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Integration of animal behaviors under stresses with different time courses

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Abstract

We used animal models of "forced swim stress" and "chronic unpredictable stress", and tried to reveal whether a passive coping style of high flotation behavior in forced swim stress predicts anhedonia behavior after chronic unpredictable stress, and whether the dopamine system regulates floating and anhedonia behaviors. Our results confirmed that depression-prone rats use "floating behavior" as a coping strategy in forced swim stress and more readily suffer from anhedonia during chronic unpredictable stress. Intraperitoneal injection or nucleus accumbens microinjection of the dopamine 2/3 receptor subtype agonist ropinirole reduced floating behaviors in depression-prone animals, but increased sucrose preference in rats showing anhedonia. These data indicate that floating behavior is a defensive mode that is preferred by susceptible individuals under conditions of acute stress. Simultaneously, these animals more readily experienced anhedonia under long-term stress; that is, they were more readily affected by depression. Our results suggest that dopamine 2/3 receptor subtypes in the nucleus accumbens play an important role in floating behaviors and anhedonia.

Key Words: *nerve regeneration; brain injury; depression; stress resistance; susceptible to depression; chronic unpredictable stress; forced swim; dopamine; nucleus accumbens; NSFC grant; neural regeneration*

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Introduction

Depression is a state of low mood, anhedonia and aversion to activity. Patients with depression frequently show a variety of physical symptoms. Their daily lives and social functions are greatly affected, which brings a heavy burden upon patients, family, and the whole community (Palermo-Neto, 1997; Cryan et al., 2001; Yarkov et al., 2003; Papakostas, 2006; Sokoloff et al., 2006; Riddle et al., 2010). Clinical and basic studies have made important progress in developing a treatment for depression, but there are still many problems. For example, the cure rate of depression with medicine is only 50% in the clinic, and almost half of depression patients show no apparent improvement after taking conventional antidepressants (Petersen et al., 2005; Taylor et al., 2006). In patients with effective drug treatment, common antidepressants take effect slowly, and protracted symptoms exist in those undergoing maintenance therapy (Nierenberg and Wright, 1999), with a high relapse rate (Mueller et al., 1999; Solomon et al., 2000).

Clinical studies have confirmed that the dopamine system, especially the mesolimbic dopamine system, exerts a vital effect on the pathogenesis of depression (Palermo-Neto, 1997; Cryan et al., 2001; Yarkov et al., 2003; Zhou et al., 2005; Antonijevic, 2006; Bertaina-Anglade et al., 2006; Papakostas, 2006; Sokoloff et al., 2006; Sekine et al., 2007), including decreased dopamine levels or dopamine metabolism (Roy et al., 1985, 1992; Lambert et al., 2000), increased dopamine receptor binding/sensitivity (D'haenen and Bossuyt, 1994; Verbeeck et al., 2001; Klimek et al., 2002) and decreased dopamine transporter activity (Meyer et al., 2001; Neumeister et al., 2001). Among depression patients who committed suicide, the content of the dopamine metabolite dihydroxy-phenyl acetic acid in the nucleus accumbens is remarkably diminished (Bowden et al., 1997). The content of another dopamine metabolite, homovanillic acid, in cerebrospinal fluid is also noticeably decreased. The content of homovanillic acid in urine is significantly lower in depressed patients who attempt suicide than in those who do not and healthy controls (Roy et al., 1992). Moreover, homovanillic acid levels in cerebrospinal fluid are negatively associated with the severity of depression (Roy et al., 1985). The above findings indicate that dopamine metabolism is strongly correlated with a patient's condition. Neuroimaging studies have revealed an increase in dopamine 2/3 receptor binding sites (Yang et al., 2008) in the corpus striatum (Shah et al., 1997) and basal ganglia (D'haenen and Bossuyt, 1994) of depressed patients. Consistent with this phenomenon, a previous study found that the sensitivity of dopamine 2 receptors

was increased in the central nervous system of depressed patients (Verbeeck et al., 2001). Interestingly, this increase in sensitivity of dopamine 2 receptors was associated with taking selective serotonin reuptake inhibitors and tricyclic preparations (Healy and McKeon, 2000). An autopsy study demonstrated an increase in the number of dopamine 2/3 receptor subtype binding sites in the basolateral amygdala and central amygdale, but numbers of dopamine transporter binding sites were obviously reduced in the central amygdala (Klimek et al., 2002). Additionally, single photon emission computerized tomography showed increased dopamine transporter binding activity in the basal ganglia and striatum (Tanda et al., 1994; Laasonen-Balk et al., 1999; Yang et al., 2008). These findings suggested that the dopamine system was strongly associated with the occurrence of depression (Laasonen-Balk et al., 1999; Healy and McKeon, 2000; Lambert et al., 2000; Cryan et al., 2001; Meyer et al., 2001; Neumeister et al., 2001; Klimek et al., 2002; Wall et al., 2003; Millan et al., 2004; Bekris et al., 2005).

