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Physical environment modulates the behavioral responses induced by chemical stimulation of dorsal periaqueductal gray in mice

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Abstract

In order to investigate the relationship between behaviors elicited by chemical stimulation of the dorsal periaqueductal gray (dorsal PAG) and spontaneous defensive behaviors to a predator, the excitatory amino acid D,L-homocysteic acid (5 nmol in 0.1 μl), was infused into the dorsal PAG and behavioral responses of mice were evaluated in two different situations, a rectangular novel chamber or the Mouse Defense Test Battery (MDTB) apparatus. During a 1-min period following drug infusion, more jumps were made in the chamber than in the MDTB runway but running time and distance traveled were significantly higher in the runway. Animals were subsequently tested using the standard MDTB procedure (anti-predator avoidance, chase and defensive threat/attack). No drug effects on these measures were significant. In a further test in the MDTB apparatus, the pathway of the mouse during peak locomotion response was blocked 3 times by the predator stimulus (anesthetized rat) to determine if the mouse would avoid contact. Ninety percent of D,L-homocysteic treated animals made direct contact with the stimulus (rat), indicating that D,L-homocysteic-induced running is not guided by relevant (here, threat) stimuli. These results indicate that running as opposed to jumping is the primary response in mice injected with D,L-homocysteic into the dorsal PAG when the environment enables flight. However, the lack of responsivity to the predator during peak locomotion suggests that D,L-homocysteic-stimulation into the dorsal PAG does not induce normal antipredator flight.

Keywords: D,L-homocysteic acid; Dorsal periaqueductal gray; Aversion; Mouse Defense Test Battery; Defense reactions; Mice

1. Introduction

The periaqueductal gray (PAG) is a midbrain structure divided along its rostro-caudal axis into dorsomedial, dorsolateral, lateral and ventrolateral columns (Carrive, 1993). The dorsal columns of the PAG are proposed to integrate behavioral and autonomic expression of defensive reactions (for reviews, see Bandler and Shipley, 1994; Graeff, 1990,1994). In rats, either electrical or chemical stimulation of these columns induces freezing behavior alternating with vigorous flight and apparently aimless vertical jumps (Di Scala et al., 1984; Bandler et al., 1985; Aguiar et al., 2006). These behaviors appear to be similar to escape reactions induced by natural aversive stimuli, such as exposure to a proximal predator (Blanchard and Blanchard, 1988). Stimulation of the dorsal PAG in humans undergoing surgery has produced reports of intense fear associated with autonomic reactions (e.g. tachycardia and hyperventilation) reminiscent of a full-blown panic attack (Nashold et al., 1969). Given the striking similarities between the autonomic and behavioral effects of dorsal PAG stimulation and the symptoms of panic attacks, it has been suggested that this site is

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involved in the genesis of panic disorder in humans and that dorsal PAG stimulation can serve as a model of panic attack (Graeff, 1990; Beckett et al., 1992; Jenck et al., 1995).

Recent ethopharmacological studies suggest that flight responses of mice to an oncoming threat stimulus are particularly responsive to propanic and antipanic drugs (Blanchard et al., 2001). Combining this focus on flight with dorsal PAG stimulation, Carvalho-Netto and Nunes-de-Souza (2004) reported that infusion of the excitatory amino acid D,L-Homocysteic into the dorsal PAG of mice produces explosive behaviors which include running and jumping; behaviors also previously reported in rats (Beckett et al., 1992). However, the behavioral responses elicited in that study were evaluated in a small enclosed apparatus (chamber with 23 cm diameter and 30 height), which may have prevented a clear analysis of the flight response. In fact, we have observed that mice under drug effect ran often into the walls chamber suggesting that behavioral response patterns induced by D,L-homocysteic infusion might depend on features of the physical environment (unpublished data). Hence, one of the objectives of the present work was to investigate the D,L-homocysteic-induced escape response in two different apparatuses, a small rectangular novel chamber and a large oval runway, the Mouse Defense Test Battery (MDTB) (e.g. Griebel et al., 1996). The first apparatus permitted only brief forward locomotion before the animal would encounter a wall. The second was an endless oval runway allowing mice to run forward for 2 m before being required to turn around a curved section of runway into another 2 m straight alley, ending in another curved section straightening into the original segment.

Following an initial 1-min postdrug period, animals from both conditions were tested using the standard MDTB procedure, which is designed to assess the defensive reactions of mice to a natural predator — the rat. A number of psychoactive drugs have been evaluated in this test (Blanchard et al., 2001 for review), and a consistent pattern of results has emerged. Among the various defensive behaviors, active defenses such as fight and escape appear to be selectively responsive to panicogenic compounds such as yohimbine (Blanchard et al., 1993), as well as to chronic administration of panicolytic compounds such as alprazolam (Griebel et al., 1995a), imipramine and fluoxetine (Griebel et al., 1995b). Observation of a selective increase in flight and active defenses would provide further and specific evidence that stimulation of dorsal PAG produces panic-like effects.

