Behavioural Processes 83 (2010) 331–339

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

Coexistence of Anhedonia and anxiety-independent increased novelty-seeking behavior in the chronic mild stress model of depression

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ARTICLE INFO

Article history:
Received 19 July 2009
Received in revised form
18 December 2009
Accepted 25 January 2010

Keywords:
Anhedonia
Anxiety
Chronic mild stress
Depression
Novelty-seeking

ABSTRACT

Previous research demonstrated excessive decreases in reward sensitivity and increases in harm avoidance in depressed individuals. These results straightly lead to a hypothesis that depressed patients should avoid novelty or express reduced novelty-seeking behavior. Nevertheless, literature in this regard is inconsistent. Furthermore, whether the potentially altered novelty-associated behavior is dependent on changed anxiety/fear or related to altered goal-directed approaching tendency is unclear. Here, we tested novel object-approaching behavior in a free-exploration paradigm in chronic mild stress (CMS)-induced anhedonic and stress-resistant rats respectively. Other CMS-induced, emotional behaviors were also examined in a battery of behavioral tests including novel cage, exploration, locomotor activity and elevated plus maze (EPM). We found that compared with controls, stress-resistant rats who consistently showed lower anxiety level in EPM (time in open arms) and, open-field (OF) test (time in central area) showed no sign of enhanced novel object approaching behavior. To the contrary, the anhedonic ones who did not express any sign of reduced anxiety showed paradoxically intensified novelty-approaching behavior. We concluded that reduced anxiety would not necessarily lead to enhanced novelty-seeking behavior; anhedonia coexists with anxiety-independent, increased novelty-seeking behavior. The salient paradox of coexistence of anhedonia and increased novelty-seeking behavior was critically discussed.

1. Introduction

Anhedonia, one of the core symptoms of major depression, is defined as "markedly diminish interest or lack of reactivity to pleasurable stimuli" (American Psychiatric and Association, 1994). Preclinical studies, which were targeted at modeling anhedonia, also demonstrated a generalized decrease in sensitivity to reward (to review, see Willner, 2005). For example, chronic mild stress model (CMS), which is a highly validated animal model of depression, resulted in a robust decline of sucrose preference/intake. Besides, CMS abolished or attenuated the conditioned place preference effect induced by sweet solution, palatable food (Papp et al., 1991), low-dose of amphetamine (Papp et al., 1991) and morphine (Valverde et al., 1997). CMS also caused an increase in the threshold current required to support ventral tegmentum self-stimulation (Moreau et al., 1992), and reduced male sexual activity (Grønli et al., 2005). All of the above evidence indicated a decrease in the reward value of a certain reinforcer. In the meantime, a number of studies consistently demonstrated that patients with major depression diagnosis scored significantly higher than healthy subjects on harm avoidance propensity (Abrams et al., 2004; Chien and Dunner, 1996; Celikel et al., 2009; Hansenne et al., 1997; Nery et al., 2009; Tanaka et al., 1998). Since depressed individuals display decreased reward sensitivity and increased harm avoidance tendency, novelty-seeking behavior should be straightly inhibited in depression. Nevertheless, data in this aspect are rather inconsistent or even contradictory. For example, although hospitalized depressed patients scored significantly lower than matched controls on sensation-seeking (Carton et al., 1992; Carton et al., 1995), a propensity to seek out varied, novel and intense sensations which was a driving construct underlying novelty-seeking behavior (Zuckerman, 1994), others demonstrated a significantly higher novelty-seeking score of depressed patients (Nery et al., 2009). In preclinical research, chronic stress-induced anhedonia in mice was reported to associate with reduced exploration to a novel object (Strekalova et al., 2004). In contrast, animals predisposed to learned helplessness with reduced sucrose intake displayed significantly increased exploration to a novel environment which has been interpreted as an increase in novelty-seeking behavior (Shumake et al., 2005). The above results meant that the alterations in novelty-seeking behavior of depressed individuals are by far unclear. This may derive from the dual properties of novelty, which contained both rewarding and aversive components (Glanzer, 1958; Bardo et al., 1996; Bevins and Bardo, 1999; Bevins

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doi:10.1016/j.beproc.2010.01.020

and Besheer, 2005), and elicited approach and avoidance conflict in organism (Hughes, 1997; Montgomery and Monkman, 1955; Montgomery, 1955; Powell et al., 2004).

The present study aimed to examine the effect of anhedonic state on novelty-seeking behavior. CMS model was employed to induce anhedonia in rats. The approach behavior towards a novel object set in the open-field (OF) was manipulated to measure novelty-seeking behavior. Since the expression of novelty-seeking behavior is suggested to be balanced between the approach and fear/anxiety-facilitated avoidance tendencies (Hughes, 1997; Kazlauckas et al., 2005; Montgomery and Monkman, 1955; Montgomery, 1955; Powell et al., 2004), animals' anxiety level in elevated plus maze (EPM) and in the central area of the OF, as well as the explorative activity in a relatively milder novel cage were also assessed.

In the present study, only a subgroup of animals displayed decreased sucrose preference, which were categorized as the anhedonic animals. The counterparts of these anhedonic animals who were exposed to the same CMS treatment but without these anhedonic expressions were categorized as stress-resistant ones. All the behavioral analyses were made in anhedonic and stress-resistant animals respectively. This methodology possessed significance in that CMS-induced anhedonic and stress-resistant animals have been evidenced to bear distinct behavioral and neurochemical alterations recently (Bergström et al., 2007; Bergström et al., 2008; Strekalova et al., 2004). Thus, if these two "kinds" of animals were examined in an undistinguished way, it is possible that the effect size would be minimized as with whether anhedonic animals showed altered novelty-seeking behavior.

