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Research report

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

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Abstract

Electrical stimulation of the dorsal periaqueductal grey matter (DPAG) and deep layers of the superior colliculus (DLSC) of the rat elicits anxiety-like reactions such as freezing and flight. The temporal course of the effects of the aversive electrical stimulation of the DPAG (5, 15 and 30 min afterward) and DLSC (5, 10 and 15 min afterward) on the defensive response of rats exposed to elevated T-maze were determined. The elevated T-maze generates two defensive behaviors, inhibitory avoidance and one-way escape, which have been related, respectively, to generalized anxiety and panic disorders. Prior electrical stimulation of the DPAG (15 min) and DLSC (5 min) enhanced inhibitory avoidance when compared to no-operated and sham animals, although not affecting escape. Therefore, stimulation of the DPAG and DLSC causes a heightened responsivity to anxiogenic stimulus, but not to panicogenic stimulus, inherent to elevated T-maze. These findings support the participation of the DPAG and DLSC in the elaboration of adaptive responses to stressful situations. Besides, the data supports the view that prior electrical stimulation of DPAG and DLSC is selective in sensitizing rats to anxiety-like behaviors, but not to panic-like behaviors in the elevated T-maze test.

Keywords: Periaqueductal grey matter; Deep grey layer of the superior colliculus; Electrical stimulation; Aversion; Anxiety-like behavior; Elevated T-maze; Rat

1. Introduction

Several results indicate that prior experience with an aversive stimulus might enhance emotional reactions to a novel and stressful situation. For example, rats exposed to a single aversive stimulus, such as a cat, increased anxiety-like responses in the elevated plus-maze [3,6]. These same patterns of results have been also observed in numerous other experiments. Accordingly, rodents submitted to restraint [22,31] can increase anxiety-like behavior in the elevated plus-maze. In the same way, social defeat in rodents may induce a nonspecific exaggeration of defensive behavior in social situations (for review see [28]).

It has been proposed that the sensitization effect of prior experience with an aversive stimulus on subsequent stressful situations might be employed as an useful animal model of anxiety disorder [2,4,6,9,44]. According to this view, exposure to aversive stimuli with sufficient duration, intensity or frequency may cause a breakdown in integrity of neural circuitry responsible for adaptive defense behaviors [27,50].

An analogous and more localized sensitization effect has been demonstrated in the rodent after direct stimulation of the neural substrates of defensive behavior. Prior exposure to an aversive electrical stimulation of the inferior colliculus produces a consistent increment of anxiety-like behavior in rats tested in the elevated plus-maze [38]. It has been also reported

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a sensitization effect in defensive responses after aversive electrical stimulation of the superior colliculus [26] and the amygdaloid complex [1,23,24]. These results are in agreement with the suggestion that the aversive stimulation of brain structures involved in defensive reactions can become more sensitive to further deal with subsequent processing of emotional information.

It is well established that electrical or chemical stimulation of the dorsal periaqueductal grey matter (DPAG) and deep layers of the superior colliculus (DLSC) of the rat elicits anxiety-like reactions such as freezing and flight responses and concomitant autonomic changes [8,12,13,14,17,18,42,45,47]. In fact, electrical stimulation of these structures in humans can also induce feelings of fear, terror and impending death, accompanied by marked neurovegetative changes [7,35,36]. However it is still unclear whether previous stimulation of these midbrain structures might increase different patterns of defensive behaviors in a subsequent stressful situation.

The present study investigated whether aversive electrical stimulation of the DPAG and DLSC could result in alteration of the animal's defensive responses to subsequent exposition to elevated T-maze. This test generates, in the same rat, inhibitory avoidance and one-way escape behaviors. So far, reported drug effects on these tasks have largely supported the proposed animal model of anxiety associates inhibitory avoidance with generalized anxiety disorder and escape response with panic disorder [19,20,40,48,52,56]. The elevated T-maze test occurred at 5, 15 and 30 min (DPAG) and at 5, 10 and 15 min (DLSC) after a single aversive electrical stimulation of these brain regions. Finally, to ensure that any differences in behavior between stimulated and nonstimulated animal could not simply be attributed to differences in activity and exploratory tendency, animal were also tested in the arena.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 200–300 g, were housed in individual Plexiglas-walled cages under 12:12 dark/light cycle (lights on at 07:00 h) at 23 ± 1 °C, and given free access to food and water throughout the experiment. All procedures were conducted in conformity with the Brazilian Society of Neuroscience and Behavior Guidelines for care and use of laboratory animals, which comply with Principles of Laboratory Animal Care (NIH, No. 85-23, 1985). All efforts were made to minimize animal suffering.