Basic studies have also revealed significant pathological changes in the dopamine system of depressed animals under stress (Taylor et al., 1982; Tossman and Ungerstedt, 1986; Simon et al., 1993; Levant, 1997; Steiner et al., 1997; Gendreau et al., 1998; Lawford et al., 2006; Perona et al., 2008; Schneier et al., 2008; van der Wee et al., 2008). After an inescapable uncontrollable electric shock, dopamine 2 receptor density was reduced in the caudate nucleus and core area of the nucleus accumbens of rats experiencing learned helplessness (Winter et al., 2007). In rats with anhedonia under chronic unpredictable stress, the release of dopamine and its metabolites was obviously altered in the prefrontal cortex and corpus striatum. The level of dopamine 2 receptor messenger RNA expression was decreased in the midbrain ventral tegmental area, substantia nigra, core area and shell area of the nucleus accumbens and caudate nucleus (Dziedzicka-Wasylewska et al., 1997; Winter et al., 2007). Additionally, the dopamine 2 receptor binding activity in the nucleus accumbens was apparently reduced (Papp et al., 1994). These changes in the dopamine system could be reversed by slowly injecting antidepressants (Dziedzicka-Wasylewska et al., 1997; Bekris et al., 2005; Yang et al., 2008; Vignisse et al., 2011). Clinical and basic studies have indicated that the dopamine system exerts a crucial effect on the pathological development of depression, but the effects of the dopamine system, especially of dopamine 2/3 receptor subtypes, on the treatment of depression, have not been examined in a comparative study using animal models.

Basic studies addressing stress-induced depression in experimental animals could investigate the relationship between stress and depression (D'haenen and Bossuyt, 1994; Papp et al., 1994; Tanda et al., 1994; Willner et al., 1994). The forced swim test and chronic unpredictable stress have been extensively used to prepare animal models of depression (Strekalova et al., 2008; Bolkunov et al., 2009; Tian et al., 2011; Varga et al., 2011; Vignisse et al., 2011; Vollenweider et al., 2011). In conditions of acute stress, animals show increased depression-like behaviors such as floating behaviors. Chronic stress causes persistent anhedonia-like behavior (Sun et al., 2004; Cryan and Holmes, 2005; Mathews and MacLeod, 2005; Remy et al., 2005). This "floating behavior" and "anhedonia" is considered to be a model of depression in behavioral neuroscience studies. Studies have verified that the sucrose preference in high floating animals (high percentage of floating behaviors) after a forced swim test is not decreased, but floating behavior is more frequently observed in animals showing anhedonia than in control animals after chronic unpredictable stress (Sun et al., 2004; Cryan and Holmes, 2005; Mathews and MacLeod, 2005; Remy et al., 2005). Therefore, the relationship between animals showing floating behaviors under acute stress and animals showing anhedonia under chronic stress remains poorly understood, as does the mechanism underlying the effects of the dopaminergic system on the above two kinds of models of depression.

In this study, we sought to explore the following three problems. (1) Whether there is a predictive relationship between floating behavior of animals after forced swim test and anhedonia behavior of animals after chronic unpredictable stress; and if there is a predictive relationship, do animals showing a high percentage of floating behavior easily suffer from anhedonia after chronic unpredictable stress? (2) Whether the dopamine 2/3 receptor subtype regulates floating behavior in models after forced swim test? If yes, does the nucleus accumbens play an important role in this regulation? (3) Whether the dopamine 2/3 receptor subtype regulates anhedonia in models after chronic unpredictable stress? If yes, does the nucleus accumbens play an important role in this regulation?

Materials and Methods

Experimental animals

One-hundred and four adult male Wistar rats were purchased from VitalRiver, Beijing, China. Their initial body weight was between 250 and 270 g. All rats were housed at 20–24°C in 15–20% humidity, with a light cycle of 8:00–20:00, in a specific-pathogen-free room. All rats were acclimated to the conditions for 1 week before experiments. During this week, an experimenter regularly caught and touched these rats to exclude non-experimental specific stress. All protocols were approved by the Animal Ethics Committee of the Chinese Academy of Sciences.

Establishment of rat models in a forced swim test

The forced swim test, also called the desperate experiment or Porsolt test, was first reported by Porsolt et al. (1978). Animals could not escape from the bad surroundings, which resulted in behavioral despair. In our experiments, a transparent cylindrical container (50 cm high, 25 cm diameter) was used. During the test, the depth of water was 35 cm, and the water temperature was 25°C. A 60 W frosted yellow light bulb provided lighting. A camera was placed in the front of the glass bucket to record animal's behaviors in water. A total of 64 rats were randomly assigned to a stress group (n = 34) and a control group (n = 30). The forced swim test was performed over

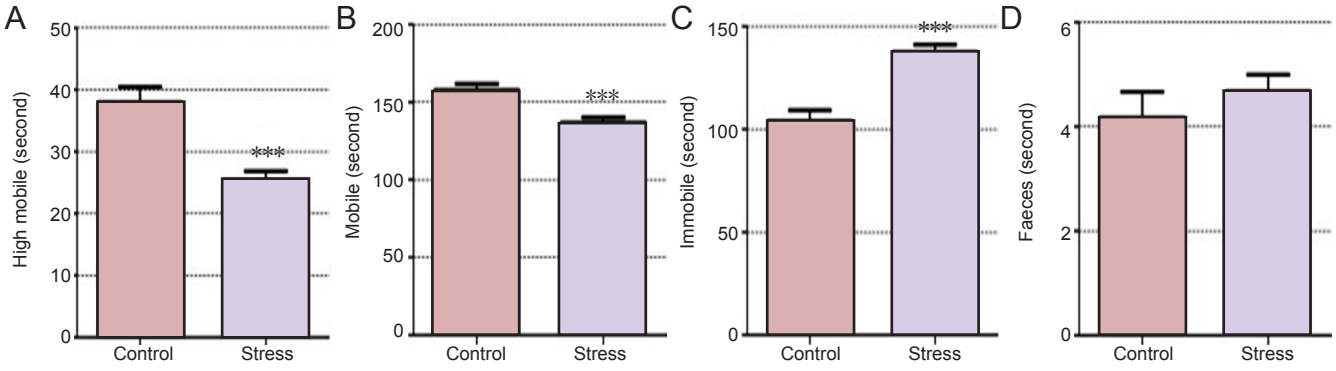

Figure 1 Effects of forced swim stress on floating behavior in rats.
Compared with the control group ($n = 30$), 15-minute forced swimming significantly reduced high-intensity exercise time (A) and movement time (B), increased floating time (C), and faeces time (D) in the stress group ($n = 34$) during a 5-minute forced swim test. ***$P < 0.001$, mean ± SEM, two sample t-test.