However, it has been well demonstrated that the main behavioral responses (running and jumping) induced by microinjection of D,L-homocysteic into the dorsal PAG, which have been considered as proximal defensive responses, remain for a brief period of 40–60 s following drug administration (Bandler and DePaulis, 1988; Beckett et al., 1992; Carvalho-Netto and Nunes-de-Souza, 2004). A further test in the MDTB apparatus was proposed to determine if the mouse would react to threat stimuli during this brief postinjection period. To determine if mice would respond appropriately to the threat, avoiding it by changing the direction of flight, the pathway of the mouse during peak behavioral responses (first minute) was blocked 3 times by the predator stimulus (anesthetized rat).

2. Materials and methods

2.1. Subjects

Subjects were male Swiss–Webster mice, obtained from Charles River Suppliers (St. Louis, MO). Upon arrival, all mice were single-housed in opaque polypropylene cages in temperature controlled room (22±1 C°) with ad lib access to food and water. The mice were acclimatized for 4–6 weeks until they reached a weight range of 30–40 g. All mice were maintained on a 12 h light/dark cycle (lights on at 07:00 am). Three male Long–Evans rats were used as predator stimuli during the course of the study.

2.2. Drugs

D,L-homocysteic acid was obtained from Sigma (St. Louis, MO) and dissolved in 0.9% sterile physiological saline. The dose used (5 nmol/0.1 μl) was based on previous studies (Carvalho-Netto and Nunes-de-Souza, 2004; Beckett et al., 1992).

2.3. Surgery

Mice were implanted unilaterally with 8 mm stainless-steel guide cannula (26-gauge) under sodium pentobarbital (90 mg/kg, i.p.) anesthesia. The guide cannula was fixed to the skull using dental cement and jewelers' screw. Stereotaxic coordinates for the dorsal PAG were 4.16 mm posterior to the bregma, 1.32 mm lateral to the midline and 2.23 mm ventral to the skull surface, with the guide cannula angled 26° to the vertical. A dummy cannula inserted into the guide cannula at the time of surgery served to reduce the incidence of occlusion. To prevent accumulation of salivatory and bronchial secretions, 0.01 mg/kg subcutaneous (s.c.) glycopyrrolate (Luitpold Pharmaceuticals, Shirley, NY), was administered 15 min before surgery. Upon removal from the stereotaxic apparatus mice were administered 1 ml 0.9% saline s.c. to prevent dehydration.

2.4. Intracerebral drug administration

Following a 1 week recovery period, mice were transferred from the main holding area to the laboratory and left undisturbed for 1 h prior to drug administration. Each mouse was lightly restrained and a 32-gauge injection cannula (1.0 mm longer than the guide cannula) was inserted into the guide cannula, the injector connected via PE-10 polyethylene tubing to a 10 μl Hamilton microsyringe. Solution administration was controlled by an infusion pump (Harvard Apparatus, Inc. USA) programmed to deliver a volume of 0.1 μl over a period of 10 s. Confirmation of successful infusion was obtained by monitoring the movement of a small air bubble in the PE-10 tubing. Immediately following drug infusion, each animal was placed in the experimental chamber or in the MDTB apparatus.

2.5. Apparatus

The behavioral responses induced by dorsal PAG chemical stimulation were evaluated in a chamber or in the MDTB apparatus.

2.5.1. Chamber

The test chamber was a black Plexiglas box (28 × 24 × 35 cm) with one side of transparent Plexiglas, which permitted recording of the experiment by a vertically mounted camera linked to a video monitor and DVD.

2.5.2. MDTB apparatus

The Mouse Defense Test battery apparatus (Fig. 1) was an oval runway, 0.40 m wide, 0.30 m high, and 4.8 m in total length, consisting of two 2.0-m straight segments joined by two 0.4-m curved segments, and separated by a median wall (2.0 m long × 0.30 m high). The apparatus was elevated to a height of 0.80 m from the floor to minimize the mouse's visual contact with the experimenter. All parts of the apparatus were made of black Plexiglas. The floor of the runway was marked with white lines every 20 cm, to facilitate distance measurement. Activity was recorded using two ceiling-mounted video cameras.