2.2. Surgery

The animals were anaesthetized with tribromoethanol (250 mg/kg, i.p.) and fixed in a stereotaxic frame (David Kopf, USA). A unipolar electrode was implanted in the midbrain, aimed at the DPAG or DLSC. The electrode wire was connected to a male pin, parallel to the outer end of the other pin. Together, they could be plugged into an amphenol socket at the end of a flexible electrical cable ad used for brain stimulation. Holding the incisor bar 2.4-mm below the interaural line, the electrode was introduced using the following coordinates with the lambda serving as the reference for each plane: (a) DPAG—postero-anterior, 0.0 mm; medio-lateral, −1.9 mm; dorso-ventral, −5,2 mm, at an angle of 22° with the sagital plane and (b) DLSC—postero-anterior, −0.9 mm; medio-lateral, −1.5 mm; dorso-ventral, −4.5 mm. The electrode was fixed to the skull by means of acrylic resin and three stainless steel screws.

2.3. Apparatus

A box of 25 cm × 20 cm × 20 cm was placed inside an insulating chest provide with a fan and indirect illumination by means of a 25-W red lamp. The grid floor consisted of stainless steel rods spaced 1.2 cm apart. Brain stimuli were generated by a sine-wave stimulator [30]. 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 box.

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

The arena was a wooden square box (60 cm × 60 cm), with walls 60 cm high and the floor divided into nine squares of 20 cm × 20 cm.

2.4. Procedure

Started at the third and fourth days after the surgery, animals were gently handled for 5 min. In the fifth day, each animal was placed in the box and its aversive threshold was determined. For that, an electrical stimulus (ac, 60 Hz, 15 s) was presented through the implanted electrode. The interstimuli interval was 15 s. The current intensify started at the level of 20 μA and was increased by steps of 8 μA until the rat started to run or jump. Aversive threshold was defined as the lowest current intensity that produced one of these defensive behaviors in three successive trials of electrical stimulation. The procedure to determine the aversive electrical stimulation threshold took nearly 5 min.

After the occurrence of escape behavior, animals were divided into groups according to the intervals of 5, 15 and 30 min (DPAG) or 5, 10 and 15 min (DLSC) before the exposure to the elevated T-maze. For both structures the first interval tested was 15 min. Since an effect was observed in the DPAG within this time frame, 5 and 30 min intervals were also investigated in order to track the time span of the sensitization effect. In the case of the DLSC, as no effect was observed within the 15 min interval shorter intervals of 10 and 5 min were tested. The study also included two control groups: a sham-operated group, which underwent the same surgery procedure, except that no electrical current was applied. The other control group consisted of unoperated rats.

To perform elevated T-maze, each rat was placed at the distal end of the enclosed arm facing the intersection of the arms. The time taken to withdraw from this arm with the four paws was recorded (baseline latency). The same measurement was repeated twice at 30-s intervals (avoidance 1 and avoidance 2). Following the avoidance training, the rat was placed at the end of the open arm and the time taken to leave the arm with the four paws was recorded (escape 1). The same measurement was repeated twice at 30 s intervals (escape 2 and 3). During the 30-s intervals between each trial, the animals were placed in a Plexigas cage (28 cm × 18 cm).

Immediately after the test, each rat was placed in the center of the arena to measure locomotor activity. For this, the total number of lines crossed during 5 min was recorded. Animals from each experiment were tested only once.

2.5. Histology

Upon completion of the experiments, the animals were deeply anaesthetized with urethane and perfused intracardially with saline 0.9% followed by formalin solution (10%). Seven days later the brains were frozen. Serial 60 μm brain sections were cut using a microtome in order to localize the positions of the electrode tips according to Paxinos and Watson's atlas [39].