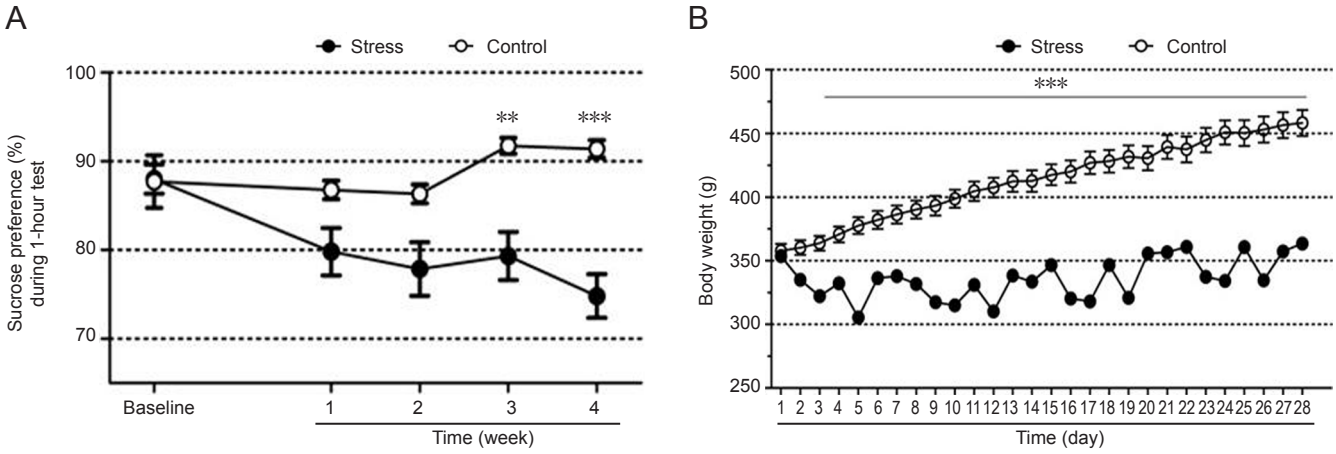

Figure 2 Effects of chronic unpredictable stress on sucrose preference and weight in rats.
Compared with the control group ($n = 10$), chronic stress for 4 consecutive weeks significantly diminished the percentage of sucrose intake in the stress group ($n = 30$) (A; **$P < 0.01$, ***$P < 0.01$, mean ± SEM, two-way analysis of variance, Bonferroni test). That is, the rats in the stress group suffered from anhedonia, and the weights of rats in the stress group were significantly reduced (B; mean ± SEM, two sample t-test).

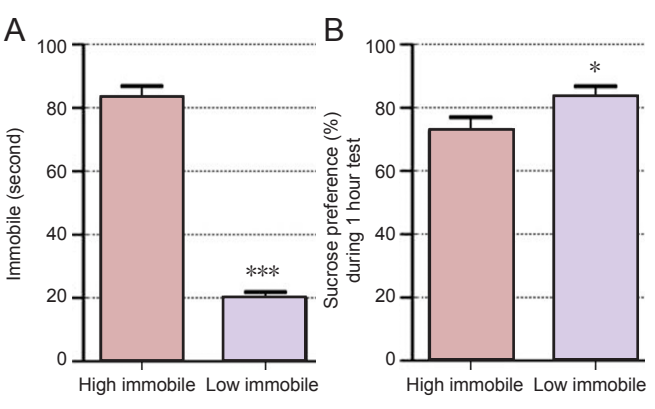

Figure 3 Predictive effects of acute floating behavior on anhedonia in rats.
(A) After identical forced swimming, significant differences in floating behavior were detectable between rats with high and low percentages of floating behaviors (***$P < 0.001$). (B) The sucrose preference level under chronic stress was lower in rats with a high percentage of floating behaviors under acute stress than rats with a low percentage of floating behaviors (*$P < 0.05$). Data are expressed as mean ± SEM ($n = 8$) and the two sample t-test was used.

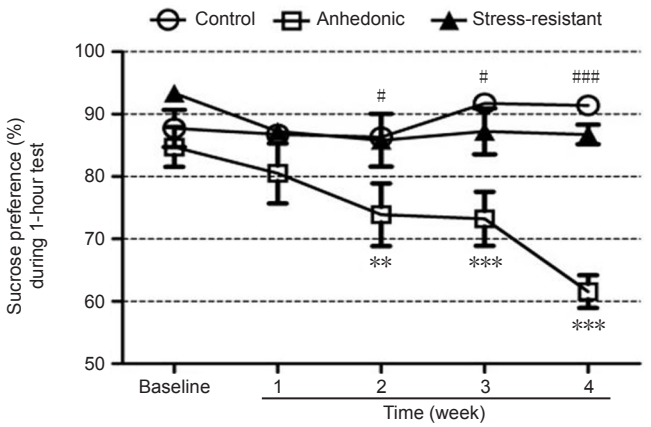

Figure 5 Individual differences in the percentages of sucrose intake under chronic unpredictable stress.
Anhedonia appeared in some rats (**$P < 0.01$, or ***$P < 0.001$). Stress resistance occurred in some rats (#$P < 0.05$, or ###$P < 0.001$). Data are expressed as mean ± SEM. Control: control group ($n = 8$); anhedonic: anhedonia group ($n = 8$); stress-resistant: stress resistance group ($n = 10$). Multivariate analysis of variance and the Bonferroni test were used.

