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## Research report

## Effects of D-cycloserine on the behavior and ERK activity in the amygdala: Role of individual anxiety levels

Shey-Lin Wu<sup>a,b,c</sup>, Li-Sung Hsu<sup>d</sup>, Wei-Ting Tu<sup>e</sup>, Wen-Fu Wang<sup>a</sup>, Yao-Ting Huang<sup>a</sup>,  
Cornelius Rainer Pawlak<sup>f,\*\*</sup>, Ying-Jui Ho<sup>g,\*</sup>

<sup>a</sup> Department of Neurology, Chang-Hua Christian Hospital, Taiwan, ROC

<sup>b</sup> Department of Bioindustry Technology, Dayeh University, Taiwan, ROC

<sup>c</sup> Center of General Education, Central Taiwan University of Science and Technology, Taiwan, ROC

<sup>d</sup> Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taiwan, ROC

<sup>e</sup> Institute of Nanoengineering and Microsystems, National Tsing Hua University, Taiwan, ROC

<sup>f</sup> Department of Psychopharmacology, Central Institute of Mental Health, Mannheim, Germany

<sup>g</sup> School of Psychology, Chung Shan Medical University, Taiwan, ROC

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### Abstract

Low dose of D-cycloserine (DCS), a partial agonist of glycine binding site on *N*-methyl-D-aspartate (NMDA) receptors, can facilitate extracellular signal-regulated kinase1/2 (ERK1/2) activity in the amygdala and modulate emotional behavior. However, the relationship between ERK1/2 activation, individual anxiety levels, and DCS is unknown. Therefore, based on open arm time in the elevated plus-maze, male Wistar rats were divided into subgroups with either low (LOA) or high open arm (HOA) time. Open arm time is usually accepted as a critical index of unconditioned anxiety-like/avoidance behavior. On the following day, DCS (30 mg/kg, i.p.) was administered 30 min before the second elevated plus-maze test. On day 8 and 9, the rats were subjected to a 2-day session of the forced swim test, receiving the DCS treatment again 30 min before the 2nd day. On the 16th day, 30 min after the administration of DCS, the rats were sacrificed in order to detect the phosphorylation of ERK1/2 (p-ERK1/2) in the amygdala by Western blots. The results showed that: (1) DCS decreased the open arm time in HOA but not LOA rats. (2) DCS suppressed the immobility in the day-2 trial of the forced swim test and increased the p-ERK1/2 level in the amygdala in LOA but not HOA rats. This is the first instance data has been found indicating different sensitivities of p-ERK1/2 and behavioral responses to the treatment of DCS between HOA and LOA rats. The results suggest that the activity of NMDA receptor-mediated ERK1/2 signaling is mediated by individual behavioral differences which are related to the antidepressant-like activity of DCS. This study provides first insight into the pathophysiological role of ERK signaling with regard to individual differences in emotional behavior.

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### 1. Introduction

Stress has been recognized as an important factor involved in the development of affective disorders [50]. Vulnerable to aversive stimuli, the amygdala has been implicated as a critical brain site in mediating the effects of stress-related emotional stim-

uli. The glutamatergic *N*-methyl-D-aspartate (NMDA) receptor in the amygdala is reported to be involved in several stress responses, for example, anxiety, fear, and depression [24,27]. The receptor may exert its effects by modulating extracellular signal-regulated kinases 1 and 2 (ERK1/2) signaling pathways [1,22]. Although much has been learned about the neurobiological mechanisms underlying affective disorders at systematic and cellular levels, relatively little is known about the molecular mechanisms of ERK1/2 underlying such diseases.

Previous observations indicate that stress alters the activity of intracellular signaling pathways [52] and aggravates the symptoms of affective disorders [50]. Recently, attention has been focused on signaling pathways mediated by ERK1/2 in the

\* Corresponding author at: No. 110, Sec. 1, Chien-Kuo N. Road, Tai-Chung City 402, Taiwan, ROC. Tel.: +886 4 24730022x11853; fax: +886 4 23248191.

\*\* Corresponding author at: J5, D-68159, Mannheim, Germany.