2.6. Analysis of results

Repeated measure analysis of variance (ANOVA) was used to analyze both inhibitory avoidance and escape results, with procedure as the independent factor and trials as the repeated measure. Significant differences with the independent factor or with the interaction between the independent and repeated

factors were followed by the multiple comparison test of Duncan. Locomotor activity, number and intensity of electrical stimulation applied to DPAG and DLSC data were submitted to one-way ANOVA, followed by post hoc test of Duncan.

3. Results

Histological examination of the brain slices indicated that all electrode tips were located inside of DPAG or DLSC. Electrical stimulation of the DPAG and DLSC caused escape behavior, which stopped as soon the electrical stimulation was switched off.

The number of electrical stimulation applied to DPAG was 9.37 ± 1.54 and to DLSC was 6.67 ± 0.69. The intensity of electric current applied to DPAG and DLSC was 68.96 ± 12.33 and 48.0 ± 5.52 μA, peak to peak, respectively. ANOVA followed by the Duncan multiple comparison test showed that the number [$F(2,27) = 4.17$, $p = 0.003$] and intensity of electrical stimulation [$F(2,27) = 4.17$, $p = 0.003$] applied to DPAG was significantly longer in 15 min group when compared to 5 min group, but not to 30 min group. ANOVA did not show significant differences to the number [$F(2,32) = 0.68$, $p = 0.52$] and intensity of electrical stimulation [$F(2,32) = 0.75$, $p = 0.48$] applied to DLSC.

For all of the analyses reported here, no-operated controls either DPAG (5, 15 and 30 min) or DLSC (5, 10 and 15 min) were collapsed due to the fact that there were no differences in performance between these three groups. The same procedure was used to sham-operated groups.

As shown in Fig. 1 (upper panel), the animals tested in the elevated T-maze after the aversive threshold determination of DPAG acquired inhibitory avoidance of the open arms [trial effect, $F(2,176) = 86.56$, $p = 0.000$]. There was significant effect of procedure [$F(4,88) = 4.83$, $p = 0.001$] and procedure × trial interaction [$F(8,176) = 2.25$, $p = 0.026$] on withdrawal latencies was found. A repeated measure analysis of variance followed by the Duncan multiple comparison test showed that in 15 min group the avoidance 1 [$F(4,92) = 4.91$, $p = 0.0013$] and avoidance 2 [$F(4,92) = 2.51$, $p = 0.048$] were significantly longer in the animals that received a aversive electrical stimulation in the DPAG when compared to a no-operated and sham-operated groups.

As shown in the lower panel of Fig. 1, repeated measure analysis of variance also has shown a significant effect of trial [$F(2,176) = 16.27$, $p = 0.000$], but a nonsignificant effect of procedure [$F(4,88) = 0.55$, $p = 0.70$] and procedure × trial interaction [$F(8,176) = 1.23$, $p = 0.29$] on latency to escape from the open arm. The locomotor activity in the arena was not altered significantly by the procedure [$F(4,89) = 1.36$, $p = 0.26$].

As illustrated in Fig. 2 (upper panel), the animals tested in the elevated T-maze after the aversive threshold determination of superior colliculus acquired inhibitory avoidance of the open arms [trial effect, $F(2,190) = 87.93$, $p = 0.000$]. There was a significant effect of both procedure [$F(4,95) = 2.42$, $p = 0.05$] and procedure × trial interaction [$F(8,190) = 2.23$, $p = 0.027$] on withdrawal latencies was found. A repeated measure analysis of variance followed by the Duncan multiple comparison test showed that in 5 min group the avoidance 1 was