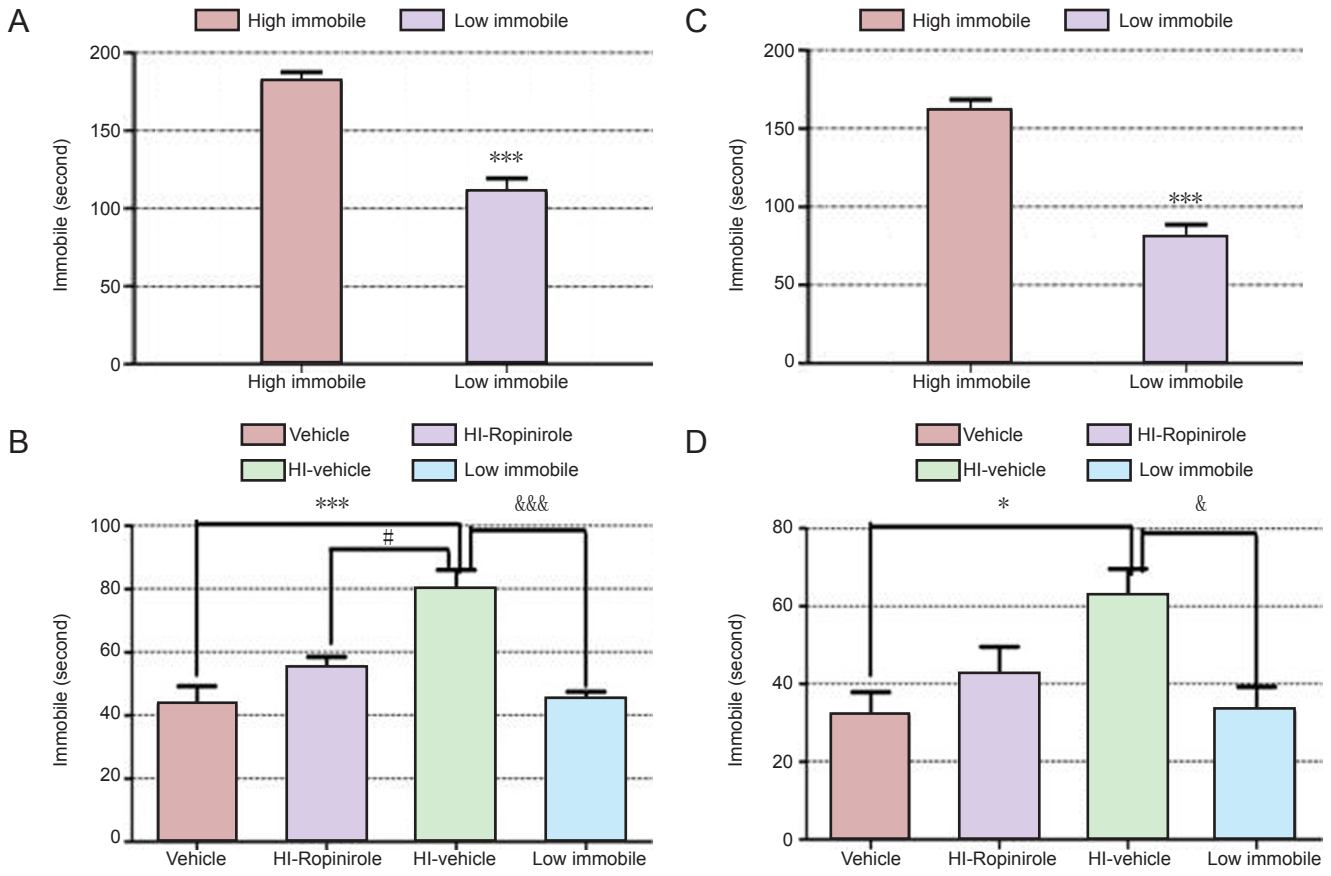

Figure 4 Effects of a dopamine 2/3 receptor agonist on floating behaviors in rats susceptible to depression under acute stress.
(A) Floating behaviors of rats confirmed to have high ($n = 16$) and low ($n = 8$) percentages of floating behaviors during 15-minute pre-processing.
(B) Chronic intraperitoneal injection of ropinirole could reduce floating behaviors in rats showing a high percentage of floating behaviors ($n = 8$) during a 5-minute test (&&&$P < 0.001$, ***$P < 0.001$). Significant differences in floating time were detectable between the saline control group and ropinirole group of rats showing a high percentage of floating behaviors (#$P < 0.05$).
(C) Floating behaviors of rats showing high ($n = 8$) and low ($n = 8$) percentages of floating behaviors during 15-minute pre-processing.
(D) Nucleus accumbens microinjection of ropinirole diminished floating behaviors in rats showing a high percentage of floating behaviors ($n = 8$) during a 5-minute test (&$P < 0.05$, *$P < 0.05$).
High immobile: group showing a high percentage of floating behaviors; Low immobile: group showing a low percentage of floating behaviors; vehicle: saline control group; HI-Ropinirole: ropinirole-treated group showing a high percentage of floating behaviors; HI-vehicle: saline-treated control group showing a high percentage of floating behaviors. Data are expressed as mean ± SEM. (A, C) Two sample *t*-test; (B, D) multivariate analysis of variance, Bonferroni test.

