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Dorsal periaqueductal gray stimulation facilitates anxiety-, but not panic-related, defensive responses in rats tested in the elevated T-maze

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Abstract

The escape response to electrical or chemical stimulation of the dorsal periaqueductal gray matter (DPAG) has been associated with panic attacks. In order to explore the validity of the DPAG stimulation model for the study of panic disorder, we determined if the aversive consequences of the electrical or chemical stimulation of this midbrain area can be detected subsequently in the elevated T-maze. This animal model, derived from the elevated plus-maze, permits the measurement in the same rat of a generalized anxiety- and a panic-related defensive response, i.e., inhibitory avoidance and escape, respectively. Facilitation of inhibitory avoidance, suggesting an anxiogenic effect, was detected in male Wistar rats (200-220 g) tested in the elevated T-maze 30 min after DPAG electrical stimulation (current generated by a sine-wave stimulator, frequency at 60 Hz) or after local microinjection of the $GABA_A$ receptor antagonist bicuculline (5 pmol). Previous electrical (5, 15, 30 min, or 24 h before testing) or chemical stimulation of this midbrain area did not affect escape performance in the elevated T-maze or locomotion in an open-field. No change in the two behavioral tasks measured by the elevated T-maze was observed after repetitive (3 trials) electrical stimulation of the DPAG. The results indicate that activation of the DPAG caused a short-lived, but selective, increase in defensive behaviors associated with generalized anxiety.

Key words: Panic disorder; Anxiety; Elevated T-maze; Dorsal periaqueductal gray stimulation

Introduction

Stimulation of the dorsal periaqueductal gray matter (DPAG) in animals generates defensive responses that resemble those displayed by these animals when confronted with natural predators (1). These reactions include vigorous escape reactions and autonomic changes such as tachycardia, exophthalmia and increased blood pressure, indicating that the animals are experiencing a markedly stressful situation. In humans, stimulation of the DPAG is reported to be extremely unpleasant. Activation of this structure in awake patients during the course of a neurosurgical procedure evokes strong feelings of fear, impending death or non-localized pain, and prominent autonomic changes similar to those occurring in a panic attack (2,3). Given the similarities between the autonomic and behavioral effects of DPAG stimulation and the symptoms of panic attacks, it has been suggested that the DPAG is involved in the genesis of panic disorder in humans and that stimulation of this midbrain area in animals can model panic attacks (4-8).

In the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-RT) (9), panic disorder is characterized not

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only by its more clearly discernable symptom, the recurrence of panic attacks, but also by worries/anxiety about having other panic attacks and their consequences. This anticipatory anxiety may ultimately lead to behavioral disturbance, such as avoidance of places where panic attacks were or could be experienced.

In order to investigate the pathophysiological basis of panic disorder in the laboratory, it is desirable to use animal models that could address all of the features of the disorder. Unfortunately, no such integrative model exists, even though apparent success has been achieved in modeling specific, in particular its core symptom, the panic attack.

For instance, the escape response evoked by electrical stimulation of the rat DPAG, which has been most commonly assumed to be an index of a panic attack, is attenuated by drugs clinically effective in treating panic disorder (for a review, see Ref. 8). As is frequently reported regarding the stress hormone cortisol in humans after a panic attack, the plasma concentration of corticosterone in rats is not altered after a long and continuous period of DPAG electrical stimulation leading to vigorous escape performance (10, but see also Ref. 11). This pattern of pharmacological and hormonal responses has been equally described in rodent models in which escape behavior is induced by naturally aversive stimuli such as a predator (12) or exposure to places that may offer risk/threat to the animal (13).

In order to further explore the validity of the DPAG stimulation model for the study of panic disorder, in the present study we determined if the aversive consequences of the electrical or chemical stimulation of this midbrain area can be detected subsequently in the elevated T-maze. This animal model, derived from the elevated plus-maze (14), allows the measurement in the same rat of an anxiety- and a panic-related defensive response, i.e., inhibitory avoidance and escape, respectively (15-17). It was of interest to determine if after single or repetitive DPAG aversive stimulation rats would show higher anxiety levels resembling the manifestation of anticipatory anxiety in panic disorder patients.