2.6. Procedure

2.6.1. Experiment 1. D,L-homocysteic-induced explosive motor response: chamber vs. MDTB apparatus

Mice were randomly assigned to four groups (n = 8–10); two groups were injected with D,L-homocysteic (5 nmol) or saline into the dorsal PAG and each animal was immediately placed in the chamber. The resultant behaviors were observed and video recorded for 1 min. The other two groups received the same pharmacological treatment and were immediately placed in the MDTB apparatus for behavioral recording for 1 min. No further manipulations or conditions were imposed during this 1-minute period. The following behavioral responses were recorded throughout the stimulation trials:

Jumping: Upward leaps directed or not to the border of the apparatus. For those groups treated with D,L-homocysteic and exposed to the chamber situation (where most of the jumps occurred), the data were presented as percentage of jumps preceded or not preceded by touch or crash into the chamber walls with the mouse's body, nose or vibrissae.
Walking: Slow locomotion with elevation of trunk and tail and out of phase stance and swing movements of contralateral limbs
Trotting: Fast locomotion with elevation of trunk and tail and out of phase stance and swing movements of contralateral limbs
Galloping: Running alternating stance and swing movements of anterior and posterior limb pairs.
Freezing: Complete absence of movement except breathing, while the animal assumes a characteristic tense posture.
Facial contact: The animal crashes or touches head first into the wall of the chamber or MDTB
Lateral contact: While galloping or trotting the animal comes into contact with the chamber or MDTB walls with its body (trunk) or its vibrissae.
Rearing: Standing on hind limbs, with both forelimbs off the floor. This measure included both unsupported rearing, and rearing against the wall.

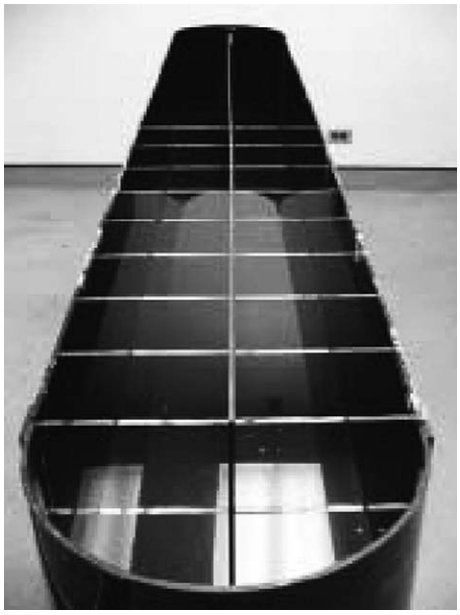

Fig. 1. An overhead view of the MDTB apparatus. The two 2-m straight segments of the oval runway are joined by two 0.4-m curved segments and separated by a median wall. The apparatus was elevated 0.8 m.

The distance traveled for each mouse was also measured during the first 20 s after intra-dorsal PAG injection.

2.6.2. Experiment 2. Standard MDTB procedure

Immediately after experiment 1 (i.e. 1 min postdrug), animals from both conditions (chamber and MDTB) were then tested using the standard MDTB procedure which is designed to assess the defensive reactions of mice to a natural predator, the rat. This procedure is described in detail in Blanchard et al., 2001, 2003). Briefly, the procedures and measures taken were as follows:

2.6.2.1. Reactions to the predator. When the subject mouse is at one end of the apparatus, a hand-held anesthetized rat is introduced into the opposite end of the open runway and brought up the subject at an approximate speed of 0.5 m/s. Approach was terminated on contact with, or movement by, the subject. Avoidance distance (the distance from rat to subject at the point of flight) was recorded. This procedure was repeated five times, with mean avoidance distance (cm) and number of avoidances calculated for each subject.

2.6.2.2. Chase. The hand-held rat is brought up to the subject at a speed of approximately 2.0 m/s. Total flight time (time taken to travel 15 m when the mouse was running away from the rat) was recorded. Overall flight speed (m/s) and maximum linear flight speed (over a 2-m linear segment of the runway) were subsequently calculated. The number of stops (pause in locomotion) and reversals (subject turned and ran in the opposite direction) were recorded.

2.6.2.3. Straight alley. Upon closing two doors (80 cm apart), one runway was converted into a closed straight alley in which

the subject was trapped. The threat stimulus (rat) was held at one end of straight alley. Subjects were given three successive 30 s trials in which both the number of approach—withdrawals and the number of voluntary contacts with the rat stimulus were recorded. Measures taken also included immobility (freezing) time and frequency of jump escapes.

2.6.2.4. Forced contact. With the alley length reduced to 40 cm the hand-held rat quickly approached and contacted the subject (five contacts). This procedure was repeated three times. During each trial, the number of vocalizations, defensive uprights, jump escapes and bites were recorded.