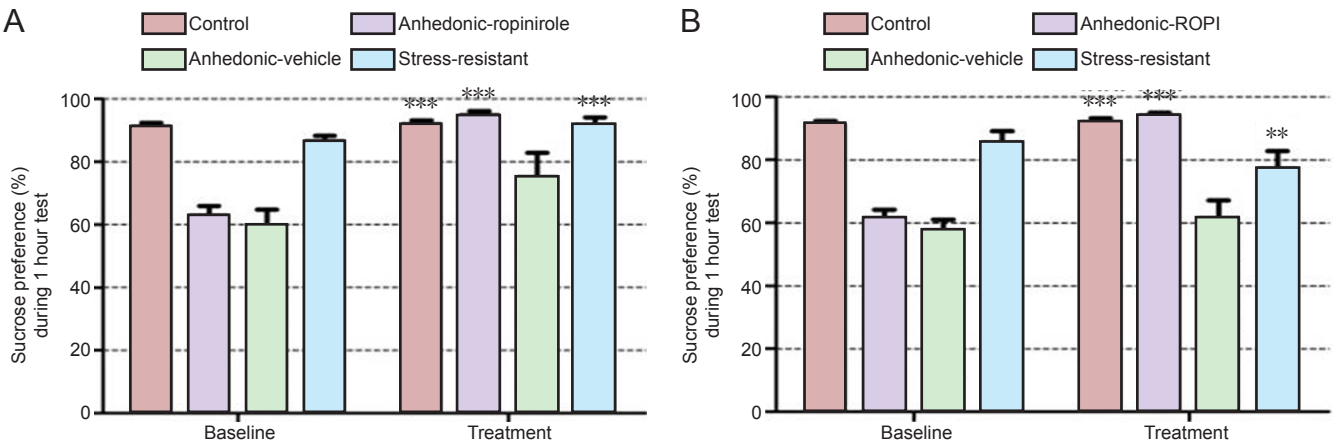

Figure 6 Ropinirole decreased anhedonia in rats susceptible to depression under chronic unpredictable stress.
Chronic (A) and acute (B) intraperitoneal injection of ropinirole significantly elevated sucrose preference levels in rats with anhedonia (***$P < 0.001$, **$P < 0.001$). Data are expressed as mean ± SEM. Control: Control group ($n = 8$); anhedonic-ropinirole: anhedonia-ropinirole group ($n = 8$); anhedonic-vehicle: anhedonia-saline control group; stress-resistant: stress resistance group ($n = 10$). Multivariate analysis of variance and the Bonferroni test were used.

2 days. On the first day, rats in the stress group were placed in the swimming pond for 15 minutes. The rats were then taken out of the swimming pond, dried and housed in cages for 24 hours. The rats in the stress and control groups were placed in the swimming pond for 5 minutes to observe their swimming behavior and floating behavior. Precise data on swimming and floating behaviors were analyzed using Ethovision software, which identified swimming and floating behaviors by analyzing the percentage of changes in animal images.

Establishment of rat models of chronic unpredictable stress

Depression models induced by chronic unpredictable stress were first established by Willner in 1987 (Abdo et al., 2010). The models were established by administering a series of chronic unpredictable mild stresses to simulate various stresses in daily life. The various stresses were given in a pseudo-random method. Stressors included: twice 2-hour restraint stress (Strekalova et al., 2005), twice 30-minute low-temperature stress (0–4°C), three times 8-hour high-temperature stress (32 ± 1°C), three times 12-hour crowded living, twice 12-hour wet floor, three times 18-hour food deprivation, twice 12-hour water deprivation, twice 1-hour empty bottle stress, three times 12-hour strong light exposure, once 5-minute cold water swimming (4°C), four times cage tilt at 45°, and three times strobe light stress (Katz et al., 1981; Valverde et al., 1997).

A total of 40 rats received tests of sucrose preference, were subjected to the elevated plus maze and were weighed. They were divided into a stress group (n = 30) and a control group (n = 10). No significant difference in the above indices was observed between the stress and control groups. Chronic unpredictable stress was performed for 4 weeks. Rats' weights were measured every day, and sucrose preference was measured every week. After stress, the sucrose preferences and weights of animals were measured again. The volumes of sweet water and water consumed within 1 hour and 12 hours were calculated. Sucrose preference (%) was calculated as the volume of sweet water/(the volume of sweet water + the volume of water) × 100%.

Stereotaxic localization of rat brain

The rats were intraperitoneally anesthetized with sodium pentobarbital (55 mg/kg), and intraperitoneally injected with atropine (0.05 mg/kg) to avoid respiratory distress (Agustin Zapata and Chefer, 2009). Rat skulls were fixed with a stereotaxic apparatus (Woruide, Shenzhen, Guangdong Province, China). In accordance with a stereotaxic atlas (Paxinos and Watson, 1997), the precise sites of the nucleus accumbens injections were + 1.7 mm posterior to the anterior fontanelle, and ± 1 mm lateral to the left and right.