Material and Methods

Animals

Male Wistar rats (Universidade de São Paulo, Campus Ribeirão Preto), weighing 200-220 g on the day of the surgery for electrode or guide-cannula implant, were housed in groups of 5 per cage (50 x 60 x 22 cm) until surgery. After surgery, animals were housed in pairs in Plexiglas-walled cages (30 x 19 x 13 cm). Room temperature was maintained at 22 ± 1°C, with lights on from 7:00 am to 7:00 pm. Food and water were freely available throughout the experiments, except during testing. The experiments reported in this article were performed in compliance with the recommendations of the Brazilian Society of Neuroscience and Behavior (SBNeC), which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals.

Apparatus

The elevated T-maze was made of wood and had three arms of equal dimensions (50 x 12 cm). One arm, enclosed by 40-cm high walls, was perpendicular to two opposite open arms. To avoid falls, the open arms were surrounded by a 1-cm high Plexiglas rim. The whole apparatus was elevated 50 cm above the floor.

The open-field test was performed in a wooden square arena (60 x 60 cm) with 30-cm high walls.

The escape response induced by DPAG electrical stimulation was evaluated in a bowl-shaped cage (Round bottom bowl, model MD1500, Bioanalytical Systems, USA; height = 35 cm, top and base diameter = 40 and 25 cm, respectively). Brain stimuli were generated by a sine-wave stimulator. The stimulation current (peak to peak) was monitored on the screen of an oscilloscope (Minipa, Brazil). The brain electrode was connected to the stimulator by means of an electromechanical swivel and a flexible cable, allowing ample movement of the animal inside the experimental cage. The electrodes were made of two twisted stainless steel wires, each 250 µM in diameter, enamel insulated except at the cross-section of the tips.

Luminosity at the level of the T-maze arms, open-field or circular arena was 60 lux. After each experimental session, the models were cleaned with 10% ethanol.

Surgery

The animals were anesthetized with 2,2,2 tribromoethanol (250 mg/kg, *ip*) and positioned in a stereotaxic frame. An electrode or a guide-cannula (0.6 mm in external diameter) was implanted in the DPAG following the coordinates of the atlas of Paxinos and Watson (18). Briefly, holding the incisor bar 2.5 mm below the interaural line, the cannula or electrode was introduced 1.9 mm lateral to lambda at an angle of 22° with the sagittal plane, until it was 3.2 or 5.2 mm below the surface of the skull, for the guide-cannula and electrode, respectively. The electrode or cannula was fixed to the skull with acrylic resin and two stainless steel screws.

At the end of surgery, all animals were injected (*im*) with 0.2 mL of an antibiotic preparation [benzylpenicillin procaine (600,000 IU) and streptomycin base (500 mg), Pentabiótico Veterinário Pequeno Porte, Forte Dodge, Brazil] to prevent possible infections. In addition, for postoperative analgesia, all animals received a single subcutaneous injection of fluxinin meglumine (2.5 mg/kg; Schering-Plough, Brazil), a drug with analgesic, antipyretic and anti-inflammatory properties.

The animals were left undisturbed for 5 days after surgery, except for normal handling for cage cleaning.

Procedure

On the 6th day after surgery, animals were gently

handled by the experimenter for 5 min in the morning and afternoon.

On the 7th day, the rats were exposed to one of the open arms of the T-maze for 30 min. A wooden barrier mounted on the border between the maze central area and the proximal end of the open arm isolated this arm from the rest of the maze.