Tel.: +49 621 1703 6262; fax: +49 621 1703 6255.

E-mail addresses: cornelius.pawlak@zi-mannheim.de (C.R. Pawlak), yjho@csmu.edu.tw, joshuayjho@yahoo.com.tw (Y.-J. Ho).

exploration of the pathophysiology of depression [45]. ERK1/2 is extensively distributed throughout the central nervous system [16]; its signaling cascade takes part in learning [58], memory [56], and neuronal plasticity [13]. Importantly, after being activated, the phosphorylation of ERK1/2 (p-ERK1/2) regulates the neuronal function [60] and is capable of suppressing depressive behavior in learned helplessness and forced swim paradigms [59]. These data suggest that p-ERK1/2 may be relevant to the molecular pathophysiology of depression and/or stress response.

Direct NMDA agonists may be neurotoxic [46]; but the glycine modulatory site of the NMDA receptor provides an opportunity to modulate the glutamatergic activity in a less potent fashion. Recent results show that D-cycloserine (DCS), a partial agonist at the glycine modulatory site on the NMDA receptor, can regulate glutamatergic activity in a more limited fashion [40]. DCS is a widely available, safe compound that has been applied in the clinic for several decades, without side effects on cardiac, respiratory, and hormone function [66]. Although DCS at a high dosage of 500–1000 mg/day was used as an antibiotic to cure tuberculosis [44,55]; it has also been found that the dosage of 500 mg/day improves several psychological symptoms, such as insomnia, distaste, and depression, in 47% of patients with tuberculosis [8,9]. Further, acute DCS as an extension of exposure-based psychotherapy accelerates the associative learning processes that contribute to ameliorating psychopathological conditions [53]. In addition, a low level of DCS, 50–100 mg/day, either administered alone [65] or combined with antipsychotics [17–21], decreases negative syndromes and increases cognitive functions in schizophrenic patients. In rats, systemic administration of DCS (3 and 30 mg/kg), enhances learning and memory function [39]. Moreover, systemic administration (15 mg/kg, SC) of and intra-amygdala infusions of DCS cause an elevation of p-ERK1/2 in the amygdala and facilitates the extinction of conditioned fear [41,42,67].

The elevated plus-maze is a widely used behavioral paradigm in the field of experimental anxiety research [54], which presumably measures anxiety-like and fear-motivated avoidance behavior [23,49]. Further, the forced swim test has been widely used as an animal model of depression [51]. During a typical forced swim test animals show increased immobility time in the day-2 trial, after being exposed to inescapable stress in the day-1 trial [25]. Apart from general patterns, however, several experiments have shown that rats, although identical in strain, sex, and age, can differ systematically in their behavioral response to an elevated plus-maze [11,57]. In addition, pharmacological experiments have shown that this paradigm yields a wide range of variance [30], suggesting that there are individual differences in the sensitivity of behavior to drug treatment [4,28,37,62]. Thus, we have recently shown that rats upon their first exposure to the elevated plus-maze can be divided into those that spent less time on the open arms (LOA; previously termed high anxiety (HA) rats), and those that spent more time on the open arms (HOA; previously termed low anxiety (LA) rats) [25,28]. These LOA and HOA rats differ in several aspects, for example, behavior [25], neurochemical functions [57,63,64], drug susceptibility [28], and molecular levels [47,48].

The present experiment was designed to obtain more detailed behavioral information on rats with individual differences in anxiety-like behavior during elevated plus-maze testing, and asked whether such individuality is related to emotional and molecular responses to DCS treatment. For this purpose, rats were initially tested in a similar way as in our previous studies [25,28,57,64], that is, they were screened in a plus-maze. Based on the first plus-maze trial, they were divided into LOA and HOA groups, and retested. One week later, all rats were tested in a forced swim test. Then, the effects of DCS on behavior and activity of ERK1/2 in the amygdala were analyzed.