2. Materials and methods

2.1. Animals and housing

Forty-eight male Wistar rats (Vital River Laboratories Inc., Beijing, China), weighing 290 to 340 g at the start of the experiment, were used (Permission No. 199036). Except as described below, animals were housed singly in steel-hanging cages ($25 \times 22.5 \times 30$ cm) with food and water ad libitum in a temperature ($23 \pm 2\,^{\circ}$C) and humidity (40–50%) controlled environment. All rats were maintained on a 12-hour light-dark cycle (7:00 light on; 19:00 light off) and all experiments were conducted during the light phase. The experimental protocols were in compliance with the National institutes of health guide for care and use of laboratory animals (Publication No. 85-23, revised 1985).

2.2. General experimental paradigm

After 1-week habituation to the colony environment, all rats were submitted to novel cage test. Then, the animals were trained to consume a weak (1%) sucrose solution, and assigned to stress ($n = 33$) or control group ($n = 15$) with a matched baseline sucrose preference score (see below). Stress animals were subjected to a modified version of CMS procedure for 6 weeks (Willner et al., 1987). Control animals were kept in a separate room and had no visual or olfactory contact with the stressed ones. After 6 weeks of CMS treatment, animals from each group were subjected to a battery of behavioral tests, including novel cage, OF (with a novel object in it) and EPM. All behavioral tests were fulfilled within 5 days after termination of the CMS treatment.

2.3. Sucrose preference test

Before the start of CMS procedure, rats were given a continuous 48-hour exposure to two drinking bottles, one with 1% sucrose solution and the other with tap water. Then, all animals received two baseline preference tests beginning at the start of the dark cycle (19:00 PM) (D'Aquila et al., 1997). The test lasted for 12-hour. Subsequently, animals were assigned to stress ($n = 33$) or control ($n = 15$) group with a matched baseline preference score in the final baseline test. The preference score was calculated as percentage of sucrose solution intake to the total amount of liquid consumed.

During the CMS treatment, sucrose preference tests were carried out under similar conditions to the baseline test at 1-week interval. To minimize the influence of metabolic factors on sucrose preference (Strekalova et al., 2004), food and water deprivation was not applied before the test. The animals were tested stress free. Duration of the test was set to 12-hour in order to reduce possible influence of neophobia and acute after-effect of prior stress (Konkle et al., 2003). Positions of the two bottles were counterbalanced across left or right in each group to prevent possible "side preference" effect. Preference score below 65% at the end of CMS treatment was taken as the criterion for appearance of anhedonia in the present study (Strekalova et al., 2004).

2.4. Chronic mild stress

The CMS regime lasted for 6 weeks and consisted of three to four different stressors each day, except on the preference test day. The stressors included soiled cage (200 ml of water spilled onto the bedding), periodic food or water deprivation, odor (sandalwood), continuous intense lighting, stroboscopic lighting (100 flashes/minute), grouped housing in a small cage (six or eight rats), paired housing (change of cage-mate), cage tilt (30°). The detailed procedure is illustrated in Table 1.

2.5. Novel cage test

The novel cage test was conducted in the dimly lit (10 lux) colony room in the animals' light cycle. Rats were individually introduced into another standard steel-hanging cage (with the same features as their home cage) underlain with fresh sawdust bedding. Any extrastress component was avoided as much as possible except transference of the animal from its homecage to the novel cage. Number of rearings was counted during a 5-minute period by visual observation (Marques et al., 2008; Reif et al., 2004; Strekalova et al., 2004). The rearing behavior was defined as vertical extensions of head, body and forelimbs, either free standing or leans against the walls, excluding those associated with grooming.

2.6. Open-field Test

The apparatus consisted of a dark blue stainless-steel circular arena (180 cm in diameter) surrounded by a 60-cm wall in height. The test room was lit by two incandescent lamps (40 watt each) placed symmetrically around the apparatus. Before the test began, a small toy (black cube cage, measuring $6 \times 6 \times 6$ cm) was secured on the center of one quadrant of the arena. A video camera suspended from the ceiling recorded the movement of the animal. Considering rats are biologically designed to seek shelter near the edge of the maze, a 10-cm-radius circular region around the arena center was artificially specified as the central area with the radius (10 cm) equal to the average body length of the tested animals. An open entry was recorded when all forepaws of the animal were placed inside the central area. Similarly, a circular area with 13-cm radius around the novel object ($6 \times 6 \times 6$ cm) was designated as the "novelty-seeking area". This made sure that the tested animal should be within the monitoring scope when it snooped around the novel object (see Fig. 1). The OF test lasted for 15 minutes. When the test began, each rat was gently placed near the edge of the maze facing against the wall. The following parameters were recorded:

Table 1
Schedule of chronic mild stress (CMS) procedure.