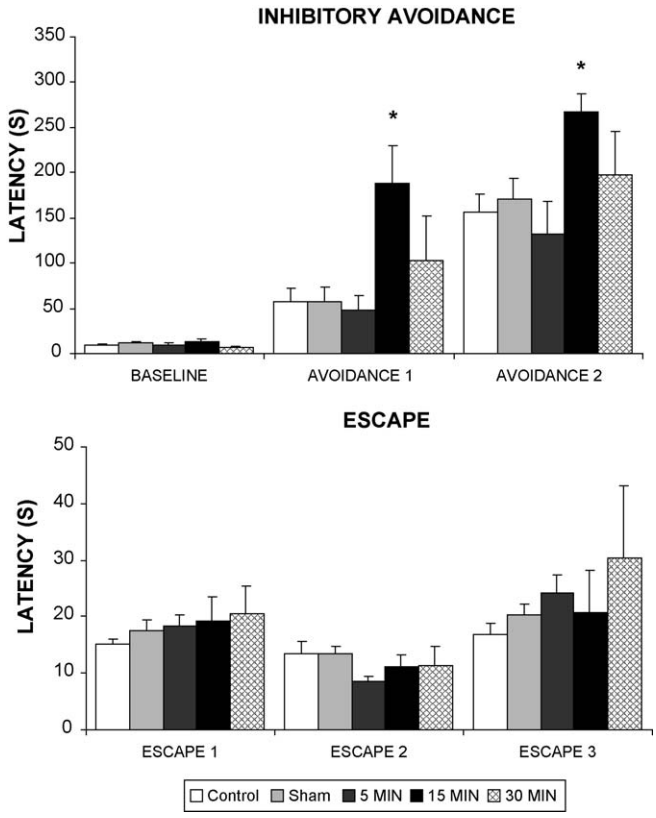

Fig. 1. Effect (mean ± S.E.M.) of electrical stimulation of the DPAG on inhibitory avoidance (upper panel) and escape (lower panel) latencies of rats tested in the elevated T-maze 5 min ($n = 11$), 15 min ($n = 11$) and 30 min ($n = 7$) after. $^{*}p < 0.05$, compared to non-operated ($n = 33$) and sham group ($n = 30$).

significantly longer [$F(4,99) = 3.98$, $p = 0.005$] in the animals that received a aversive electrical stimulation in the superior colliculus when compared to a no-operated and sham-operated groups.

As shown in the lower panel of Fig. 2, repeated measure analysis of variance has shown that electrical stimulation of the superior colliculus did not alter the one-way escape performance. It was found a significant effect of trial [$F(2,188) = 8.33$, $p = 0.000$], but a nonsignificant effect of procedure [$F(4,94) = 0.59$, $p = 0.67$] and procedure × trial interaction [$F(8,188) = 0.44$, $p = 0.897$] on latency to escape from the open arm. As shown in Table 1, the locomotor activity in the arena was not altered significantly by the procedure [$F(4,95) = 2.27$, $p = 0.07$].

Table 1
Effect (mean ± S.E.M.) of electrical stimulation of the DPAG and DLSC on locomotor activity measured in the arena

DPAG		DLSC	
Treatment	Crossing	Treatment	Crossing
Control	24.56 ± 2.90	Control	31.26 ± 2.08
Sham	24.97 ± 3.30	Sham	32.03 ± 2.85
5 min	19.09 ± 4.41	5 min	19.36 ± 3.27
15 min	13.64 ± 1.59	10 min	24.82 ± 3.74
30 min	23.86 ± 4.94	15 min	26.70 ± 5.22

