernan et al., 2000; Löw et al., 2000) experiments. Besides GABA neurotransmission system, the neurotransmitter serotonin is also involved in psychosocial stress and stress-related emotional/behavioral disorders (D’Aquila et al., 1994; Deakin and Graeff, 1991; Graeff et al., 1996). As was expected, in the present study, 5-weeks of CMS caused decreases in 5-HT and GABA concentrations in frontal cortex and hippocampus. These neurochemical changes might suggest a possible involvement of GABA (Adell et al., 1988; Julio-Pieper et al., 2012) and 5-HT (Finlay et al., 1995; Otero Losada, 1988) neurotransmission in the behavioral and mood alterations induced by CMS.

In addition, both GABA and serotonin have been shown to impact risk assessment behaviors. For instance, in rat elevated plus maze, diazepam and 5-HT1A receptor antagonists have been shown to modify the number of SAPs (Griebel et al., 2000). The 5-HT1A receptor partial agonist buspirone and 5-HT2A/2C receptor antagonists increase the counts of HDs. The serotonin reuptake inhibitor zimelidine reduced HDs and total entries in the EPM task (Griebel et al., 1997). For GABA, previous research has also demonstrated that GABA receptor modulator diazepam changes risk assessment behaviors in an anxiety/defense test battery (Blanchard et al., 1990). In the present study, the CMS-induced changes of specific ethological measures for risk assessment were accompanied by neurochemical responses to CMS, including decreased 5-HT, 5-HIAA and GABA concentrations in frontal cortex and hippocampus. Moreover, selective serotonin reuptake inhibitor fluoxetine reversed the CMS-induced alterations of anxiety and risk assessment behaviors and ameliorated the CMS-caused changes of 5-HT, 5-HIAA and GABA concentration levels.
Fig. 9. Correlation analysis for SAPs and frequency of light/dark transitions in LDT task. A: non-stressed control group; B: CMS-stressed group; C: fluoxetine treatment (20 mg/kg); D: diazepam treatment (4 mg/kg).

Fig. 10. Correlation analysis for SAPs and time spent in the light chamber in LDT task. A: non-stressed control group; B: CMS-stressed group; C: fluoxetine treatment (20 mg/kg); D: diazepam treatment (4 mg/kg).
GABA concentrations. Compared with fluoxetine, GABA-A receptor modulator diazepam ameliorated the CMS-induced changes of GABA concentration in frontal cortex and hippocampus but had no effects on 5-HT and 5-HIAA concentrations. Previous studies have suggested inconsistent effects for diazepam treatment on GABA concentrations under non-stressed conditions. While an acute or single dose of diazepam treatment facilitates GABA release, repeated diazepam treatment may induce a drug-tolerance and prolonged decline of GABA concentrations in brain (Gonsalves and Gallager, 1987; Heninger and Gallager, 1988; van Rijnsoever et al., 2004). The detailed mechanism for reversal effects of chronic diazepam treatment on CMS-induced decline of GABA concentration in the present study was still under investigation. In addition, accompanied by these neurochemical changes, diazepam ameliorated CMS-induced conventional spatiotemporal activities but failed to reverse the CMS-affected risk assessing behaviors. All these behavioral data and neurochemical analysis indicated that CMS-induced emotional and behavioral changes, including anxiety level and risk assessment, may be mediated by different neurotransmission systems with different sensitivities to different pharmacological treatments. While both GABA and 5-HT neurotransmissions appear to be involved in CMS-induced ethological behavioral changes, the 5-HT system might be much more involved in CMS-induced risk assessment changes than GABA.

4.4. Neuroendocrinal basis of CMS-induced risk-assessment alteration

Previous research indicates that over-activated HPA axis function might be involved in stress-induced behavioral alterations of risk assessment (Rodgers et al., 1999; Mikics et al., 2005). In the present study, behavioral changes of risk assessment in CMS-stressed mice were accompanied by a significantly higher level of neuroendocrine...
response to stress. The elevated plasma corticosterone concentration in stressed animals might contribute to the CMS-altered risk assessment. In addition, the elevated plasma corticosterone concentration together with the CMS-altered risk assessment behaviors was blocked by fluoxetine, one selective serotonin reuptake inhibitor. In contrast, consistent with previous research report (Barlow et al., 1979), GABA receptor modulator diazepam had no impacts on CMS-elicited corticosterone levels and failed to reverse CMS-altered risk assessment behaviors.

5. Conclusion

In the present study, the results of neuroendocrine and neurochemical analysis as well as behavioral measurements extend the usefulness of risk assessment for a better understanding of emotional reactivity and decision making under stressful situations. The present findings also suggest possible roles of serotonergic neurotransmission on CMS-induced emotional and neuroendocrine responses and CMS-affected risk assessment and decision making.

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Fig. 13. Correlation analysis for SAPs and durations/entries in OFT. A, E, I: non-stressed control group; B, F, J: CMS-stressed group; C, G, K: fluoxetine treatment (20 mg/kg); D, H, L: diazepam treatment (4 mg/kg).
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