2.6.3. Experiment 3. Evaluating the ability of mice to react to the threat stimulus during D,L-homocysteic-induced flight

In order to evaluate the ability of mice to react to environmental stimuli following D,L-homocysteic infusion, the previous control groups (i.e. saline groups from experiments 1–2) were injected intra-dorsal PAG with D,L-homocysteic (5 nmol) 24 h following the initial test. Each animal was immediately placed in the MDTB apparatus. During the peak running response induced by dorsal PAG stimulation (i.e. first 50 s after injection) the pathway was blocked once by a closed door at the end of a runway and 3 times by an anesthetized predator stimulus (rat). To assess changes in the subject's speed and its response to the introduced block of its pathway, a frame-by-frame analysis of the DVD (1/30) was conducted. This analysis was carried out 60 cm before the animal reached the introduced block of the runway (either the door or the Rat). The scoring was divided into three equal squares (20 cm each) of the MDTB apparatus and then a mean for each animal was calculated. The procedure evaluated whether the mice would avoid contact with the interposed stimulus.

2.7. Histology

Mice were sacrificed with an overdose of sodium pentobarbital and received an infusion of 10% methylene blue intra-dorsal PAG, according to the microinjection procedure described above. The animals were perfused intra-cardially with 10 cc 0.9% formalin and their brains were removed from the cranial cavity and stored in 10% formalin/30% sucrose solution for at least 48 h before histological analysis. Mouse brains were coronally sectioned by cryostat (50 μm) and microscopically verified with reference to the atlas of Paxinos and Franklin (2001). Data from animals with injection sites outside the dorsal PAG were excluded from analysis.

2.8. Statistical analyses

The behavioral data from experiments 1 and 2 were analyzed by factorial analysis of variance (ANOVA) with saline vs. D,L-homocysteic treatment and chamber vs. MDTB apparatus as between-subjects factors. Where significant main effects (drug) or interactions (drug × apparatus) were obtained, data were further analyzed by Newman–Keuls multiple comparisons test. Repeated measures analysis of variance (ANOVA) was used to analyze the data from experiment 3. A p value ≤ 0.05 was considered significant.

2.9. Ethics

All procedures were run in accord with protocols approved by the University of Hawaii Institutional Animal Care and Use Committee.

3. Results

Histological analysis demonstrated that 36 mice had accurate cannula placements in the PAG (Fig. 2). Final sample sizes ranged from $n=8$–10 animals per group.

3.1. Experiment 1. D,L-homocysteic-induced explosive motor response: Chamber × MDTB apparatus

As shown in Figs. 3, 4 and 5 ANOVA revealed a main effect of drug for most behavioral responses [frequency of jump $F(1,31)=25.7$, $p<0.001$; frequency of facial contact $F(1,31)=10.3$, $p<0.05$; frequency of lateral contact $F(1,31)=34.9$, $p<0.001$ (Fig. 3); duration galloping $F(1,31)=66.9$, $p<0.001$;

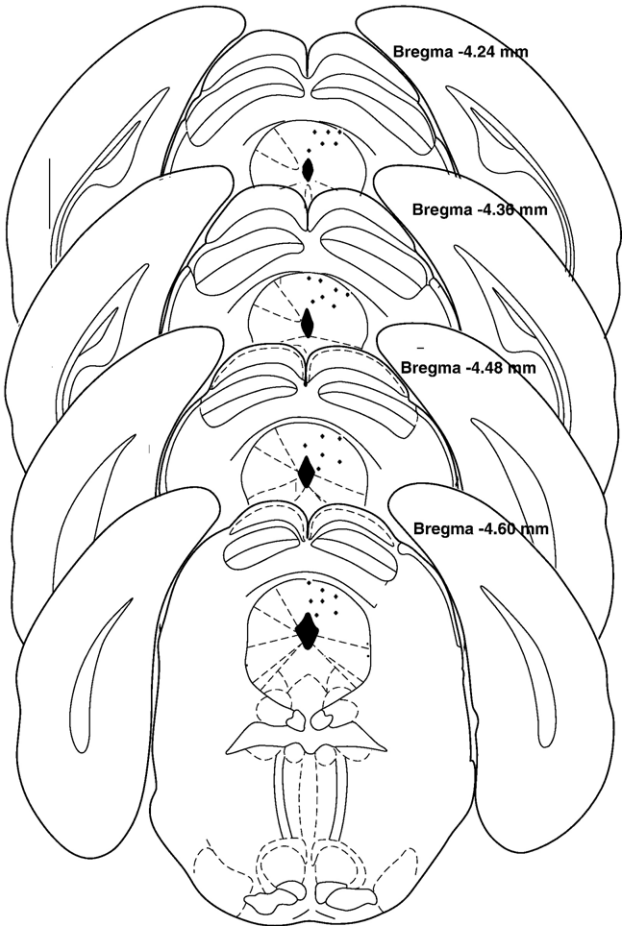

Fig. 2. Schematic representation of microinfusion sites within the midbrain periaqueductal gray (PAG) of the mouse. The number of the points in the figure is less than the total number of mice because of the overlaps.