On the next day, rats in Experiment 1 were randomly allocated to groups of DPAG-stimulated or control subjects. In the former, the animals were placed in a bowl-shaped cage and the escape threshold was determined by applying electrical stimuli (AC, 60 Hz, 10 s) through the implanted electrode. The inter-stimulus interval was 10 s. The current intensity started at 20 µA and was increased by steps of 4 µA until the rat presented running or jumping reactions, characterizing the escape behavior. When these behaviors were observed, application of the electrical stimulation to the DPAG was interrupted by the experimenter. The escape threshold was defined as the lowest current intensity that evoked escape in three successive trials of electrical stimulation. Animals with basal thresholds above 152 µA were not utilized.

Control animals were placed in the same experimental cage for 5 min (the average time for escape threshold determination), but no electrical current was delivered through the DPAG-implanted electrode.

The animals were tested in the elevated T-maze, either 5 (Experiment 1A; N = 12), 15 (Experiment 1B; N = 10-11), 30 min (Experiment 1C; N = 9), or 24 h (Experiment 1D; N = 8) after escape threshold determination.

In Experiment 2, animals were injected (0.2 µL) into the DPAG with the $GABA_A$ receptor antagonist bicuculline (5 pmol; Sigma, USA; N = 8), or saline (N = 9) and tested in the elevated T-maze 10 min later.

In Experiment 3, the animals were tested in the elevated T-maze after either 1 or 3 experimental sessions for escape threshold determination. While in Experiment 3A (N = 9-11), the time interval between the DPAG stimulation trials was 24 h, in Experiment 3B (N = 11-12) it was 3 days. In the group of animals with a single session of electrical stimulation, the escape threshold was always evaluated in parallel to the last stimulation session of the 3-day-stimulated groups. In the two preceding stimulation sessions, animals of the single stimulation group were placed in the experimental cage for 5 min, but no electrical current was delivered through the DPAG-implanted electrode. Control animals were placed in the experimental cage three times, for 5 min each time, with inter-trial intervals of 24 h (Experiment 3A) or 3 days (Experiment 3B). No electrical current was delivered through the DPAG-implanted electrode in these sessions. In both Experiment 3A and B, tests in the elevated T-maze were performed as described below 24 h after the last session of DPAG stimulation.

The test in the elevated T-maze was initiated by measurement of inhibitory avoidance acquisition. To this end, each animal was placed at the distal end of the enclosed arm of the elevated T-maze facing the intersection of the arms. The time taken by the rat to leave this arm with all four paws was recorded (baseline latency). The same measurement was repeated in two subsequent trials (avoidance 1 and 2) at 30-s intervals. Following avoidance training (30 s), rats were placed at the end of the same previously experienced open arm and the latency to leave this arm with four paws was recorded 3 consecutive times (escape 1, 2, and 3) at 30-s inter-trial intervals. A cutoff time of 300 s was established for the avoidance and escape latencies. Immediately after being tested in the elevated T-maze, each animal was placed for 5 min in the open-field for the evaluation of locomotor activity. The total distance traveled was analyzed with a video tracking system (Ethovision; Noldus, Holland).

Histology

After the experiments, animals were sacrificed under deep urethane anesthesia. The brain was perfused intracardially with saline solution (0.9%) followed by 10% formalin solution before being removed and fixed in 10% formalin. Brain sections of 60 µm were obtained with a microtome in order to localize the electrode tip or drug infusion site, according to the Paxinos and Watson atlas (18). Only animals with electrodes located inside the DPAG (dorsomedial and dorsolateral columns) were included in the statistical analysis.

Statistical analysis

Two-way ANOVA with repeated measures was used to analyze both avoidance and escape data (Experiments 1-3), with procedure (DPAG stimulation, drug injection, or control) as the independent factor and trials as the repeated measure. When appropriate, *post hoc* comparisons were performed by the Duncan test. Locomotor activity in the open-field was submitted to the Student *t*-test (Experiments 1 and 2) or one-way ANOVA, followed by the Duncan test (Experiment 3).