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
7:00 – 21:00	Soiled cage	11:00 – 17:00	Stroboscopic lighting	9:00 – 19:00	Paired housing	9:00 – 21:00	Cage tilt	13:00 – 19:00	Stroboscopic lighting	9:00 – 19:00	Cage tilt
19:00 – next day 11:00	Food deprivation	17:00 – next day 9:00	Water deprivation	9:00 – 19:00	Cage tilt	9:00 – 21:00	Paired housing	21:00 – next day 13:00	Odour	19:00 – next day 9:00	Paired housing	19:00 – next day 7:00	Sucros preference test
19:00 – next day 11:00	Odour	17:00 – next day 9:00	Grouped housing (6 rats)	19:00 – next day 9:00	Soiled cage	19:00 – next day 11:00	Continuous intense lighting	21:00 – next day 13:00	Grouped housing (8 rats)	19:00 – next day 9:00	Soiled cage		
19:00 – next day 11:00	Continuous intense lighting					21:00 – next day 13:00	Food deprivation						

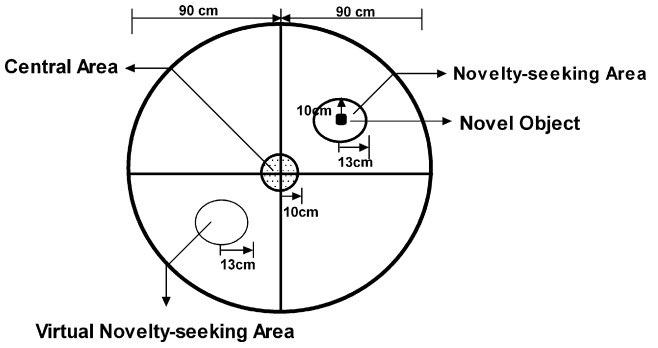

Fig. 1. Schematic map of open-field test.

- total distance traveled;
- number of fecal boli laid during the test;
- time spent in the central area;
- number of entries into the central area;
- time spent in the "novelty-seeking area";
- number of entries into the "novelty-seeking area".

To avoid the possible contamination of "novelty-seeking behavior" by enhanced locomotor activity after CMS treatment (Grønli et al., 2005; Strekalova et al., 2005), a symmetrical region with completely equal size to the above "novelty-seeking area" was defined as the "virtual novelty area" (without a novel object in it) (see Fig. 1). The duration in and number of entries into this "virtual novelty-seeking area" were also recorded. The parameters used for final analyses were calculated by the subtraction of "novelty-seeking" data to the "virtual novelty-seeking" data, for duration and number of entries respectively.

2.7. Elevated plus maze test

The EPM (ENV-560, Med Associates, Lafayette, IN) was constructed of black Plexiglas and consisted of two opposing open arms (50 × 10 cm, surrounded by a 1-cm high Plexiglas ledge), perpendicular to two opposing closed arms (50 × 10 × 40 cm). Connecting these arms was a junction area measuring 10 × 10 cm. Two infrared (I/R) photo beams were positioned at the entrance of each of the four arms and tracked the subject as it explored each arm of the maze. The maze was elevated to a height of 50 cm above the floor. The experiment was carried out in a dimly illuminated room and performed between 8:00 to 12:00 A.M. Individual rat was gently placed at the junction area, facing one of the open arms. During 5-minute test session, the following measures were taken:

- number of entries into open or closed arms;
- time spent in open or closed arms;
- time in the junction area;
- total arm entries.

At the end of each test session, the maze was carefully cleaned (10% alcohol) before the next animal was tested (Walf and Frye, 2007).

2.8. Data analysis

Data were analyzed with SPSS statistical software (13.0). To evaluate the effects of CMS upon sucrose preference and body weight change, two-way ANOVA with repeated measures was used with "time" as within-subject factor and "group" (stress vs control or anhedonic vs stress-resistant vs control) as between-subject factor. Paired *t* test was used to examine the changes of rearing

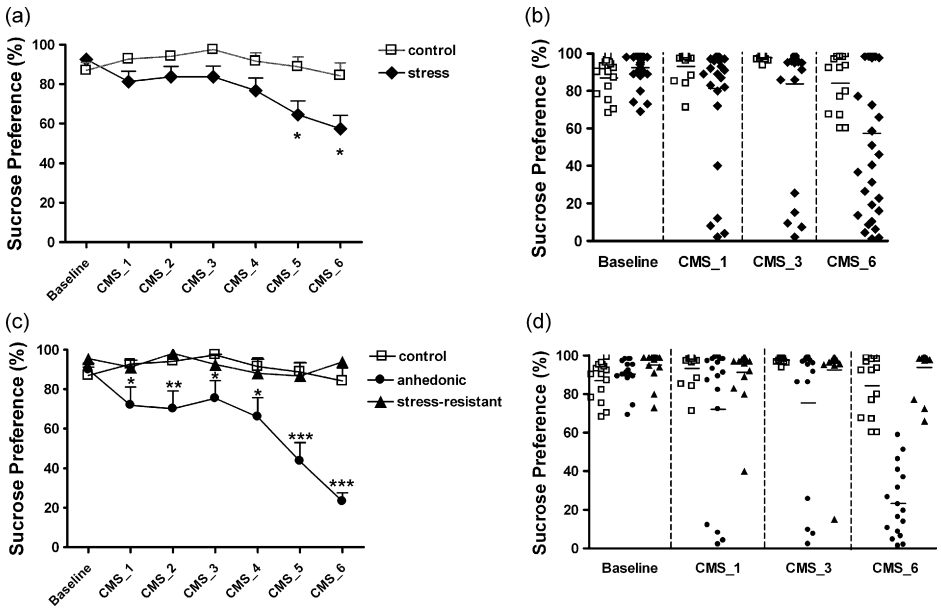

Fig. 2. Effect of chronic mild stress (CMS) on sucrose preference alterations. Data were expressed as mean (%) ± SEM (2a, 2c) or individual (2b, 2d). Comparisons were made between stress ($n = 33$) and control ($n = 15$) group. * $p < 0.05$ denoted significant differences relative to control group (2a); Comparisons were made between anhedonic ($n = 17$), stress-resistant ($n = 16$) and control ($n = 15$) group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ denoted significant differences relative to stress-resistant and control group (2c).

behavior before and after CMS. When comparisons were made more than two groups, one-way ANOVA and post-hoc examination (LSD or Dunnett's T3 depending on results of homogeneity of variance tests) were applied where appropriate. Since the number of fecal boli in the OF was not normally distributed, Chi^2 test was used. A probability of $p < 0.05$ was considered to be statistically significant.