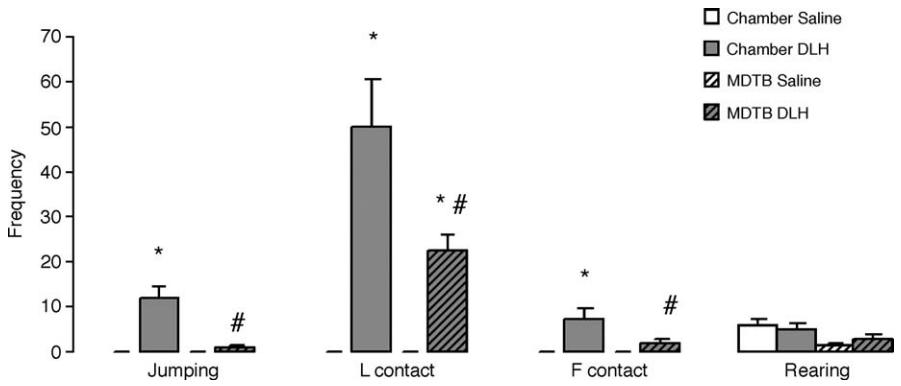

Fig. 3. Effect of D,L-homocysteic injected into the dorsal PAG on the behaviors (frequency) of mice evaluated in two different situations: chamber × MDTB (n=8–10). Each bar represents the mean±. *p<0.05 compared to respective control group and # p<0.05 compared to D,L-homocysteic Chamber group by Newman–Keuls multiple comparisons test. Lateral and facial contact behaviors are represented by L contact and F contact, respectively.

duration trotting $F(1,31)=93.7$, $p<0.001$; duration walking $F(1,31)=234.6$ $p<0.001$; duration of freezing $F(1,31)=7.13$, $p<0.01$(Fig. 4); and distance traveled $F(1,31)=185.1$, $p<0.001$ (Fig. 5)]. ANOVA for the situation/apparatus factor revealed significance for frequency of jump [$F(1,31)=19.6$, $p<0.001$]; lateral contact [$F(1,31)=5.13$, $p<0.05$]; rearing [$F(1,31)=5.54$, $p<0.05$]; duration of galloping [$F(1,31)=3.93$, $p<0.05$; and distance traveled [$F(1,31)=16.3$, $p<0.001$]. ANOVA also indicated a drug vs. apparatus interaction for jumps [$F(1,31)=19.65$, $p<0.001$]; lateral contact [$F(1,31)=5.13$, $p<0.05$] (Fig. 3); galloping [$F(1,31)=3.93$, $p<0.05$]; walking [$F(1,31)=7.06$, $p<0.01$] (Fig. 4); and, distance traveled [$F(1,31)=15.11$, $p<0.001$] (Fig. 5). This interaction was not significant for facial contact, rearing, trotting, or freezing. For treated mice in the chamber compartment, 87% of these jumps were preceded by a contact of the animal's body, nose or vibrissae with one or more chamber walls.

3.2. Experiment 2. Standard MDTB procedure

All behavioral data (means±S.E.) from the standard MDTB procedure, evaluated beginning 1 min after drug administration, are presented in Table 1. The factorial ANOVA did not indicate a reliable effect of drug for any task evaluated in the standard MDTB procedure, except jump escapes [$F(1,31)=5.92$, $p<0.05$]. The t-test for independent samples indicated that frequency of jumps decreased in the straight alley for the drugged animals that had been run in the MDTB apparatus during the first minute after D,L-homocysteic infusion, compared to controls run in the same situation ($t=2.18$, $df=15$, $p<0.05$), but it did not reveal any significant difference in comparison to animals from the Chamber situation ($t=1.02$, $df=16$, $p>0.05$).

3.3. Experiment 3. Evaluating the ability of mice to react to the threat stimulus during D,L-homocysteic-induced flight responses

Table 2 presents the mean number of frames (at 30 fps) required for animals of each group to pass through each of 3, 20-cm squares that were progressively closer to either a closed door, or an anesthetized rat, placed in the MDTB runway. The number of frames translates to flight speed, with 4 frames per second equaling 1.5 m/s, and 8.4 frames (the highest number of frames required for any group) equaling .71 m/s. ANOVA for repeated measures did not reveal any trend of increase of the number of frames required to pass through each square at 60, 40, or 20 cm while moving toward the closed door [$F(2,14)=1.0$ $p>0.05$] or the three rat-blocked pathway trials [trial 1 $F(2,14)=$