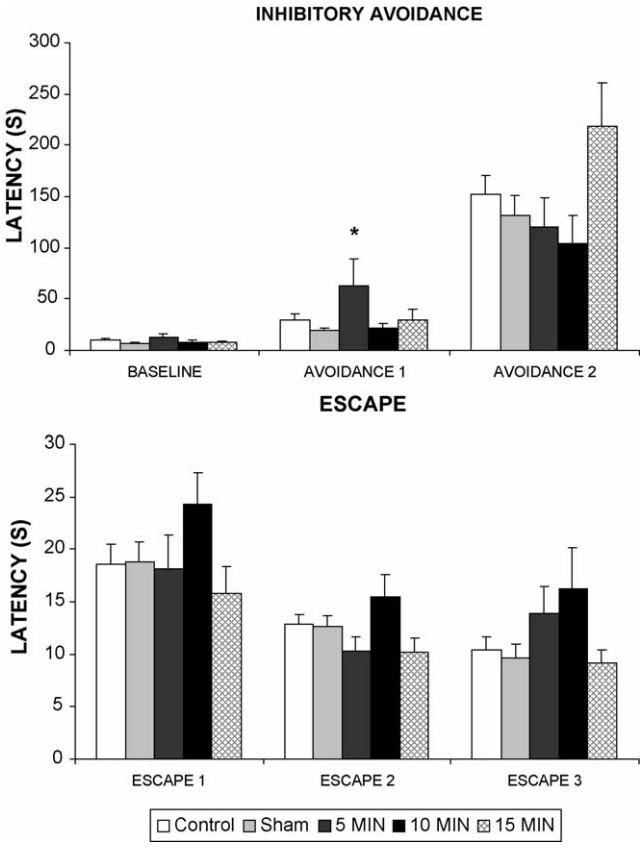

Fig. 2. Effect (mean ± S.E.M.) of electrical stimulation of the DLSC on inhibitory avoidance (upper panel) and escape (lower panel) latencies of rats tested in the elevated T-maze 5 min ($n = 12$), 10 min ($n = 11$) and 15 min ($n = 10$) after. $^{*}p < 0.05$, compared to non-operated ($n = 37$) and sham group ($n = 30$).

4. Discussion

Electrical stimulation of the mesencephalic tectum has been described as aversive in animal and human causing anxiety-related responses (for review see [12,13,14,18,42,45]). In agreement with these reports, present results found that electrical stimulation of the DPAG or DLSC of rats was able to induce active defensive behavior such as running. Stimulation within these midbrain areas also enhanced inhibitory avoidance but not escape behavior in the elevated T-maze test.

The fact that aversive electrical stimulation of the DPAG and DLSC did not produce any effect in the arena test indicated that the facilitator effect of electrical stimulation within these brain areas in subsequent inhibitory avoidance in the elevated T-maze test was not due to general motor activity. Therefore, this effect seems to be particularly linked to defensive reactions, and could be the result of an increase of anxiogenic-like responses.

Aversive electrical stimulation of the DPAG or DLSC did not change animal escape behavior in the elevated T-maze test. In line with these results, King [25,26] found that escape responses sensitized by electrical stimulation of the superior colliculus were very unstable and occurred only under the most extreme conditions. Besides, Adamec et al. [5] related that vulnerability to lasting changes in different behaviors in response to predator stress may differ or have different thresholds. The most consistent findings with predator stress were its effects on behavior in the plus-maze (open arm exploration and risk assessment) and on startle than effects on light/dark box or social interaction.

Considering the rather close localization of DPAG and DLSC, the minor effects of DLSC stimulation could be attributed to current spreading to the DPAG. Schenberg et al. [45] have proposed two main criterions in defining the defensive repertoire of tectum structures: (i) the stimulus-dependent elicitation of defensive behaviors with stimulation of low-magnitude and (ii) the production of a given behavior following the high-resolution stimulation with frequency-varying square-wave pulses of fixed intensity. In the present study, it was used the lowest current intensity that produced escape behavior. Besides, the aversive threshold detected to both midbrain structures was similar. Consistent with this, Schenberg et al. [45] showed that intensity-varying sine-wave stimulation of the DPAG and DLSC produced repertoires virtually identical in both their threshold and hierarchy and that defensive behaviors as immobility, trotting, exophthalmus, defecation and micturation are elicited by frequency-varying square-wave stimulation applied to the DPAG and DLSC using stimulation intensity comparable, supporting a genuine participation of DLSC in these defensive responses.

The midbrain periaqueductal grey [54] and DLSC [53] are also implicated in the network subserving audiogenic seizures that consist of a sequence of discrete behavioral phases (i.e., wild running, clonus–tonus seizures, and post-ictal depression). Thus, the avoidance behavior facilitated by either DPAG or DLSC stimulation might be due to fatigue of the neurons of these midbrain areas instead of a sensitization effect. However, a fatigue explanation of the present results seems to be improbably since the animals did not present any seizures. Moreover, there are several reports showing effect of inhibitory and excitatory drugs administered into these midbrain structures after electrical stimulation with similar characteristic as used in the present study (for review see [12,13,14,18,42,45]), suggesting that neurons within DPAG and DLSC are not fatigued by the electrical stimulation with this characteristics.