## 2. Materials and methods

### 2.1. Animals

All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of the Chung Shan Medical University. Forty-three male Wistar rats ( $242 \pm 4$  g; National Laboratory Animal Center, ROC) were housed in groups of five rats in acrylic cages (35 cm  $\times$  56 cm  $\times$  19 cm). The housing room was maintained on a 12-h light:dark cycle (lights on: 7:00–19:00 h), with food and tap water provided *ad libitum*. Each animal was handled and gentled for 5 min on 3 consecutive days prior to the experiment.

### 2.2. General procedure

Behavioral tests were performed in the following order: a 2-day session of an elevated plus-maze test began with the 1st day, a 2-day session of a forced swim test started on the 8th day, and thereafter the rats were sacrificed on the 16th day. The procedure of behavioral observation was consistent with our previous report [26]. Briefly, the behavioral tests were begun 3 h after the start of the light cycle on. First, the animals were weighed in the animal room, placed individually in a clean cage, and transported to a dim observation room (28 lux). The test equipment was thoroughly cleaned by using 20% alcohol before each rat was tested. DCS (Sigma, USA) was dissolved in saline (0.9% NaCl) immediately before usage and administered by intraperitoneal (i.p.) injection in a volume of 1 ml/kg. The animals received three injections of either DCS or saline, 30 min before each of the second trial of plus-maze and forced swim testing, respectively, and before killing.

### 2.3. Behavioral tests

#### 2.3.1. Elevated plus-maze test

The elevated plus-maze apparatus was made of plastic and consisted of two opposed open arms (50 cm  $\times$  10 cm), two opposed closed arms with no roof (50 cm  $\times$  10 cm  $\times$  40 cm), and an open square (10 cm  $\times$  10 cm) in the center and was located 50 cm above the floor. Behavior in the elevated plus-maze was observed for 5 min as described previously [25]. The following measures were analyzed from videotapes: (1) the number of entries into and (2) the time spent on open or closed arms; (3) open arm latency; and (4) within-arm activity, that is, the number that an animal crossed a virtual line which divided an arm into a proximal and a distal half. Each rat was tested on two consecutive days (5 min each). The open arm time in the first elevated plus-maze test (trial 1) was used to screen individual anxiety-like levels and to establish high (HOA) and low (LOA) open arm responders of the same size. On the following day, 30 min prior to the plus-maze test, the rats received intraperitoneal injections of either saline or DCS (30 mg/kg),  $n=10-11$  for each group (trial 2). The rationale of a single dosage used in the present study was based on the fact that the dosage of DCS used in behavioral studies is ranging from 0.5 to 30 mg/kg [39,67]. In our previous study, the treatment of 5–30 mg/kg DCS caused dose-related behavioral changes, with the most pronounced changes induced by 30 mg/kg DCS in the elevated plus-maze [26].

### 2.3.2. Forced swim test

This test was carried out in a clear glass tank (25 cm × 25 cm × 60 cm) containing 39 cm of clean water at 26 °C. The apparatus was cleaned thoroughly and the water changed between tests on different rats. A swimming test was performed on two consecutive days (15 min on day 1, and 5 min on day 2) and videotaped as described previously [26,29]. Thirty minutes before the day-2 session, rats received the injection treatment that was identical to what they had received in the plus-maze test. Immobility was measured from the videotapes and was defined as when the rats remained motionless or floating (including small limb movements to keep their heads above the water) [2]. Data from the first 5 min of each session were analyzed.