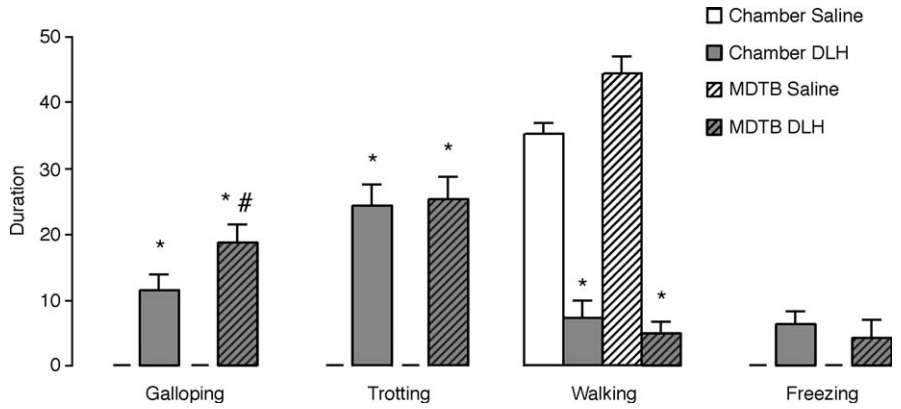

Fig. 4. Effect of DLH injected into the dorsal PAG on the behaviors (duration) of mice evaluated in two different situations: chamber × MDTB (n=8–10). Each bar represents the mean±SEM. *p<0.05 compared to control group and # p<0.05 compared to D,L-homocysteic Chamber group by Newman–Keuls multiple comparisons test.

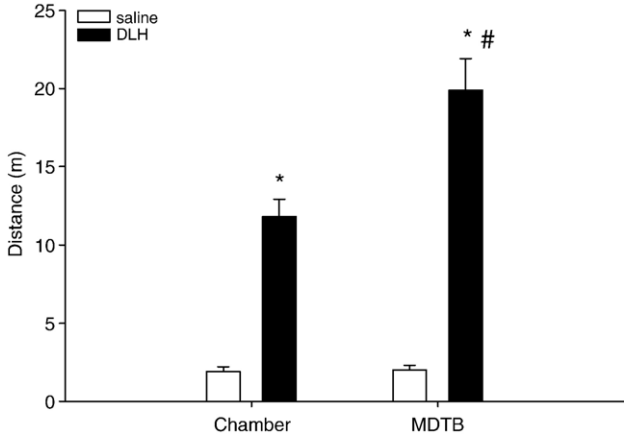

Fig. 5. Effect of DLH injected into the dorsal PAG of mice on the distance moved either in the chamber or in the MDTB apparatus (n=8–10) following 20 s initial after administration of drug. *p<0.05 compared to respective control group and #p<0.05 compared to chamber group.

0.81 p>0.05]; [trial 2 F(2,14)=1.36 p>0.05]; [trial 3 F(2.14)= 1,43 p>0.05], suggesting that the animals did not reduce speed to avoid contact with either a barrier (door) or an aversive stimulus.

4. Discussion

The present results are in agreement with previous findings in rats and mice (e.g. Beckett et al., 1992; Carvalho-Netto and Nunes-de-Souza, 2004) that microinjection of the excitatory amino acid D,L-homocysteic into the dorsal PAG produces explosive motor behaviors characterized by running (galloping and trotting) and jumping. However, this study further demonstrates that the type of motor response to D,L-homocysteic infusion is strongly influenced by features of the environment in which animals are tested. In the chamber compartment, where animals often ran into the walls, jumping was the overwhelmingly predominant response during a brief period of 40–60 s following drug administration. In the MDTB, with its endless oval runway, mice exhibited galloping, rather than jumping, as a primary response to D,L-homocysteic. In fact, in the MDTB apparatus D,L-homocysteic-treated mice failed to make significantly more jumps than controls.

These findings contrast with some previous reports that physical characteristics of the test conditions do not impact responsivity to dorsal PAG stimulation. Schenberg et al. (2005) reported that neither test arena size (20 vs. 50 cm diameter) or the presence or absence of a roof had any effects on the thresholds of somatic defensive behaviors, including jumping. Although different animal species (rat vs. mouse) and different methods (electrical or chemical stimulation) and specifics of the test arena used in these studies were different, the major difference may be between measures: thresholds for responses may be less sensitive to situational characteristics than is the expression of the responses themselves.