Screening of anhedonia and stress-resistant animals under chronic unpredictable stress and drug intervention

In accordance with sucrose preference at 4 weeks of chronic unpredictable stress, 16 anhedonia rats and 10 stress-resistant rats were selected. Anhedonia rats were further assigned to an administration group (n = 8) and a control group (n = 8). In the ropinirole experiment, the rats were intraperitoneally injected with the dopamine 2/3 receptor subtype agonist ropinirole (1 mg/kg, 0.65 mg/kg), once a day, for 7 consecutive days. On the 7th day, sucrose preference was tested. In the administration test in the nucleus accumbens, all rats received intubation, and were allowed to recover for 6 days after surgery. Sucrose preference was then measured. Ropinirole (1.625 μg/μL) was also injected into the nucleus accumbens of anhedonia rats 30 minutes before the test, while an equal volume of physiological saline was injected in control rats.

Screening of floating susceptibility and stress-resistant animals in the forced swim stress and with drug intervention

Desipramine is a tricyclic antidepressant. Its main mechanism of action is inhibiting the reuptake of norepinephrine, but the effects of desipramine on reuptake of serotonin are weak. We observed the effects of desipramine on floating behavior during 15-minute pre-processing (forced swimming), selected the most sensitive time for drug treatment, and finally identified the depression index, which could be used as a standard to select rats with a high or low percentage of floating behavior in the subsequent tests. Thus, 16 rats with a high percentage of floating behavior and 8 rats with a low floating percentage were selected. The 16 rats with high percentage of floating behavior were assigned to a ropinirole administration group (n = 8) and a control group (n = 8), and subjected to testing.

Statistical analysis

All data were expressed as mean ± SEM, and were analyzed using GraphPad prism 4.0 and SPSS 17.0 software (SPSS, Chicago, IL, USA). Multivariate analysis of variance was applied when experimental data contained two or three factors (two- or three-way analysis of variance. Multiple comparisons of the differences in intergroup data were performed using Duncan's method or Bonferroni test. Data were compared between groups using the two sample t-test. A value of $P < 0.05$ was considered statistically significant.

Results

Forced swim stress apparently increases floating behavior of rats in the stress group

Fifteen-minute pre-processing before the forced swim test resulted in significant model effects, with significantly diminished high-intensity exercise and movement time in the forced swim test in the stress group, and increased floating time (**Figure 1A–C**, $P < 0.001$). No significant difference in fecal excretion was observed between the stress group and control group (**Figure 1D**), which indicated that the increase in floating behavior was not induced by non-specific emotional changes in the stress group.

Chronic unpredictable stress obviously decreases sucrose preference in rats in the stress group

The sucrose preference level began to decrease 1 week after chronic unpredictable stress and maintained until the end

of the stress (4 weeks) in the stress group. Two-way analysis of variance demonstrated a significant intergroup main effect ($F_{(1, 37)}$ = 19.23, P < 0.0001), and a significant interaction ($F_{(4, 37)}$ = 3.505, P < 0.05), but there was no main effect of time (P = 0.11). The Bonferroni test revealed that the sucrose preference level reduced at 3 weeks after stress in the stress group (P < 0.01). Significant differences in sucrose preference level were visible between the stress and control groups at 4 weeks (P < 0.001; **Figure 2A**). The decreased sucrose preference level was a major index of successful model establishment. That is, the rats suffered from anhedonia, which is a key symptom of depression.

Under the initial state, no significant difference in weight was detected between the stress and control groups (337 g *vs*. 338 g, P = 0.8676). It is clearly observed that the increase in weight in the stress group was significantly slower than that in the control group, and the weights of rats in the stress group even diminished. Repeated-measures analysis of variance revealed significant differences in weight between the stress and control groups from day 2 of stress ($F_{(1, 37)}$ = 88.54, P < 0.001; **Figure 2B**).

Acute floating behavior is predictive of anhedonia in rats

The rats were sorted according to floating time in the forced swim group. Eight rats with a high percentage of floating behavior and eight with a low percentage of floating behavior were screened (P < 0.001; **Figure 3A**). After chronic unpredictable stress, sucrose preference levels were significantly lower in rats showing a high percentage of floating behavior than in those with a low percentage at 4 weeks (P < 0.05; **Figure 3B**). Data analysis revealed that rats showing a high percentage of floating behavior under conditions of acute stress also showed anhedonia under the chronic stress.

A dopamine 2/3 receptor agonist reduces floating behaviors in depression-susceptible rats under acute stress conditions

As shown in **Figure 4**, no significant difference in floating behaviors was detected between ropinirole-treated (intraperitoneal injection of ropinirole 0.65 mg/kg per day) and non-model rats showing a high percentage of floating behaviors (P > 0.05). Significant differences in floating time were observed between the high-percentage floating behavior saline control group (HI-vehicle) and the saline control group (vehicle) (P < 0.001). Significant differences in floating time were also observed between the ropinirole- and saline-treated rats showing a high percentage of floating behaviors (P < 0.05). Significant differences in floating time were detectable between the high-percentage floating behavior saline control group (HI-vehicle) and the low-percentage floating behavior group (low-immobile) (P < 0.001). These findings suggested that intraperitoneal injection of a dopamine 2/3 receptor agonist decreased floating behaviors (**Figure 4B**).

By contrast, following injection of ropinirole into the nucleus accumbens, no significant difference in floating behaviors was detected between the ropinirole- and saline-treated rats showing a high percentage of floating behaviors (P > 0.05). Significant differences in floating time were detected between high-floating saline control group (HI-vehicle) and saline control group (vehicle) (P < 0.05). Significant differences in floating time were observed between the high-percentage floating behavior saline control group (HI-vehicle) and the low-percentage floating behavior group (low immobile) (P < 0.05). These findings indicated that nucleus accumbens microinjection of a dopamine2/3 receptor agonist could reduce floating behaviors (**Figure 4D**).