Results

Experiment 1

Figure 1 shows that electrical stimulation of the DPAG facilitated inhibitory avoidance of the open arms [procedure effect: $F(1,16) = 6.62$, $P < 0.05$, trial effect: $F(2,32) = 3.75$, $P < 0.05$] in animals tested 30 min after the escape threshold determination. This anxiogenic effect was not observed in animals tested 5, 15 min, or 24 h after the stimulation session (Table 1). In these last experiments, only trial effects [$F(2,44) = 14.97$, $P < 0.05$; $F(2,38) = 8.21$, $P < 0.05$; $F(2,28) = 10.67$, $P < 0.05$, respectively], but not procedure x trial interactions were detected.

In none of the experiments performed did electrical stimulation of the DPAG at the escape threshold affect escape

expression or locomotion in the open-field (Table 1).

Experiment 2

Five of 8 animals injected with bicuculline in the DPAG attempted to escape (mainly by jumps) from a top-covered Plexiglas-walled cage (30 x 19 x 13 cm) where they were left after injection and before testing in the elevated T-maze. Only the scores of these animals were computed in the statistics.

As shown in Figure 2, intra-DPAG injection of bicuculline

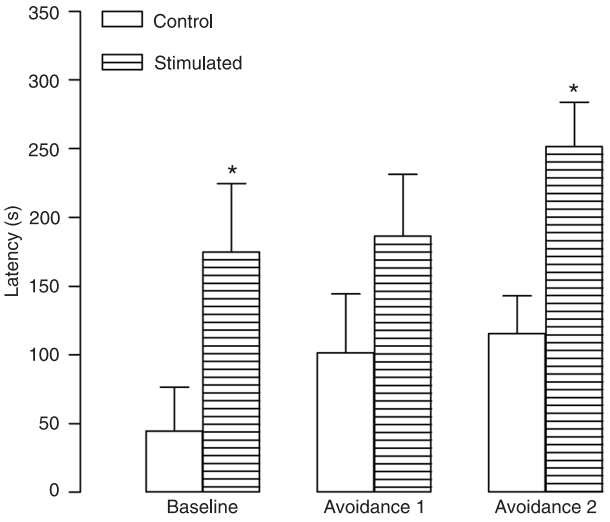

Figure 1. Effect (means ± SEM) of electrical stimulation of the dorsal periaqueductal gray matter (DPAG) at the escape threshold on avoidance latencies measured in the elevated T-maze. Animals were tested in the elevated T-maze 30 min after DPAG electrical stimulation (N = 9). *P < 0.05 compared to the control group in the same trial (repeated measures ANOVA, followed by the Duncan test).

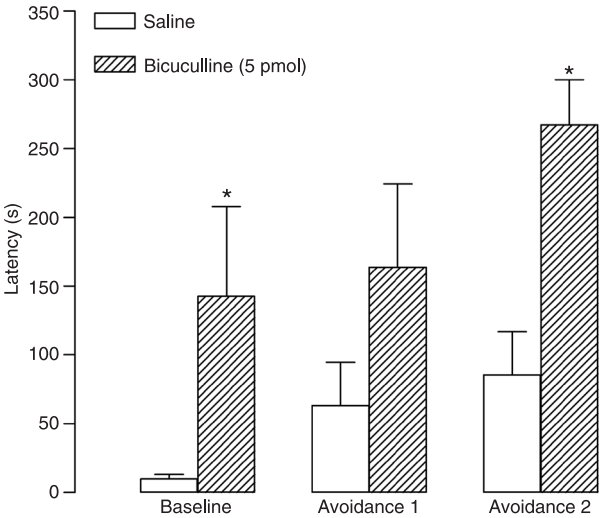

Figure 2. Effect (means ± SEM) of intra-dorsal periaqueductal gray matter injection of bicuculline (5 pmol) or saline on avoidance latencies measured in the elevated T-maze (N = 9-5). *P < 0.05 compared to the control group in the same trial (repeated measures ANOVA, followed by the Duncan test).

Table 1. Effect of the dorsal periaqueductal gray matter electrical stimulation on the behaviors measured in the elevated T-maze and open-field.