Our results further suggest that the switch from running to jumping immediately after D,L-homocysteic stimulation, for animals run in the chamber compared to the MDTB apparatus, may be modulated by tactile contact. In the chamber, 87% of either vertical or horizontal jumps were preceded by a contact (touch or crash) of the animal's head or vibrissae to the chamber wall. Consonant with the predominance of jumps in the chamber, more lateral and facial wall contacts occurred in this situation than in the MDTB situation. Based on findings that visual stimuli (i.e., approach of a brush or glove) did not readily evoke defensive reaction in rats that had been infused with kainic acid within the dorsal PAG, Bandler and DePaulis (1991) suggested that chemical stimulation of dorsal PAG induces a deficit in the rat's visual perception of environmental stimuli. The results of Experiment 1 suggest that D,L-homocysteic infusion provoked a

Table 1
Effect of DLH acid infusions in the DPAG on behavioral responses of mice confronted with a rat in the standard MDTB procedure following the initial 1-min post-drug period (first minute on the chamber or on the MDTB apparatus)

Behaviors	Chamber		MDTB		F(1,31)
	Control	DLH	Control	DLH	
Reaction to the predator					
Avoidance distance (cm)	60.2±15.2	22.0±10.5	53.2±13.1	46.0±12.5	3.18
Avoidance frequency	2.0±0.5	0.6±0.2	1.9±0.4	1.9±0.6	2.22
Chase/flight test					
Flight speed (m/s)	0.51±0.04	0.57±0.07	0.59±0.05	0.62±0.08	0.45
Max speed (m/s)	0.89±0.05	0.83±0.04	0.96±0.06	0.94±0.12	0.30
Stops	5.4±1.1	2.6±0.6	3.9±1.2	3.2±1.0	2.70
Reversals	5.1±1.1	2.8±0.8	3.4±1.1	3.0±1.4	1.43
Straight alley test					
Approaches/ withdrawals	2.1±0.4	1.9±0.5	2.6±0.3	2.6±0.5	0.23
Contacts	1.3±0.4	1.4±0.4	1.6±0.6	1.3±0.5	0.97
Jump escapes	1.1±0.6	0.5±0.1	4.3±0.7	2.1±0.6*	5.92
Freezing (s)	4.8±1.0	3.7±0.9	2.1±0.8	2.8±1.4	0.54
Forced contact test					
Uprights	9.5±1.9	6.4±1.3	5.6±2.1	7.6±1.2	0.13
Vocalization	11.0±1.4	7.7±1.8	11.5±1.8	11.1±0.9	1.45
Bites	0.0±0.0	0.8±0.4	0.5±0.3	1.4±0.7	3.54
Jump escapes	4.7±2.2	7.0±1.4	9.1±2.0	7.6±1.2	0.55

Values represent means±S.E.M. *p<0.05 compared to control.

Table 2
Response of the animals under DLH-initial effect peak (explosive flight) to environmental stimuli (door or rat)

Target	Frames in the first square (60 cm from target)	Frames in the second square (40 cm from target)	Frames in the third square (20 cm from target)	Percentage of the contact
Closed door	4.0±0.3	4.0±0.3	3.9±0.2	100
Rat block trial 1	5.8±0.6	6.4±1.0	5.8±0.6	90
Rat block trial 2	6.0±0.4	6.1±0.5	6.5±0.6	90
Rat block trial 3	6.8±0.4	7.3±0.4	8.4±1.4	80

The values represent the means of the number of frames required to pass through each square at 60, 40, or 20 cm while moving toward a closed door or one of three rat blocked pathway trials. It was tallied to demonstrate differences in speed based on the location the target. Each square represents a 20 cm block in the MDTB apparatus. Contact was considered when the subject crashed or touched its face into the target (door or rat).

similar effect in mice, along with a concomitant increase in the role of tactile stimuli in modulating the behavioral responses elicited by D,L-homocysteic acid infusion into the dorsal PAG.

Previous studies have emphasized that jumping, running, or galloping induced by dorsal PAG stimulation are related to natural defensive responses elicited by aversive situations (Beckett et al., 1992; Keay and Bandler, 2001; Vianna and Brandao, 2003; Bittencourt et al., 2004; Schenberg et al., 2001), as they are similar to escape reactions induced by natural aversive stimuli, such as the exposure to a proximal predator (Blanchard and Blanchard, 1988; Blanchard et al., 2001). They have also been recently proposed as indices of panic-like behaviors in rats (Vargas and Schenberg, 2001).