Factor analysis was employed to further clarify the "factors" underlying measures of emotional alterations after CMS treatment in each individual group (stress vs control, or anhedonic vs stress-resistant vs control). The following measures were included: locomotor activity in the first 3, 5 and the total 15 minutes of the OF test, duration in central area/number of entries into the central area, duration in novel area/number of entries into novel area. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity were conducted to ensure that data were adequate for factor analysis. A principal components analysis with orthogonal (varimax) rotation was performed. Only factors with eigenvalues greater than one were accepted.

3. Results

3.1. Changes of sucrose preference following CMS treatment

For the comparisons among stress ($n = 33$) and control ($n = 15$) group, two-way ANOVA revealed significant effects of "group" (stress vs control) ($F[1, 46] = 5.646$, $p < 0.05$), "time"($F[6,276] = 4.347$, $p < 0.001$), and "group" × "time" interaction ($F[6,276] = 2.173$, $p < 0.05$) (Fig. 2a). Simple effect analyses demonstrated a significant decrease of preference score in stress group ($F[6, 276] = 9.17$, $p < 0.001$) but not in control group (NS) throughout the CMS treatment. The difference between stress and control group was apparent after 5 weeks of CMS ($F[1,46] = 5.03$, $p < 0.05$) and persisted thereafter (6th week) ($F[1,46] = 5.99$, $p < 0.05$).

At the end of the CMS treatment, 51.5% of stress animals exhibited a robust decline of sucrose preference (with preference score below 65%), and were regarded as anhedonic animals ($n = 17$); whereas 48.5% of stress animals ($n = 16$) did not display decreased sucrose preference, and were indicated as stress-resistant animals (Fig. 2b). Repeated ANOVA demonstrated significant effects of "group" ($F[2,45] = 23.482$, $p < 0.001$), "time" ($F[6,270] = 7.999$, $p < 0.001$), and "group" × "time" interaction ($F[12, 270] = 5.369$, $p < 0.001$) among anhedonic, stress-resistant and control animals

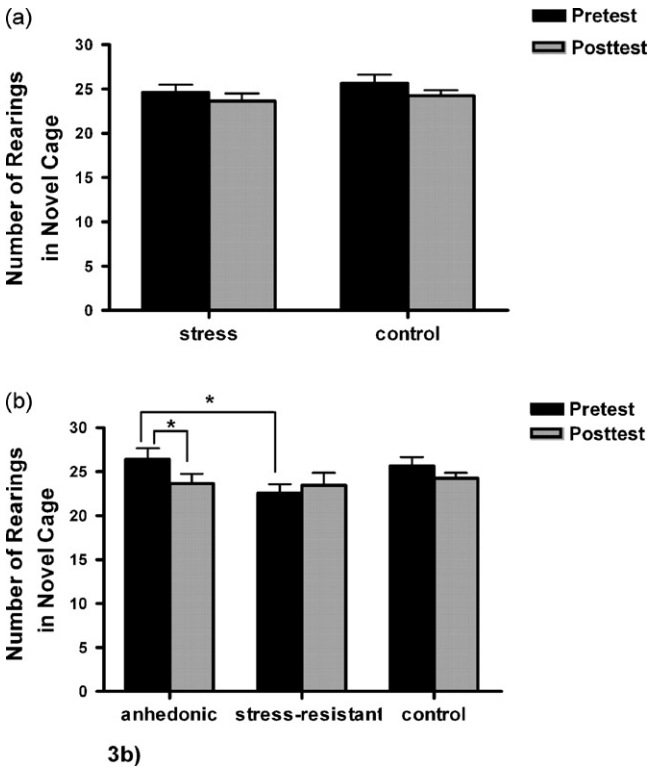

Fig. 3. Effects of chronic mild stress (CMS) on rearing behavior in novel cage test before and after CMS treatment. Data were expressed as mean (number) ± SEM. No significant difference of rearing behavior was found between stress ($n = 33$) and control ($n = 15$) group before and after CMS (3a); Anhedonic rats ($n = 17$) showed more rearing behavior than stress-resistant animals ($n = 16$) before CMS treatment and the rearing behavior of anhedonic rats was significantly decreased after CMS (3b) (* $p < 0.05$).

(Fig. 2c). The preference score for anhedonic group decreased significantly ($F[6,270] = 18.28$, $p < 0.001$), while stress-resistant and control group did not change (NS) (Fig. 2d).

3.2. Changes of body weight following CMS treatment

The animals in stress group gained less body weight compared with controls throughout the CMS treatment. Significant effects of "group" (stress vs control) ($F[1,46] = 27.376$, $p < 0.001$), "time" ($F[6,276] = 1168.442$, $p < 0.001$), and "group" × "time" interaction ($F[6,276] = 49.157$, $p < 0.001$) were found. For the comparisons among anhedonic, stress-resistant and control group, significant effects of "group" ($F[2,45] = 13.757$, $p < 0.001$), "time" ($F[6,270] = 1165.643$, $p < 0.001$), and "group" × "time" interaction ($F[6,270] = 24.106$, $p < 0.001$) were found. Both anhedonic and stress-resistant animals weighted less than control, but the former two groups did not differ (data not shown).