Experiment (time after stimulation)	N	Baseline	Avoidance 1	Avoidance 2	Escape 1	Escape 2	Escape 3	Distance traveled in the open-field (m)
Experiment 1A (5 min)								
Control	12	30.33 ± 8.01	152.33 ± 38.58	199.08 ± 36.72	6.75 ± 1.12	5.25 ± 0.76	6.83 ± 1.22	20.82 ± 2.11
Stimulated	12	65.83 ± 8.25	137.83 ± 11.00	154.67 ± 9.75	8.25 ± 1.07	11.00 ± 2.86	9.75 ± 2.38	19.71 ± 1.32
Experiment 1B (15 min)								
Control	10	29.00 ± 6.60	45.10 ± 15.71	142.20 ± 38.24	6.10 ± 1.23	5.00 ± 0.97	6.50 ± 1.42	19.79 ± 2.08
Stimulated	11	68.73 ± 34.53	150.73 ± 43.33	158.36 ± 41.55	7.09 ± 1.22	4.91 ± 0.97	6.73 ± 1.50	14.96 ± 1.42
Experiment 1D (24 h)								
Control	8	80.25 ± 39.77	129.50 ± 41.75	173.63 ± 39.09	10.50 ± 1.12	10.63 ± 1.63	8.75 ± 2.43	14.09 ± 1.64
Stimulated	8	91.63 ± 31.36	213.25 ± 43.18	250.25 ± 33.20	8.13 ± 1.25	8.38 ± 1.66	5.63 ± 1.00	14.07 ± 1.23
Experiment 3A (24-h intervals)								
Control	9	48.78 ± 29.65	116.28 ± 44.74	134.72 ± 39.53	6.58 ± 1.12	7.58 ± 2.09	5.86 ± 1.80	15.23 ± 2.94
One stimulation	11	54.32 ± 24.71	110.56 ± 34.59	122.38 ± 29.67	6.46 ± 0.84	5.75 ± 1.1	4.59 ± 1.08	15.82 ± 1.47
Three stimulations	10	86.53 ± 35.01	159.49 ± 39.56	149.87 ± 34.49	10.12 ± 2.8	6.02 ± 1.4	5.53 ± 1.4	16.42 ± 1.96
Experiment 3B (3-day intervals)								
Control	12	75.58 ± 31.64	136.00 ± 35.93	137.75 ± 36.22	5.83 ± 0.67	5.17 ± 0.84	5.75 ± 0.76	13.12 ± 0.59
One stimulation	11	23.55 ± 5.19	139.36 ± 35.68	152.18 ± 36.07	5.45 ± 0.64	4.64 ± 0.86	4.91 ± 0.78	14.90 ± 1.76
Three stimulations	11	72.09 ± 34.05	70.27 ± 24.54	112.91 ± 36.92	5.36 ± 0.72	4.45 ± 0.94	5.45 ± 1.19	15.47 ± 1.75

Data are reported as means ± SEM.

facilitated inhibitory avoidance of the open arms [procedure effect: $F(1,12) = 17.62$, $P < 0.01$, trial effect: $F(2,24) = 4.0$, $P < 0.05$], without affecting escape performance or locomotion in the open-field (data not shown).

Experiment 3

Repetitive electrical stimulation of the DPAG, either at inter-trial intervals of 24 h (Experiment 3A) or 3 days (Experiment 3B), did not change the behaviors measured in the elevated T-maze or in the open-field (Table 1). In these two experiments there were significant trial effects [$F(2,54) = 11.33$, $P < 0.05$; $F(2,62) = 7.82$, $P < 0.05$, respectively], but not procedure x trial interactions.

In the repetitively stimulated groups, the intensity of electrical current required to evoke escape did not differ significantly among the 3 trials nor was it different from that needed to induce the same behavior in the groups with a single-stimulation session (data not shown).

Discussion

The present results showed that either electrical or chemical stimulation of the DPAG at a level capable of evoking escape behavior facilitated inhibitory avoidance of the elevated T-maze open arms, suggesting an anxiogenic effect, without affecting escape performance.