### 2.4. Western blotting

Animals were sacrificed 1 week after the forced swim test by exposure to CO<sub>2</sub> 30 min after injection of DCS and their brains immediately removed. The amygdala was dissected out on an ice-bath plate. The protein in the tissue was extracted by homogenizing the tissue in ice-cold lysis buffer, containing 20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium orthovanadate, leupeptin (1 µg/ml), and 1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 2900 × g for 15 min at 4 °C (Hermle Z323K centrifuge, Gosheimerstr, Germany), the supernatant re-centrifuged under the same conditions, and the final supernatant taken and its protein concentration measured using a Bio-Rad protein assay kit (Bio-Rad laboratories, CA, USA). The protein was mixed with loading dye and denatured at 100 °C for 10 min. Thereafter equivalent amounts (35–50 µg per lane) of protein for each sample were separated in 10% sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE). Proteins in the gel were electrotransferred onto polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked over night at 4 °C with phosphate-buffered saline (PBS) containing 5% skim milk, and then incubated with the primary antibodies, a mouse monoclonal antiphospho-ERK antibody (Santa Cruz Biotechnology, CA, USA), for 1 h at room temperature. The membrane was washed with PBS containing 0.1% Tween-20, followed by reacting with horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotechnology, CA, USA), for 1 h at room temperature. After washing with PBS the reactive signals were detected by using an enhanced chemiluminescence (ECL) kit (Amersham Pharmacia Biotech, UK). The total amount of ERK1/2 was detected with rabbit polyclonal antibodies against p44/42 ERK (1:4000) (Promega, USA). Tubulin expression was used as the internal control.

#### 2.4.1. Densitometry

Densitometric analysis of band intensity in the blots was carried out with an imaging system (Fuji Film Las-3000) and an image processing software (Image-Gauge version 3.46, Science Lab 99 for Windows; Fuji Film, Tokyo, Japan). Band intensity (mean intensity minus background intensity) was measured for ERK1/2 and p-ERK1/2. For each blot, the relative protein level was calculated from the ratio of absorbance, ERK1/2 or p-ERK1/2 by tubulin, to correct for small difference in protein loading. Samples for  $n = 10$ – $11$  rats in each treatment group were analyzed in a Western blot. Values are expressed as percentage of mean of the vehicle-treated HOA group.

### 2.5. Statistical analysis

As in our previous studies [26,47,48,57,64], 43 animals were ranked using the open arm time in the first elevated plus-maze. Then, the rats were assigned via median split into two subgroups with either high (21 animals with less open arm time; LOA rats), or low anxiety-like levels (22 animals with more open arm time; HOA rats). These subgroups were used to examine the effects of DCS on behavior and ERK activity. Statistical testing was performed to compare within or between groups using  $t$ -tests for paired or unpaired data. For the data of forced swim test, when we detected  $p$ -values  $> 0.05$  in the ANOVAs, we further analyzed the data with post hoc comparisons ( $t$ -tests corrected with Bonferroni). The rationale is that a significant overall  $F$  is not required in order to conduct multiple comparisons [68], since the multiple comparison tests were initially designed without regard to the overall  $F$  [31]. Further, since the forced

swim test is a 2-day session and the changes of immobility between day-1 and day-2 are used as an index of despair behavior, paired  $t$ -test was employed for within group comparison. Statistical analysis of ERK1/2 and p-ERK1/2 data was carried out with the nonparametric Mann–Whitney  $U$ -test. All results are expressed as the mean ± S.E.M. The level of significance was defined as  $p < 0.05$ .

## 3. Results

### 3.1. Elevated plus-maze test (trial 1)

Based on the measure of time spent in the open arm of the elevated plus-maze on the day-1 session, animals were assigned to the HOA and LOA subgroups. These subgroups had the following profiles (Table 1): The latency to the first entry into an open arm was significantly higher in LOA rats ( $t = -5.26$ ,  $p < 0.001$ ), compared to HOA rats. The LOA rats spent less time in the open arm ( $t = 7.53$ ,  $p < 0.001$ ) but longer in the closed arm ( $t = -7.34$ ,  $p < 0.001$ ), compared to the HOA rats. The number of open arm entry and open arm activity were lowered in LOA rats (both  $p < 0.001$ ), compared to HOA rats; but the closed arm activity did not differ between the two subgroups.