3.3. Emotional deficits induced by CMS treatment

3.3.1. Novel cage test

No significant difference of rearing behavior was found between stress and control group before and after CMS (NS) (Fig. 3a). When differences among anhedonic, stress-resistant and control group were considered, one-way ANOVA revealed a significant difference at baseline condition ($F[2,45] = 3.338$, $p < 0.05$) with anhedonic rats showing more rearing behaviors than stress-resistant rats ($p < 0.05$). Moreover, paired t test showed significantly decreased rearing behavior in anhedonic rats after CMS ($t[16] = 2.119$, $p < 0.05$). Stress-resistant and control group did not show any salient change with this measure before and after CMS (NS) (Fig. 3b).

3.3.2. Open-field test

Compared with controls, stress animals showed increased total locomotor activity in the OF ($t[43] = 3.354$, $p < 0.01$) (Fig. 4a). As for differences among anhedonic, stress-resistant and control group, a main effect of "group" was found ($F[2,44] = 5.517$, $p < 0.01$). Both anhedonic ($p < 0.01$) and stress-resistant ($p < 0.01$) rats displayed increased locomotion compared with controls (Fig. 4b).

Compared with controls, stress animals spent more time in the central area ($t[34.551] = 3.509$, $p < 0.01$) (Fig. 4c) and made more entries into it ($t[41] = 3.123$, $p < 0.01$) (Fig. 4e). Meanwhile, stress-resistant ($p < 0.01$) rats spent more time in the central area than controls ($F[2,43] = 4.936$, $p < 0.05$), while the anhedonic and control rats did not differ (NS) (Fig. 4d). Stress-resistant rats made more entries into the central area ($F[2,42] = 9.763$, $p < 0.001$) relative to anhedonic ($p < 0.01$) and control group ($p < 0.001$), while the later two groups did not differ from each other (NS) (Fig. 4f).

Stress animals spent more time ($t[41] = 2.910$, $p < 0.01$) in and made more entries ($t[42] = 3.019$, $p < 0.01$) into the novelty-seeking area than controls (Fig. 4g and i). Anhedonic rats stayed for a longer duration ($F[2.43] = 5.725$, $p < 0.01$) in novel area and made more entries into this area ($F[2.43] = 4.857$, $p < 0.05$) compared with controls. However, stress-resistant group did not differ from controls with the above measures (NS) (Fig. 4h and j).

Relative to controls, stress animals had a lower defecation incidence in the OF ($\chi^2[1] = 8.927$, $p < 0.01$) (Fig. 4k). Meanwhile, no difference was found between anhedonic and stress-resistant rats (NS) (Fig. 4l) though both groups had a lower defecation incidence than control group ($p < 0.05$).

Factor analysis of the all measures of OF activities further clarified the "factors" underlying the emotional alterations after CMS treatment in each individual group (stress vs control, or anhedonic vs stress-resistant vs control). As shown in Table 2, two factors