Noticeable "individual difference" in rats under chronic unpredictable stress

Chronic unpredictable stress can be used to induce depression models of strong and persistent stress. The reduction in sucrose preference did not appear in all rats in the stress group. The sucrose preference level began to decrease from 1 week after stress in some rats, and was maintained until anhedonia appeared. By contrast, the sucrose preference level was high during the test, which was called stress resistance (**Figure 5**).

During chronic unpredictable stress, the sucrose preference level gradually diminished with time in rats with anhedonia. Conversely, the sucrose preference level remained at a high level in rats with stress resistance. Repeated measures analysis of variance results demonstrated a significant main effect among the anhedonia, stress-resistance and control groups ($F_{(2, 26)}$ = 27.715, P < 0.001), a significant main effect of time ($F_{(4, 104)}$ = 4.23, P < 0.001), and a significant interaction of "group" × "time" ($F_{(8, 104)}$ = 4.938, P < 0.001). The Bonferroni test indicated that, from 2 weeks, significant differences were observed between the anhedonia group and the stress resistance group (P < 0.05 or P < 0.001). Simultaneously, significant differences were also apparent between the anhedonia group and control group from 2 weeks (P < 0.001 or P < 0.001). These results indicated that, because of innate differences in susceptibility, sucrose preference levels were quite different among groups.

A dopamine 2/3 receptor agonist diminishes anhedonia in rats susceptible to depression under conditions of chronic unpredictable stress

Intraperitoneal injection of ropinirole for 1 week significantly increased sucrose preference in rats with anhedonia (P < 0.001, **Figure 6A**). Nucleus accumbens microinjection of ropinirole significantly increased the sucrose preference level in rats with anhedonia (P < 0.001; **Figure 6B**).

Discussion

The forced swim test is characterized by a short stress time, easy to identify behavioral output, and sensitivity to antidepressants, and has been extensively used to induce animal models of depression (Porsolt et al., 1977). If forcing rats to swim in a limited space, the rats will finally stop trying to escape, a show of floating behavior. The 15-minute pre-processing before forced swim test is an inevitable stress. During the test, their behavior will alter after struggling for a time. That is, their behavior changes from a positive status (vio-

lent struggle) to a negative status (keeping the head above the surface), which is associated with rats' recognition about their own state. The first time the rats entered the pool, they attempted to get out of this predicament. After failure, behavioral inhibition appeared. After re-entering the same environment (they cannot escape from the pool), the sooner they understood this situation, the earlier the passive avoidance behavior occurred: rats showed a short struggling time, early appearance of immobile status, and a long duration of immobility. Many investigators believe that this typical stable floating behavior reflects a desperate state in rats. Moreover, multiple effective depression treatment reduced floating behavior. This model-induced depression-like behavior can be relieved by effective "non-drug treatments", including electroconvulsive therapy, REM sleep deprivation and rich environmental exposure (Porsolt et al., 1978).

In the present study, 15-minute pre-processing obviously enhanced floating behavior in the stress group (24 hours later). Some investigators believe that floating behavior possibly benefits survival and is an adaptive behavior. In a long-term forced swim test, animals with more floating behaviors could float in the water, and did not sink (Nishimura et al., 1988); these animals could better cope with negative stress. Nevertheless, many researchers believe that 15-minute pre-processing would cause a negative perception of the environment by animals, believing they cannot escape from the negative stress no matter how to struggle (Tian et al., 2011). A previous study verified that defecation frequency increased in rats during two forced swim tests (Armario et al., 1988). Defecation reflects the emotional reactions of animals. A clinical study on depression confirmed that negative perception was strongly associated with depression (Mathews and MacLeod, 2005). However, sucrose preference levels did not decrease in rats with a high percentage of floating behaviors, but their sucrose intake could be increased because of the large consumption of physical energy. These findings indicated that animals with a high percentage of floating behavior screened in the 15-minute forced swimming did not display anhedonia, but their depression was temporary, so it was called state depression. Therefore, their floating behavior was elevated remarkably during the forced swim test. Animals sensitive to antidepressants possibly experience negative perception most readily when facing acute, inescapable, uncontrollable stress. These animals probably suffered from anhedonia under repeated stresses.

Models of chronic unpredictable stress are very typical and commonly used animal models of depression. A series of depression-like behaviors in rats are possibly induced by giving long-period unpredictable stresses and simulating human uncertain stress events during daily life. The main change in behaviors is the decrease in sucrose preference. Animals generally prefer sweet water, but this preference in depressed rats becomes weak, with the presence of anhedonia. Experimental results verified that, after chronic unpredictable stress, the sucrose preference level was noticeably lower in the stress group compared with the control group at 2 weeks, and the decreased sucrose preference persisted until the end of the stress. A previous study verified that chronic unpredictable stress decreased the response of animals to reward, including reducing the approach of mice to food in a new environment (Tannenbaum et al., 2002), and decreasing the nose touch response to predictable sugar reward (Phillips and Barr, 1997). The above results suggest that animals present with anhedonia under conditions of chronic unpredictable stress. Anhedonia is a core symptom of depression. This kind of depression is persistent, difficult to recover from, and can lead to other kinds of depression, *e.g.*, constitutional depression. Some studies have shown that chronic unpredictable stress can increase floating behaviors in rats. What is the relationship between floating behavior and anhedonia?