### 3.2. Elevated plus-maze behavior after drug injection (trial 2)

Independent  $t$ -test showed that the open arm latency and closed arm time of LOA rat were significantly longer but the open arm time, open arm entry, and open arm activity were lower than that of HOA rats ( $df = 41$ , all  $t$ -values  $> 5.26$ ,  $p < 0.001$ ) (Table 1). Most behavioral differences between HOA and LOA rats in the plus-maze test were still present after injection of vehicle on the 2nd day, since open arm time, open arm entries, and open arm activity were still lower but closed arm time was higher in LOA rats ( $df = 20$ , all  $t$ -values  $> 3.13$ ,  $p$ -values  $< 0.01$ ), compared to HOA rats (Table 2). DCS 30 mg/kg did not influence the behavior of LOA rats in the plus-maze. In contrast, the treatment of DCS significantly changed the behavior of HOA rats by increasing the closed arm time but decreasing the open arm time, open arm entry, and open arm activity ( $df = 20$ , all  $t$ -values  $> 2.02$ ,  $p$ -values  $< 0.05$ ), compared to the vehicle-treated group (Table 2).

Table 1  
Behavior of rats in the elevated plus-maze test (1st trial)

	HOA rats ( $n = 22$ )	LOA rat ( $n = 21$ )
Open arm latency (s)	12.7 ± 5.1	167.5 ± 29.7***
Open arm time (s)	105.3 ± 12.0	10.1 ± 3.2***
Open arm entry (no.)	7.0 ± 0.6	1.0 ± 0.3***
Open arm activity (no.)	16.3 ± 1.8	1.3 ± 0.5***
Closed arm time (s)	126.8 ± 10.6	232.3 ± 9.6***
Closed arm entry (no.)	10.8 ± 0.9	10.9 ± 0.7
Closed arm activity (no.)	29.9 ± 2.5	33.3 ± 1.7

Abbreviations: HOA: high open arm time; LOA: low open arm time. \*\*\* $p < 0.001$ , compared to the HOA rats, two-tailed  $t$ -test. Data are expressed as mean ± S.E.M.

Table 2  
Effects of D-cycloserine on the behavior in the elevated plus-maze test (2nd trial)

	HOA rats		LOA rats	
	DCS 0 mg/kg (n = 11)	DCS 30 mg/kg (n = 11)	DCS 0 mg/kg (n = 11)	DCS 30 mg/kg (n = 10)
Open arm latency (s)	31.8 ± 26.9	56.8 ± 29.7	116.5 ± 43.1	187.5 ± 41.0
Open arm time (s)	86.4 ± 16.9	43.4 ± 9.6*	22.0 ± 7.5 <sup>###</sup>	16.6 ± 9.1
Open arm entry (no.)	7.8 ± 1.2	4.7 ± 1.0*	1.7 ± 0.5 <sup>###</sup>	1.7 ± 0.9
Open arm activity (no.)	19.4 ± 3.2	8.9 ± 2.6*	3.2 ± 1.3 <sup>###</sup>	3.4 ± 2.3
Closed arm time (s)	103.2 ± 16.1	172.6 ± 9.9**	190.5 ± 22.8 <sup>###</sup>	232.6 ± 15.0
Closed arm entry (no.)	9.6 ± 1.2	9.3 ± 0.6	9.0 ± 1.1	11.7 ± 1.2
Closed arm activity (no.)	24.8 ± 3.5	25.5 ± 2.0	24.6 ± 3.1	32.7 ± 3.2

Abbreviations: DCS: D-cycloserine; HOA: high open arm time; LOA: low open arm time. \* $p < 0.05$ , \*\* $p < 0.01$ , compared to the vehicle control group in the same anxiety category. <sup>###</sup> $p < 0.01$ , <sup>####</sup> $p < 0.001$ , differences between LOA and HOA rats. All analyses are two-tailed  $t$ -tests. Data are expressed as mean ± S.E.M.

3.3. Forced swim test

Immobility time was significantly increased from day-1 to day-2 session in all groups (df = 10, all  $t$ -values > 3.71,  $p$ -values < 0.05), except for LOA rats receiving 30 mg/kg of DCS treatment (Fig. 1). Finally, the correlations of open arm time in the day-1 trial of elevated plus-maze with the immobility time in the forced swim test on day-1 and that on day-2 after vehicle injection were not significant (data not shown).