The sensitization effect of inhibitory avoidance behavior induced by DPAG and DLSC electrical stimulation was observed over a short period of time: 15 min after DPAG stimulation and 5 min after DLSC stimulation. Comparable finding have been obtained by Pandossio et al. [38]. These authors found that prior electrical stimulation of the inferior colliculus caused an increment in defensive behavior in the elevated plus-maze in rats tested 5 and 10 min after the electrical stimulation but not after 15 min post-stimulation.

In contrast to this short-lived sensitization effect, King [26] found that repetitive electrical stimulation of the superior colliculus lead to an increase in defensive behaviors over a 3-month period. These different long- and short-term sensitization effects observed in defensive behavior after aversive electrical stimulation of the midbrain might be related to the time and the nature of the aversive electrical stimulation parameters used, such as duration, intensity and frequency.

Long-lasting sensitization effects have been also observed when natural aversive stimuli are employed to sensitize defensive responses. For example, Zangrossi and File [55] found that

rats previously exposed to cat odor for 5 min increased defensive responses in the social interaction and elevated plus-maze tests that could be detected 1 h after odor exposure. Furthermore, Adamec and Shallow [3] also reported that rats exposed to a cat presented an enhancement of defensive behavior up to 3 weeks in several anxiety tests. In this case, it might be possible that natural aversive stimuli are able to activate several brain areas involved with defensive behavior.

It has been shown in the hippocampus that a single stressful event elicits responses with different time-spans ranging from rapid changes in glutamatergic neurotransmission, activation of second messenger cascades by corticotropin-releasing factor (CRF) to long-lasting transcriptional changes of acetylcholinesterase [10]. Besides, it has already been shown that restraint and other forms of stress induce expression of immediate early genes such as *c-fos* or *c-jun* in several brain regions [29,32,46,49]. These genes are proposed to act as "third messengers", leading to alteration of target genes expression related to some stress-induced long-lasting changes in animal and human behavior [41]. However, these alterations may delay days or weeks to be installed. In this line, there is a growing body of evidence that neural plasticity and long-term potentiation of amygdala afferent and efferent transmission is produced by stress and may underlie increased anxiety-like behaviors following severe stress (for review see [6]).

The neurophysiological mechanisms underlying the short temporal sensitization effect on subsequent defensive behaviors in the elevated T-maze test caused by the electrical stimulation of the DPAG or DLSC is unknown. It has been shown that behavioral and autonomic consequences of electrical stimulation of the mesencephalic tectum can be attenuated by enhancing gamma-aminobutyric acid (GABA)-mediated neurotransmission, which exerts a tonic inhibitory control on the neural circuits responsible for the defense behavior repertoire (for review see [12,13]). Besides GABA, serotonin, opioids, neuropeptides, histamine and excitatory amino acids have all been implicated in the regulation of anxiety-related behaviors induced by stimulation of midbrain tectum (for review see [8,12,13,14,18,45]). This suggests that, following electrical stimulation of the DPAG or DLSC, all of these neurotransmitters are candidates for the short temporal sensitization mediating the increase in animal defensive responses.

Another possibility to explain this short-term effect could be an acute alteration such as endocrine signals. The main endocrine response to anxiety-evoking stimulus include the synthesis and the release of CRF from cells of the paraventricular nucleus, into the portal blood, initiating the hypothalamo-pituitary-adrenal response to stressors [15,37,43]. However, it has been shown that, in spite of its marked aversive properties, DPAG stimulation did not increase plasma levels of the stress hormones adrenocorticotropin and prolactin [51]. Furthermore, considering that electrical stimulation of DPAG is an animal model of panic attack, several evidences indicate that the occurrence of panic attack does not activate the hypothalamic-pituitary-adrenal axis (for review see [21]). Thus, a neurohormonal mechanism might not be responsible for the short-term anxiogenic effect of electrical stimulation of DPAG.