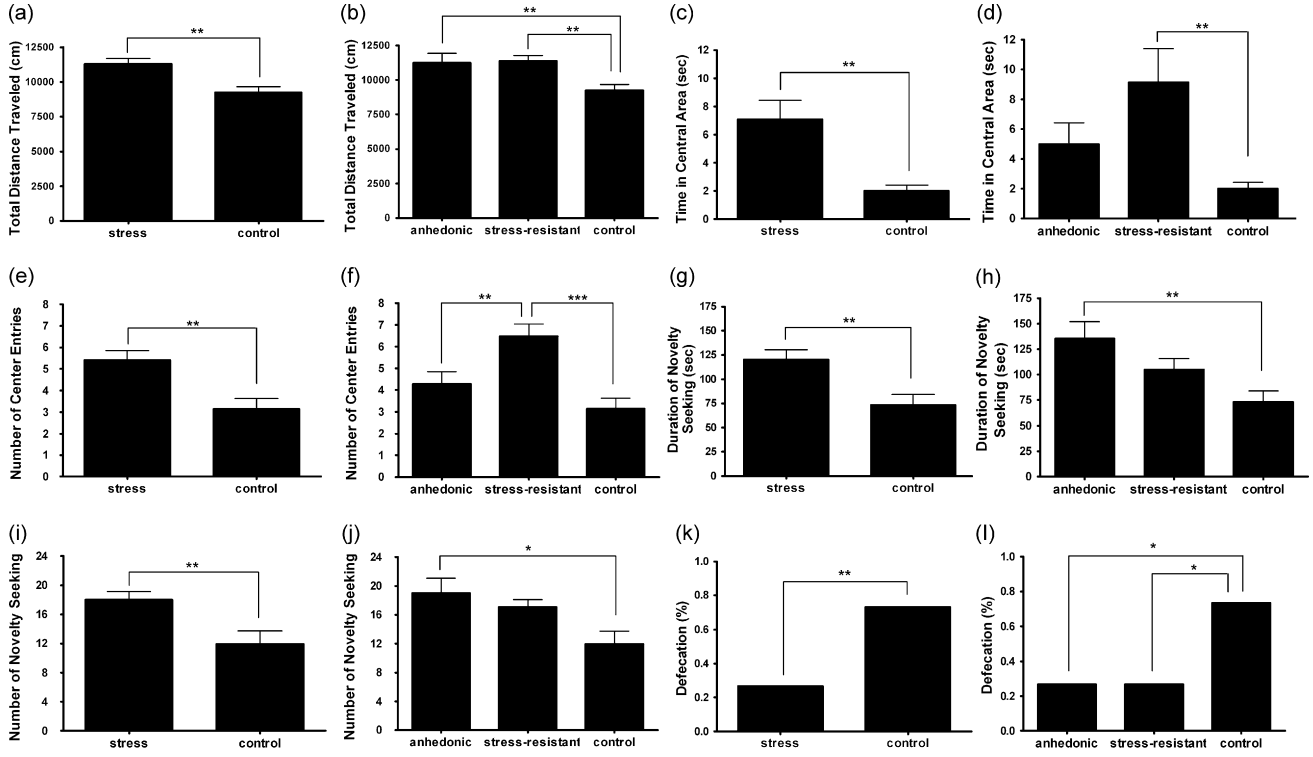

Fig. 4. Effects of chronic mild stress (CMS) on measures of emotional behaviors in open-field test (containing novel object). Comparisons were made between stress ($n = 33$) and control ($n = 15$) group or between anhedonic ($n = 17$), stress-resistant ($n = 16$) and control ($n = 15$) group. The following dependent measures were compared: total distance traveled in the open-field (a-b); duration in the central area (c-d); number of entries into the central area (e-f); duration in novelty-seeking area (g-h); number of entries into novelty-seeking area (i-j); % defecation incidence (k-l). Except for defecation incidence, data were expressed as mean ± SEM respectively. * $p < 0.05$, ** $p < 0.01$ denoted significant differences between corresponding groups as shown in the figures.

Table 2
Factor Loadings for open-field measures in control and stress rats.

Behavior	Control		Stress		
	Factor 1 42.90%	Factor 2 33.20%	Factor 1 42.80%	Factor 2 22.90%	Factor 3 17.70%
Locomotor in 3 minutes	0.812		0.923		
Locomotor in 5 minutes	0.974		0.966		
Locomotor in 15 minutes	0.824		0.853		
Number of central entries	0.668		0.687		
Duration in central area		0.835			0.963
Number of novelty-seeking		0.766		0.853	
Duration of novelty-seeking		0.819		0.884	
Sampling adequancy	Control: 0.665 Stress: 0.630				
Bartlett's test of sphericity	Control: $\chi 2(21) = 55.7$, $p = 0.000$ Stress: $\chi 2(21) = 107.6$, $p = 0.000$				

Notes: Only behavioral parameters with loadings greater than 0.6 were shown.

explained 76.1% of total variance of control animals. The factor with the largest amount of variance explained appeared to reflect "locomotor activity" (Factor 1), with the highest loadings for locomotor activity (3, 5, 15 minutes) and the number of central entries on this "factor". Factor 2 seemed to relate to "anxiety", with the highest loadings for duration in the central area and novelty-seeking area as well as number of entries into novelty-seeking area. In contrast, a 3-factor solution was identified in stress group, which accounted for 83.4% of the total variance. The parameters of locomotor activity (3, 5, 15 minutes) and number of central entries loaded on Factor 1 (locomotor activity). Duration in and number of entries into novelty-seeking area loaded on Factor 2 while duration in central area loaded on Factor 3.

Factor analysis conducted in anhedonic animals also identified three factors explaining 90.8% of the total variance (Table 3). Locomotor activity (3, 5, 15 minutes), number of central entries loaded on Factor 1. Novelty-seeking behaviors (duration and number of entries) loaded on Factor 2 and duration in central area on Factor 3. These results fell in contrast with control animals (two factors only) and indicated that for anhedonic rats, novelty-seeking behaviors dissociated from central activity. In stress-resistant group, 3 factors were also extracted. However, the results were somewhat unorderly and hard to explain, probably due to its marginal sampling adequacy for factor analysis.

3.3.3. *Elevated plus maze test*

Compared with control group, stress animals spent more time in the open arms ($t[43] = 2.255$, $p < 0.05$) (Fig. 5a). Meanwhile, stress-resistant rats stayed in the open arms for a longer duration ($F[2,44] = 4.842$, $p < 0.05$) than both anhedonic ($p < 0.05$) and control rats ($p < 0.01$) (Fig. 5b). Although no difference was found with the number of closed arm entries between stress and control group (NS) (Fig. 5c), stress-resistant rats made more closed entries ($F[2,45] = 3.707$, $p < 0.05$) than anhedonic ($p < 0.05$) and control animals ($p < 0.05$) (Fig. 5d).

4. Discussion

The main aim of the present study was to investigate the impact of anhedonic state on novelty-seeking behavior. Here, we reported

Table 3
Factor loadings for open-field measures in control, anhedonic and stress-resistant rats.

Behavior	Control		Anhedonic			Stress-resistant		
	Factor 1 42.90%	Factor 2 33.20%	Factor 1 46.90%	Factor 2 24.20%	Factor 3 19.70%	Factor 1 31.40%	Factor 2 29.70%	Factor 3 18.40%
Locomotor in 3 minutes	0.812		0.962			0.689		
Locomotor in 5 minutes	0.974		0.968				0.805	
Locomotor in 15 minutes	0.824		0.886				0.787	
Number of central entries	0.668		0.766			0.818		
Duration in central area		0.835			0.964			0.924
Number of novelty-seeking		0.766		0.899		0.852		
Duration of novelty-seeking		0.819		0.885			−0.707	
Sampling adequancy	Control: 0.665 Anhedonic: 0.597 Stress-resistant: 0.523							
Bartlett's test of sphericity	Control: $\chi 2(21) = 55.7$, $p = 0.000$ Anhedonic: $\chi 2(21) = 78.5$, p = 0.000 Stress-resistant: $\chi 2(21) = 32.7$, $p = 0.049$							

Notes: Only behavioral parameters with loadings greater than 0.6 were shown.