Our experimental results demonstrated that susceptible animals using floating behavior as a coping strategy during a forced swim test easily suffer from anhedonia following chronic unpredictable stress. This study established a connection in terms of behavioral indicators between acute stress models and chronic stress models, and found that state depression animals with floating behavior under the acute stress are more readily affected by anhedonia during chronic stress. These data indicate that floating behavior is a defensive mode that susceptible individuals tend to use under conditions of acute stress. However, this negative coping strategy probably causes anhedonia, because these animals could not effectively cope with the subsequent chronic stress.

Considering the key effect of the dopaminergic system in stress-related mental disorders, the present study further investigated the effects of the dopamine system on floating behaviors and anhedonia in model animals subjected to the forced swim stress and chronic unpredictable stress. After screening animals with high-floating level and anhedonia, this study demonstrated that 1-week intraperitoneal injection of ropinirole effectively reduced floating behaviors during the forced swim test, and reversed the reduction in sucrose preference level in animals showing anhedonia. Simultaneously, we also explored the effect of the nucleus accumbens-which has crucial effects on mood and reward-part of the dopamine system, on depressive behaviors. The results from this study suggest that, after screening animals with a high percentage of floating behaviors and anhedonia, microinjection of ropinirole into the nucleus accumbens before the forced swim test and sucrose preference test could effectively diminish floating behaviors under acute forced swim stress, and reversed the decrease in sucrose preference level in animals showing anhedonia. The above results suggest that the dopamine system exerts a crucial antidepressant effect on state depression and constitutional depression induced by acute stress and chronic stress, for which dopamine 2/3 receptor subtypes and the nucleus accumbens are important.

Numerous studies have demonstrated that, in a learned helplessness model, chronic injection of the dopamine receptor agonist quinpirole, the dopamine 1 receptor subtype agonist SKF38393, the dopamine 2 receptor subtype agonist quinpirole, or the dopamine 2/3 receptor subtype agonists ropinirole or S32504 could reverse helpless behaviors in-

duced by uncontrollable shock (Takamori et al., 2001; Millan et al., 2004; Bertaina-Anglade et al., 2006). In chronic unpredictable stress models, the dopamine 2 receptor subtype agonists pramipexole and quinpirole increased the reduction in the amount of sugar consumed and sucrose preference level in the stress group (Muscat et al., 1992; Willner et al., 1994). However, the above antidepressant effect disappeared after withdrawal of quinpirole, but re-administration of quinpirole in a subsequent sucrose test could normalize sucrose preferences (Muscat et al., 1992). In a previous study, injection of a dopamine 2/3 receptor subtype agonist, twice a day, could restore sucrose preferences in rats with depression induced by withdrawal of metamphetamine (D'Aquila et al., 1994), suggesting that dopamine 2/3 receptor subtypes could be a key target in the treatment of withdrawal-induced depression. Our findings show that 1-week administration of ropinirole diminishes floating behaviors during a forced swim test, reverses sucrose preference levels in rats with anhedonia under chronic unpredictable stress, and further confirmed that dopamine 2/3 receptor subtypes are significant in the treatment of depression.

A clinical study found that the content of the dopamine metabolite dihydroxy acid was significantly reduced in the nucleus accumbens of depression patients who committed suicide (Bowden et al., 1997). Long-term use of most antidepressants and repeated electroconvulsive therapy would obviously upregulate dopamine receptor messenger ribonucleic acid receptor expression in the nucleus accumbens shell (Meyer et al., 2001). The studies mentioned above verified that dopamine 2/3 receptor subtypes in the nucleus accumbens had important effects on the pathogenesis of depression. Our results confirmed that nucleus accumbens microinjection of a dopamine 2/3 receptor subtype agonist could effectively diminish floating behaviors under acute forced swim stress, and reverse sucrose preference levels in animals showing anhedonia under chronic unpredictable stress, which indicates that dopamine 2/3 receptor subtypes in the nucleus accumbens have important significance and clinical application prospects for the treatment of depression.

In this study, we explored the relationship between depression and the dopamine system from the perspective of individual differences, and, in parallel, compared acute stress and chronic stress. This approach is very rare in the field, with most studies using models of forced swim stress or chronic stress (Bekris et al., 2005; Gronli et al., 2005; Vignisse et al., 2011). We established a connection in terms of behavioral indicators between acute stress models and chronic stress models, and found that state depression animals showing floating behaviors under acute stress are more readily susceptible to constitutional depression (anhedonia) during chronic stress. This connection has not previously been shown. Additionally, we focused on individual differences in different animals under the same stress condition in terms of aspects of their behavior. We screened animals based on floating behaviors under acute stress after pre-processing with antidepressants and anhedonia under chronic stress. Animals showing real depression in the stress group were selected for subsequent drug treatment in this study. We did not neglect individual differences like most similar studies (Steimer and Driscoll, 2003; Wall et al., 2003; Yeritsyan et al., 2003). While behavioral research was conducted, this study further employed behavioral pharmacology by injecting drugs into the abdominal cavity and nucleus accumbens, investigated the regulatory effects of dopamine 2/3 receptor subtypes on depression in susceptible animals under acute and chronic stresses, and found that dopamine 2/3 receptor subtypes mediated the changes in the above behaviors. Our findings are significant for the integration of animal behaviors under stresses with different time courses and for further identifying the effect of the dopamine system on the onset and treatment of depression.

We primarily showed that acute floating behavior as a passive coping strategy is a main reason for anhedonia under conditions of long-term stress. Nevertheless, other indices and effects of coping strategies on behaviors after long-term stress still need to be investigated. Further studies will investigate the effects of the dopamine system on different stages of depression from the aspects of transmitter release, receptor changes and morphological changes using microdialysis, polymerase chain reaction and immunohistochemistry.

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