3.4. p-ERK1/2 Western blot

The presence of p-ERK1/2 in the amygdala was not different between the LOA and HOA rats treated with vehicle. The administration of 30 mg/kg DCS increased the level of p-ERK1/2 in the amygdala only in the LOA ( $U = 8.00$ ,  $p < 0.001$ ) but not the HOA rats (Fig. 2A, B). The expression of total ERK1/2 was not different between all the subgroups (data not shown). It's similar with previous findings where intraperitoneal injection of

DCS (15 mg/kg) did not affect the expression of ERK1/2 in the basolateral amygdala [70].

4. Discussion

The present study demonstrated that 30 mg/kg of DSC significantly decreased the open arm time in the elevated plus-maze

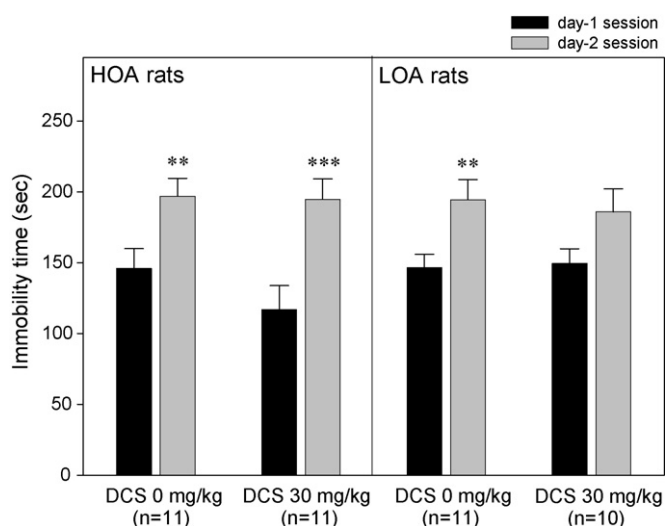


Fig. 1. Role of anxiety level in the effects of D-cycloserine (DCS) on the immobility of rats in forced swim test. Abbreviations: HOA = high open arm time; LOA = low open arm time. The immobility of LOA rats is suppressed by the treatment of DCS (30 mg/kg, i.p.) that was administered 30 min before the day-2 session. Data are expressed as mean ± S.E.M. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , compared to the day-1 session, paired  $t$ -test.

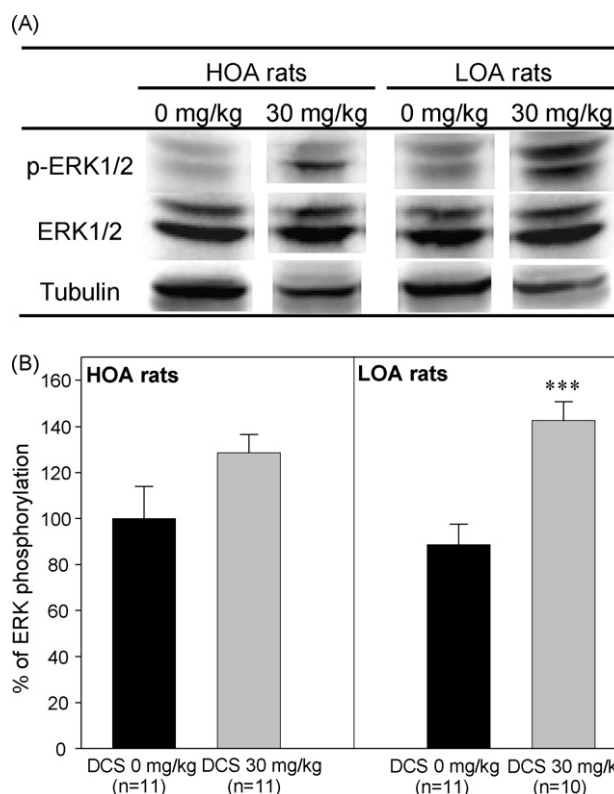


Fig. 2. Effects of D-cycloserine (DCS) on the activation of ERK1/2 (p-ERK1/2) in the amygdala. Representative Western blots showing the activation of ERK1/2 (A). Protein extracts from the amygdala are loaded in each lane. Densitometric analysis of p-ERK1/2 is shown in the histogram (B). Densitometric measures of band intensity are normalized by the corresponding tubulin band intensity to correct for differences in the amount of protein loaded in the gels. Abbreviations: HOA = high open arm time; LOA = low open arm time. Values are presented as percentage of the mean of the vehicle-treated HOA group. Data are expressed as mean ± S.E.M. \*\*\* $p < 0.001$  compared to the rats treated by vehicle in the identical anxiety category.