The anxiogenic effect detected in the elevated T-maze does not seem to be due to a nonspecific motor interference provoked by DPAG stimulation, since no effect on the distance traveled in the open-field was observed. Besides, escape performance in the elevated T-maze, also dependent on locomotion, was not changed in any of the studies performed.

The current finding agrees with the results of a previous study by our group showing that a single-electrical stimulation session of the DPAG or superior colliculus at current intensities that evoke escape facilitated inhibitory avoidance acquisition in the elevated T-maze (19). As observed in the present analysis, the anxiogenic effect evoked by DPAG activation in that study was also short-lived. Although no change in escape performance was observed in the two studies, a consistent conclusion on the selectivity of the effect found in the T-maze was only possible after the present analysis. In our former study, escape was evaluated in animals without previous exposure to the open arm, in accordance with the experimental protocol originally developed for this test (16,17). Other studies, however, have shown that a 30-min long exposure of the animals to the open arm one day before testing, as presently performed, increases the pharmacological validity of the escape task as an index of panic attack. For instance, chronic administration of the antipanic drug imipramine inhibited escape performance, suggesting a panicolytic-like effect, only in animals with this pre-exposure (20). Using this modified protocol, the elevated T-maze also revealed the anti-escape effect of other clinically effective panicolytic drugs such as fluoxetine, clomipramine and escitalopram (21,22). Based on these arguments, the present findings offer more compelling evidence that the state of anxiety generated by stimulation of the DPAG selectively generalizes to an anxiety-, but not a panic-related defensive response.

The results of Experiments 3A and B showed that three repetitive electrical stimulation sessions of the DPAG at current intensities that generate escape, either with 1- or 3-day inter-trial intervals, did not alter rat behavior in the elevated T-maze. At odds with this finding, King (23) showed that the repetitive electrical stimulation of the superior colliculus, another midbrain area whose stimulation induces escape behavior (24), enhanced the expression of defensive behaviors designed to escape the aversive conditions of an unstable, elevated and exposed plus-maze. This sensitizing effect on escape was long-lasting and was not accompanied by changes in anxiety-related defensive responses measured by tests such as the elevated plus-maze and light-dark arena. It is noteworthy that, similar to the present study, three stimulation sessions of the superior colliculus were performed at 3-4-day inter-trial intervals. Further studies are still required in order to investigate whether under different experimental protocols (e.g., number of stimulation sessions, inter-trial intervals, etc.) repetitive stimulation of the DPAG may also produce long-term changes in reactions to threat. Also of interest will be to evaluate whether repetitive stimulation of the superior colliculus, under the experimental parameters followed in the study by King (23), may affect rat behavior in the elevated T-maze.

Taken together, our results indicate that stimulation of the DPAG at an escape threshold causes a short-lived, but selective, increase in defensive behaviors associated with generalized anxiety. This might be of relevance for the study of the anxiogenic consequences of a panic attack.

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References

1. Fernandez de Molina A, Hunsperger RW. Central representation of affective reactions in forebrain and brain stem: electrical stimulation of amygdala, stria terminalis, and adjacent structures. *J Physiol* 1959; 145: 251-265.
2. Amano K, Tanikawa T, Iseki H, Kawabatake H, Notani M, Kawamura H, et al. Single neuron analysis of the human