It must be noted that CRF also functions as a neurotransmitter mediating stress-related behavioral responses by action at extrahypothalamic sites [16]. CRFergic neurons and CRF receptors are distributed in a variety of locations outside of the hypothalamus [37]. In the midbrain periaqueductal grey, CRF has a predominant excitatory effect on neurons [11]. Intra-DPAG administration of CRF has an anxiogenic effect that can be prevented by previous microinjection of alpha-helical-CRF9-41, a CRF antagonist [33]. Moreover, the anxiogenic behavior of rats previously stressed by forced immobilization was reversed by intra-DPAG injection of alpha-helical-CRF9-41, suggesting that this stress effect might involve facilitation of CRF-mediated neurotransmission in the DPAG [34]. Thus, it might be possible that changes in neuronal system that produce anxiogenic behaviors mediated by CRF neurotransmitter might be also involved in the enhancement of the defensive behavior measured in the elevated T-maze after electrical stimulation of the DPAG.

Finally, it has been suggested that that inhibitory avoidance and one-way escape in the elevated T-maze reflect different types of fear/anxiety, that may be related to generalized anxiety and panic disorder, respectively [19,20,40,48,52,56]. Since the present study found that electrical stimulation of the DPAG or DLSC induced a selective effect on inhibitory avoidance, it might be possible that exposure to these aversive stimuli might have an modulatory influence on generalized anxiety but not on panic disorders.

In conclusion, our results showed that previously electrical stimulation of DPAG or DLSC produces an increase of inhibitory avoidance, but not of the escape response, in the elevated T-maze, over a short period of time. These results indicate that these midbrain structures play a selective role on regulatory processes involved in defensive behavior to threatening situations.

References

[1] Adamec RE. Does kindling model anything clinically relevant? Biol Psychiatry 1990;27:249–79.
[2] Adamec RE. Transmitter systems involved in neural plasticity underlying increased anxiety and defense—implications for understanding anxiety following traumatic stress. Neurosci Biobehav Rev 1997;2:755–65.
[3] Adamec RE, Shallow T. Lasting effects on rodent anxiety of a single exposure to a cat. Physiol Behav 1993;54:101–9.
[4] Adamec RE, Kent P, Anisman H, Shallow T, Merali Z. Neural plasticity, neuropeptides and anxiety in animals—implications for understanding and treating affective disorder following traumatic stress in humans. Neurosci Biobehav Rev 1998;23:301–18.
[5] Adamec RE, Blundell J, Burton P. Phosphorylated cyclic AMP response element binding protein expression induced in the periaqueductal gray by predator stress: its relationship to the stress experience, behavior and limbic neural plasticity. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:1243–67.
[6] Adamec RE, Blundell J, Burton P. Relationship of the predatory attack experience to neural plasticity, pCREB expression and neuroendocrine response. Neurosci Biobehav Rev 2006;30:356–75.
[7] Amano K, Tanikawa T, Iseki H, Kawabatabe 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.