test, an index of anxiety-like behavior, in the HOA but not in the LOA rats. However, in the forced swim test DCS treatment suppressed immobility, which is often interpreted as “despair-like” behavior, in LOA but not HOA rats. These results suggest that DCS has differential anxiogenic-like and antidepressant-like effects in LOA and HOA rats, respectively. Further, the open arm time in the elevated plus-maze test was not related to the immobility time in day-1 or day-2 of the forced swim session, suggesting that physiological mechanisms underlying anxiety and despaired behavior are not the same. The basal levels of p-ERK1/2 in the amygdala were not different between LOA and HOA rats. However, we show for the first time that systemic DCS increased the activation of ERK1/2 in the amygdala only in the LOA but not in the HOA rats. This indicates that the sensitivity of ERK1/2 signaling in the amygdala after DCS treatment depends on individual behavioral differences, which may be involved in individual susceptibility to stress and/or pharmacological manipulation.

Systematic individual behavioral differences of drug sensitivity need to be taken into account in pharmacological manipulations. Fear-motivated avoidance behavior, i.e. spending most of the time in the closed arms, was observed during the elevated plus-maze test [23,49]. Anxiogenic drugs have been found to enhance this natural aversion towards the open arms [49]. Further, animals showed less escape activity by increasing the immobility time in the forced swim test [25]. The current study shows that DCS caused anxiogenic-like effect in HOA rats but left LOA rats unaffected; this coincides with previous pharmacological experiments showing that behavior yields a wide range of often seemingly contradictory results [30]. Furthermore, behavioral individuality was reported to determine the antidepressant-like activity of fluoxetine in the forced swim test, where only the low locomotor responders were affected [61].

Although the role of other transmitters cannot be ruled out, the glutamatergic NMDA receptors appear relevant for the present results, taking into account the considerable experimental evidence implicating a relationship between stress response and glutamatergic activity in the amygdala [24,27]. The signaling pathway of ERK1/2 is known to have an important role underlying NMDA receptor-mediated long-term potentiation (LTP), synaptic plasticity [14,33,60], and spatial learning [35]. Moreover, it has been reported that stimulation of NMDA receptors by specific agonists induces ERK1/2 phosphorylation in the limbic system [7,38], as well as cultured hippocampal [34,35] and striatal neurons [69]. These brain areas are all known to be relevant in affective behaviors. The present results show for the first time that DCS can differentially mediate ERK1/2 activity in the amygdala (part of the limbic system), when the p-ERK1/2 levels of LOA but not HOA rats were increased. In addition, the antidepressant-like activity (i.e. no significant increase in immobility time in LOA but in HOA rats in day-2 session of the forced swim test, compared to day-1 session) supports the view that NMDA receptor-ERK interaction in the amygdala may be involved in mechanisms underlying the activity of the glutamatergic system in controlling emotional processes. Bi-directional modulation of ERK1/2 signaling by

NMDA receptors has been shown in cortical neuronal cultures; low levels of NMDA receptor activation stimulate phosphorylation of ERK1/2 whereas high levels inhibit it [6]. In the present study, we found that DCS increased the level of p-ERK1/2 which suggests that the dosage used here (i.e. 30 mg/kg) is rather low.

DCS-induced suppression of immobility in the forced swim test was observed in only the LOA but not the HOA rats, indicating that DCS exerts differential depression-related effects depending on the basal anxiety-like levels measured in the plus-maze. These data provide further evidence supporting our previous conclusion that unselected (“normal”) male outbred Wistar rats show systematic individual differences in behavior [25], neurochemical function [57,63,64], drug susceptibility [28], and even molecular levels [47,48]. Since the NMDA receptor and p-ERK1/2 pathway are involved in learning and emotional behavior [35], the different sensitivity of ERK1/2 phosphorylation to the DCS manipulation between LOA and HOA rats might imply differences in behavioral adaptation during stress situations between those rats. Understanding the mechanisms behind such dichotomous signaling might be an important area of molecular neuroscience with direct clinical implications.