midbrain tegmentum. Rostral mesencephalic reticulotomy for pain relief. *Appl Neurophysiol* 1978; 41: 66-78.
3. Nashold BS Jr, Wilson WP, Slaughter DG. Sensations evoked by stimulation in the midbrain of man. *J Neurosurg* 1969; 30: 14-24.
4. Graeff FG. Brain defense systems and anxiety. In: Roth M, Burrows GD, Noyes R (Editors), *Handbook of anxiety. The neurobiology of anxiety*. Amsterdam: Elsevier; 1990. p 307-354.
5. Graeff FG, Zangrossi H Jr. Animal models of anxiety disorders. In: D'Haenen H, den Boer JA, Westenberg H, Willner P (Editors), *Textbook of biological psychiatry*. London: Wiley; 2002. p 879-893.
6. Jenck F, Moreau JL, Martin JR. Dorsal periaqueductal gray-induced aversion as a simulation of panic anxiety: elements of face and predictive validity. *Psychiatry Res* 1995; 57: 181-191.
7. Lovick TA. Panic disorder - a malfunction of multiple transmitter control systems within the midbrain periaqueductal gray matter. *Neuroscientist* 2000; 6: 48-59.
8. Schenberg LC, Bittencourt AS, Sudre EC, Vargas LC. Modeling panic attacks. *Neurosci Biobehav Rev* 2001; 25: 647-659.
9. American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders. 4th edn. Washington: APA Press; 2000.
10. Schenberg LC, Dos Reis AM, Ferreira Povoa RM, Tufik S, Silva SR. A panic attack-like unusual stress reaction. *Horm Behav* 2008; 54: 584-591.
11. Lim LW, Blokland A, van Duinen M, Visser-Vandewalle V, Tan S, Vlamings R, et al. Increased plasma corticosterone levels after periaqueductal gray stimulation-induced escape reaction or panic attacks in rats. *Behav Brain Res* 2011; 218: 301-307.
12. Blanchard DC, Griebel G, Blanchard RJ. The Mouse Defense Test Battery: pharmacological and behavioral assays for anxiety and panic. *Eur J Pharmacol* 2003; 463: 97-116.
13. Graeff FG, Zangrossi H Jr. The hypothalamic-pituitary-adrenal axis in anxiety and panic. *Psychol Neurosci* 2010; 3: 3-8.
14. Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *Naunyn Schmiedebergs Arch Pharmacol* 1984; 327: 1-5.
15. McNaughton N, Zangrossi Jr H. Theoretical approaches to the modeling of anxiety in animals. In: Blanchard RJ, Blanchard DC, Griebel G, Nutt D (Editors), *Handbook of anxiety and fear*. Oxford: Elsevier Academic Press; 2008. p 11-27.
16. Viana MB, Tomaz C, Graeff FG. The elevated T-maze: a new animal model of anxiety and memory. *Pharmacol Biochem Behav* 1994; 49: 549-554.
17. Zangrossi H Jr, Graeff FG. Behavioral validation of the elevated T-maze, a new animal model of anxiety. *Brain Res Bull* 1997; 44: 1-5.
18. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th edn. San Diego: Elsevier Academic Press; 2007.
19. de Almeida LP, Ramos PL, Pandossio JE, Landeira-Fernandez J, Zangrossi H Jr, Nogueira RL. Prior electrical stimulation of dorsal periaqueductal grey matter or deep layers of the superior colliculus sensitizes rats to anxiety-like behaviors in the elevated T-maze test. *Behav Brain Res* 2006; 170: 175-181.
20. Teixeira RC, Zangrossi H, Graeff FG. Behavioral effects of acute and chronic imipramine in the elevated T-maze model of anxiety. *Pharmacol Biochem Behav* 2000; 65: 571-576.
21. Poltronieri SC, Zangrossi H Jr, de Barros V. Antipanic-like effect of serotonin reuptake inhibitors in the elevated T-maze. *Behav Brain Res* 2003; 147: 185-192.
22. Pinheiro SH, Zangrossi H Jr, Del-Ben CM, Graeff FG. Elevated mazes as animal models of anxiety: effects of serotonergic agents. *An Acad Bras Cienc* 2007; 79: 71-85.
23. King SM. Escape-related behaviours in an unstable, elevated and exposed environment. II. Long-term sensitization after repetitive electrical stimulation of the rodent midbrain defence system. *Behav Brain Res* 1999; 98: 127-142.
24. Brandao ML, Cardoso SH, Melo LL, Motta V, Coimbra NC. Neural substrate of defensive behavior in the midbrain tectum. *Neurosci Biobehav Rev* 1994; 18: 339-346.