While present results suggest that the choice of running or jumps after D,L-homocysteic stimulation is largely based on environmental stimuli, specifically tactile contact with walls, these results are not counter to a view that the dorsal PAG contains elements of defense systems that are normally active when confronting aversive stimuli. However, in Experiment 2, animals tested in the MDTB following the initial 1-minute postdrug period did not show any reliable changes in their defensive reactions to a predator (rat). The failure of D,L-homocysteic to enhance any of these defensive behaviors is likely related to the brevity of effects observed with intra-PAG injections of this excitatory amino acid (Beckett et al., 1990; Carvalho-Netto and Nunes-de-Souza, 2004). Even though the initial motor reaction induced by D,L-homocysteic has a short duration, another behavioral response, immobility, may last at least 5 min (Beckett et al., 1990; Carvalho-Netto and Nunes-de-Souza, 2004). However, it is not clear if such immobility is part of the behavioral defense repertory or is a sign of exhaustion or quiescence following the dramatic locomotor activity burst elicited by D,L-homocysteic as an initial response. Bandler and DePaulis (1988) have shown that microinjections of low doses of another excitatory amino acid, kainic acid, in the PAG region evoked a significant increase in both defensive and immobile behaviors in rats tested in a social interaction test situation. The elicited reactions appeared identical to the rat's natural reactions to attack by a conspecific. The long duration and natural appearance of the kainic acid evoked reactions stands in contrast to the short explosive reactions provoked by injections in the PAG of the other excitatory amino acids such as D,L-homocysteic.

Further contrasting with kainic acid results from Bandler and DePaulis (1988), these D,L-homocysteic-stimulated animals did not show potentiated responsivity to an animated threat stimulus. The D,L-homocysteic-induced flight response was neither initiated nor guided by the introduction of the predator. It always began immediately on placement of animals into the MDTB (Experiment 1) and when a rat was introduced into the path of a running mouse, D,L-homocysteic-stimulated mice ran toward it and typically into it, or to an interposed door, without slowing (Experiment 3). Although low doses of kainic acid in the PAG may have degraded visual processing, they nonetheless produced a significant increase in both defensive and immobile behaviors in rats in the social interaction test (Bandler and DePaulis, 1988). According to Bandler and DePaulis (1988), defensive responses to a conspecific were evoked by contact with another rat during these social encounters, suggesting that the rats were usually sensitive to stimuli that might be interpreted as an indicating threat.

These differences suggest that D,L-homocysteic stimulation of the dorsal PAG may reflect high level reflex activation of locomotor systems in addition to, or instead of, activation of defensiveness or emotionality. It is notable that, in the absence of the rat, the maximum flight speed induced by D,L-homocysteic was 1.5 m/s. In the context of the 0.8 m/s maximum flight speed exhibited by untreated lab mice, or the 1.5 m/s speed of wild mice, each when chased by a predator (Blanchard et al., 1998), the speeds attained in this study by lab mice under D,L-homocysteic stimulation of the dorsal PAG appear to represent a maximal running response, and one that was not altered by the presence of the predator.

It has been firmly established that the PAG is one of the fundamental brain regions involved in the elaboration and expression of emergency defense responses (Bandler and DePaulis, 1991; Graeff, 2004). Large lesions in the area of the PAG dramatically reduce defensive behaviors to a potential predator in wild rats (Blanchard et al., 1981), although lesions of the dorsal PAG have much lesser effects on contextual conditioned fear responses (Leman et al., 2003). A striking c-Fos activation of the dorsal PAG has been reported in rats exposed to a predator (cat) or to cat odor (Canteras and Goto, 1999; Dielenberg et al., 2001), but, interestingly, not to trimethylthiazoline, a synthetic fox anal gland odor that is often used to elicit defensiveness in laboratory studies (Day et al., 2004).

While the present results do not indicate that the jumping and running behaviors initially elicited by D,L-homocysteic stimulation of the dorsal PAG reflect enhancement of defensiveness to a predator, they should not be taken as counter indicating a role for the dorsal PAG in defense. The dose given of D,L-homocysteic —albeit not outside the range of doses used in similar studies and far less than many— may have had highly unphysiological effects on the activity of the area stimulated. Also, it is by no means certain that D,L-homocysteic optimally activates the PAG elements that are normally involved in defensive behaviors elicited by threatening stimuli, and even less certain that such activation is relatively specific to these systems. What this study does indicate is a necessity for caution in interpreting jumping and running — or other— behaviors elicited by regional brain stimulation as direct analogues to the defensive behaviors that they may somewhat resemble. This research suggests that the dependence of behaviors on specifics of the test situation may be a useful feature in determining how an elicited behavior relates to responses to relevant natural stimuli. However, such dependence requires sensitive analysis. Here, differences in type of response (jump vs. run) in the chamber compared to the MDTB runway were apparently mediated by the ability of tactile stimuli to elicit jumps; a relationship that is of much less importance in the normal running response of animals to threat stimuli which are more visually mediated. Conversely, the lack of normal visual control of behavior in these animals was likely involved in their failure to slow or stop running into threatening or neutral but unyielding stimuli. These considerations suggest that a fuller analysis

of behavior, and of the circumstances in which it occurs, should accompany attempts to determine the brain mechanisms controlling defensive and other natural behavior patterns.

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