We suggest that ERK1/2 activation in the amygdala may influence the emotional and adaptive response to aversive stimulus. However, the effects are not general in nature, but depend on individual levels of anxiety-like behavior. Exposure to an aversive stimulus has behavioral and molecular consequences: a facilitation of the retention performance and a decrease in ERK1/2 phosphorylation in the amygdala [5]. Recent results showed that either systematic or intra-amygdala administration of DCS increases the ERK1/2 activation [70], and facilitates the extinction of conditioned fear [10]. In addition to the above findings, our present data suggest that increasing the ERK1/2 activity in the amygdala is relevant to the suppression of despair behavior in forced swim test. Local drug manipulation could provide a more direct evidence to address the mechanisms. For example, by using microinjections to deliver selective ERK1/2 inhibitors into the amygdala may be helpful to further elucidate the role of the ERK1/2 signaling cascade accompanying behavioral effects of DCS. ERK signaling may be associated with affective disorders. It has been reported that the p-ERK1/2 levels in the hippocampus and prefrontal cortex are decreased in depressed suicide subjects [12]. Further, depressed animals show decreased p-ERK1/2 in the prefrontal cortex [15,52]. Interestingly, the antidepressant-like activity of DCS was observed only in LOA but not HOA rats, consistent with previous findings in the clinical study, where the treatment of DCS improved the psychological symptoms in about half of patients with tuberculosis [8,9]. In line with previous results showing that ERK1/2 activation in the amygdala plays a role in the regulation of immobility behavior in rats during the forced swim test [32], the results demonstrated that activating the ERK1/2 in the amygdala using DCS suppresses the immobility in the forced swim test, suggesting that ERK1/2 activation in the amygdala is relevant to antidepressant-like activity. It is noteworthy that depressive-like behaviors during chronic forced swim tests are accompanied

by decreased levels of p-ERK2. Conversely, expression of p-ERK in the hippocampus and prefrontal cortex is positively correlated to hedonic behavior [52]. It has been inferred that a decrease of p-ERK2 might be at least one of the mechanisms underlying depression induced by stress. Thus, modulating the ERK1/2 activity may be a target for the treatment of affective disorders.

Stress-induced NMDA receptor activation has been reported to last for only 24 h [3,36]. Further, the ERK signaling cascade in the amygdala is transiently activated during Pavlovian fear conditioning within 60 min [56]. The response of ERK and immobility in the forced swim test after pharmacological manipulation returned to basal levels 48 h later [32]. Therefore, in the present study, we killed the rats a week after the last behavioral test to avoid the handling stress caused from behavioral testing, and took the brains 30 min after the DCS administration when p-ERK could have been provoked but not yet declined [70]. Although one might argue that the repeated administration of DCS in the present study, 7 days apart, may have produced residual effects or behavioral tolerance in the swim test, the fact that DCS in plasma and brain is rapidly eliminated, with plasma half-life about 70 min [43], indicates that this is most likely not the case.

In summary, DCS at 30 mg/kg increased the anxiety-like level in HOA rats and suppressed the despair behavior in LOA rats. The treatment of DCS significantly increased the activity of ERK1/2 in the amygdala in LOA rats. These data show for the first time that there are different sensitivity of ERK1/2 phosphorylation and behavioral responses to the treatment of DCS between LOA and HOA rats, suggesting that the activity of NMDA receptor-mediated ERK1/2 signaling facilitates individual differences that may be related to the antidepressant-like activity of DCS. Modulation of NMDA receptors and the ERK1/2 activity in the amygdala by using DCS implicates a new target for the development of drugs that prevent learned helplessness and/or depression. Taking individual differences of behavior and drug susceptibility into account in psychopathological studies is expected to improve our understanding of the mechanisms underlying anxiety and depression, and may help to further clarify the sometimes variable outcome of psychoactive drugs.

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