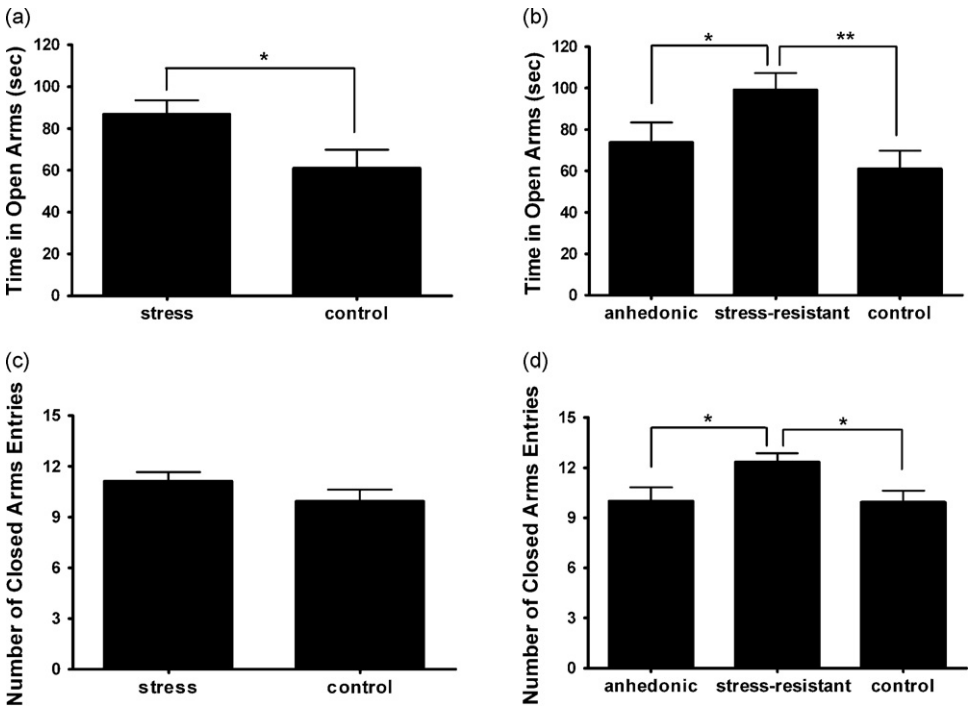

Fig. 5. Effects of chronic mild stress (CMS) on emotional behaviors in elevated plus maze (EPM). Comparisons were made between stress ($n = 33$) and control ($n = 15$) group or between anhedonic ($n = 17$), stress-resistant ($n = 16$) and control ($n = 15$) group. Time in the open arms (sec) (5a, 5b) and number of close entries (5c, 5d) were compared. Data were expressed as mean ± SEM. * $p < 0.05$, ** $p < 0.01$ denoted significant differences between corresponding groups as shown in the figures.

that anhedonic animals expressed significantly increased exploration towards the novel object compared with controls. However, the stress-resistant animals did not. The enhanced novel object exploration of anhedonic rats seemed to be goal-directed. Data for analyzing the novel object exploration (time in and number of entries into novelty-seeking area) was collected by subtracting the virtual approaching indices from actual novel-object exploration indices (see procedure section). This guaranteed that the novel-object exploration data reflected the nature of goal-directedness rather than being contaminated by enhanced locomotor activity after CMS treatment (Grønli et al., 2005; Strekalova et al., 2005). As a consequence of this analysis methodology, we found that, though both anhedonic and stress-resistant rats expressed identically increased forced novelty-induced locomotor activity, these two "kinds" of animals did express different propensity/attitude towards the novel object in the OF. Compared with controls, anhedonic rats spent more time in and made more entries into the novel object area but not in/into the central area; To the contrary, the stress-resistant rats spent more time and made more entries into the central area but not in/into the novel object area. These behavioral spatiotemporal distinctions between anhedonic and stress-resistant animals suggested that the OF apparatus provided enough room for animals to choose freely whether to approach the novel object or not, and, further supported that the enhanced novel object exploration of the anhedonic rats was an expression of goal-directed behavior towards the novel object rather than a simple expression of increased locomotor activity. In addition, the increased novel object exploration in the anhedonic rats seemed to be goal-directed since this behavior was independent of their anxiety level. Both time in the open arm of the EPM test and time in the central area of the OF test, two classically putative indices of anxiety consistently showed unaltered anxiety level of anhedonic rats relative to controls. Considering previous suggestion that novelty exploration in rodents was primarily balanced between approach and anxiety-facilitated avoidance tendencies (Hughes, 1997; Kazlauckas et al., 2005; Montgomery and Monkman, 1955; Montgomery, 1955; Powell et al., 2004), the fact that anhedonic animals who showed unaltered anxiety level explored the novel object more further suggested an interpretation of an increase in intrinsically goal-directed novelty-seeking tendency rather than a simple expression of reduced anxiety (disinhibition).

Our factor analysis data provided additional support for the interpretation of increased goal-directed novelty-seeking behavior in anhedonic rats. In control animals, the "novel object exploration indices" (time and entries) and "central activity" (time and entries), a putative fear/anxiety-inhibited behavioral index (Ramos and Mormede, 1998; Ohl et al., 2008), were loaded on the same "factor". This meant that in control animals, higher novel object exploration was a direct manifestation of lower anxiety/fear level. This result was in line with previous studies demonstrating that non-stressed animals, which displayed higher level of novelty-seeking behavior, were less anxious in several model of anxiety, such as EPM and light-dark box test (Blankstein, 1975; Kabbaj et al., 2000; Kazlauckas et al., 2005; Mällo et al., 2007; Stead et al., 2006). However, for the anhedonic animals, the "novel object exploration indices" (time and entries) were loaded on a separate "factor" which was statistically away from the "central activity" (Table 3). The independence of novel object exploration with "central activity" in anhedonic rats indicated that, under a pathological state after CMS, higher novel object exploration was no longer a manifestation of lower anxiety (disinhibition) but may reflect a higher level of goal-directed novelty-approaching tendency, that is, the increased goal-directed novelty-seeking behavior as we suggested.

It seemed somewhat peculiar that anhedonic rats would show enhanced novelty-seeking behavior since anhedonia has been critically linked to decreased functioning of the reward system (Di Chiara et al., 1999; Di Chiara and Tanda, 1997; Stamford et al., 1991; Willner et al., 1991). However, the previously evidenced hypofunctioning of the reward system might readily be linked to reward "sensitivity" rather than "reward itself". Indeed, many studies evi-

denced the decrease in consumption of sucrose solutions after CMS, but only at low sucrose concentrations (0.5–2%). This effect was not seen at higher sucrose concentrations (to review, see Willner, 1997) or with highly palatable food (Sampson et al., 1992). In addition, Willner et al. (1998) found that CMS-treated animals even worked harder to obtain sucrose solution at 95% concentration. In line with the above, the fact that depressed individuals readily show stronger tendency to the use of abusive drugs (for review, see Markou et al., 1998) also indicates that they may selectively choose to approach things with significantly higher reward/reinforcing value (e.g. sweet solution with higher concentration, highly palatable food, or abusive drugs). This interpretation was also compatible with prominent symptom of "far less interest in daily life", which may probably be due to their "hyposensitivity" of the reward system rather than no response to reward at all. In other words, for depressed individuals, the reactions towards external stimuli may be "dose-dependent" upon the reward/reinforcing value. As evidenced in the present study, anhedonic rats showed reduced novelty-induced rearing behavior in novel cage (Fig. 3b), an environment with relatively low-degree novelty, but showed enhanced interest towards the salient novel object set in the OF. With a free exploration paradigm, Rebec et al. (1997) found a briefly but significantly enhanced dopamine release when rats approached into a novel environment, with this neurotransmitter hypothesized to be reinforcing. Indeed, various kinds of novelty-seeking or so-called risk-taking behaviors have been suggested in depressed patients (Kosunen et al., 2003; Leas and Mellor, 2000; Pesa et al., 1997; Spittle et al., 1976) and hypothesized to be a means of self-medication to alleviate depressive state (Ben-Amos, 1992; Carton et al., 1995; Fishbain, 1987; Herpertz and Sass, 2000; Kandel and Davies, 1982; Markou et al., 1998).

It is noteworthy that in the present study, if we took the whole CMS group to represent the "anhedonic animals" as most of the previous studies suggested (note that the whole CMS group showed significant decreased sucrose preference score compared with control group; Fig. 2a), we could find that these "anhedonic animals" expressed both reduced anxiety (OF [Fig. 4c]/EPM [Fig. 5a]) and enhanced novelty-seeking behavior (Fig. 4g and i). This made us ready to infer that increased novelty-seeking may be caused by decreased anxiety. However, when the anhedonic and stress-resistant rats were examined separately, we could find that reduced anxiety did not necessarily lead to increased novelty-seeking behavior as evidenced with the comparisons between stress-resistant rats and controls (Fig. 4d, 4h, 4j, 5b). To the contrary, the anhedonic rats, which showed no sign of reduced anxiety, expressed significantly enhanced novelty-seeking behavior (Fig. 4d, 4h, 4j, 5b). In other words, the relationship between novelty-seeking and anxiety would be confounded if anhedonic and stress-resistant animals were examined in an undistinguished way. This highlighted the importance of setting CMS-induced anhedonic animals apart from stress-resistant ones for drawing accurate conclusions in this regard. Indeed, this "cut-off" methodology seems as a must. As Strekalova et al. (2004) have found that, only CMS-induced anhedonic mice showed increased floating behavior and decreased exploration than controls. If the whole CMS animals were compared with controls, the above differences disappeared. In addition, the respective examinations between anhedonic and stress-resistant animals could help explain a paradoxical CMS-induced anxiolytic effect evidenced in at least seven reports until now, which had been considered to be anomalous and difficult to explain (D'Aquila et al., 1994; Ducottet and Belzung, 2004; Ducottet et al., 2003; Kompagne et al., 2008; Kopp et al., 1999; Li et al., 2007; Rossler et al., 2000; Schweizer et al., 2009). Here, we found that in both OF and EPM tests, CMS-induced anxiolytic effect mainly derived from and was confounded by significantly decreased anxiety level of the stress-resistant animals (Fig. 4d, 5b). To the contrary, the anhedonic rats did not show any sign of reduced anxiety (Fig. 4d, 5b). In all, the present study clearly demonstrated that anhedonic rats, rather than stress-resistant ones, showed intrinsically enhanced goal-directed novelty-seeking behavior and this behavior was independent of changed anxiety level.

Acknowledgements

This work was supported by National natural science foundation grants (30770722, 30470578), Promotive funding for research excellency of Chinese academy of sciences (O7CX191019), and IPCAS science development fund (O8CX093009) to X.G. Zheng.

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