[8] Bandler R, Depaulis A. Midbrain periaqueductal gray control of defensive behavior in the cat and in the rat. In: Depaulis A, Bandler R, editors. The midbrain periaqueductal gray matter: functional, anatomical, and neurochemical organization. New York: Plenum Press; 1991. p. 175–98.
[9] Blanchard RJ, Nikulina JN, Sakai RR, McKittrick C, McEwen B, Blanchard DC. Behavioral and endocrine change following chronic predatory stress. Physiol Behav 1998;63:561–9.
[10] Blank T, Nijholt I, Spiess J. Molecular determinants mediating effects of acute stress on hippocampus-dependent synaptic plasticity and learning. Mol Neurobiol 2004;29:131–8.
[11] Bowers LK, Swisher CB, Behbehani MM. Membrane and synaptic effects of corticotropin-releasing factor on periaqueductal gray neurons of the rat. Brain Res 2003;981:52–7.
[12] Brandão ML, Anseloni VZ, Pandossio JE, De Araujo JE, Castilho VM. Neurochemical mechanisms of the defensive behavior in the dorsal midbrain. Neurosci Biobehav Rev 1999;23:863–75.
[13] Brandão ML, Troncoso AC, de Souza Silva MA, Huston JP. The relevance of neuronal substrates of defense in the midbrain tectum to anxiety and stress: empirical and conceptual considerations. Eur J Pharmacol 2003;463:225–33.
[14] Carobrez AP, Teixeira KV, Graeff FG. Modulation of defensive behavior by periaqueductal gray NMDA/glycine-B receptor. Neurosci Biobehav Rev 2001;25:697–709.
[15] De Souza EB, Grigoriadis DE. Corticotropin-releasing factor: physiology, pharmacology and role in central nervous system and immune disorders. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the fourth generation of progress. New York: Raven Press; 1995. p. 505–17.
[16] Dunn AJ, Berridge CW. Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? Brain Res Brain Res Rev 1990;15:71–100.
[17] Eichenberger GC, Ribeiro SJ, Osaki MY, Maruoka RY, Resende GC, Castellan-Baldan L, et al. Neuroanatomical and psychopharmacological evidence for interaction between opioid and GABAergic neural pathways in the modulation of fear and defense elicited by electrical and chemical stimulation of the deep layers of the superior colliculus and dorsal periaqueductal gray matter. Neuropharmacology 2002; 42:48–59.
[18] Graeff FG. Serotonin, the periaqueductal gray and panic. Neurosci Biobehav Rev 2004;28:239–59.
[19] Graeff FG, Viana MB, Tomaz C. The elevated T maze, a new experimental model of anxiety and memory: effect of diazepam. Braz J Med Biol Res 1993;26:67–70.
[20] Graeff FG, Netto CF, Zangrossi Jr H. The elevated T-maze as an experimental model of anxiety. Neurosci Biobehav Rev 1998;23:237–46.
[21] Graeff FG, Garcia-Leal C, Del-Ben CM, Guimarães FS. Does the panic attack activate the hypothalamic-pituitary-adrenal axis? An Acad Bras Cienc 2005;77:477–91.
[22] Guimarães FS, Del Bel EA, Padovan CM, Netto SM, de Almeida RT. Hippocampal 5-HT receptors and consolidation of stressful memories. Behav Brain Res 1993;58:133–9.
[23] Helfer V, Deransart C, Marescaux C, Depaulis A. Amygdala kindling in the rat: anxiogenic-like consequences. Neuroscience 1996;73:971–8.
[24] Kellett J, Kokkinidis L. Extinction deficit and fear reinstatement after electrical stimulation of the amygdala: implications for kindling-associated fear and anxiety. Neuroscience 2004;127:277–87.
[25] King SM. Escape-related behaviours in an unstable elevated and exposed environment. I. A new behavioural model of extreme anxiety. Behav Brain Res 1999;98:113–26.
[26] 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;98:127–42.
[27] Kolb LC. A neuropsychological hypothesis explaining posttraumatic stress disorders. Am J Psychiatry 1987;144:989–95.
[28] Koolhaas JM, De Boer SF, De Rutter AJ, Meerlo P, Sgoifo A. Social stress in rats and mice. Acta Physiol Scand Suppl 1997;640:69–72.
[29] Lino de Oliveira C, Guimarães FS, Del Bel EA. c-jun mRNA expression in the hippocampal formation induced by restraint stress. Brain Res 1997;753:202–8.
[30] Marseillan RF. A solid state sine-wave stimulator. Physiol Behav 1977;19:339–40.
[31] Martijena ID, Calvo N, Volosin M, Molina VA. Prior exposure to a brief restraint session facilitates the occurrence of fear in response to a conflict situation: behavioral and neurochemical correlates. Brain Res 1997;752:136–42.
[32] Martinez M, Calvo-Torrent A, Herbert J. Mapping brain response to social stress in rodents with c-fos expression: a review. Stress 2002;5:3–13.
[33] Martins AP, Marras RA, Guimaraes FS. Anxiogenic effect of corticotropin-releasing hormone in the dorsal periaqueductal grey. Neuroreport 1997;8:3601–4.
[34] Martins AP, Marras RA, Guimaraes FS. Anxiolytic effect of a CRH receptor antagonist in the dorsal periaqueductal gray. Depress Anxiety 2000;12:99–101.
[35] Nashold Jr BS, Wilson WP, Slaughter DG. Sensations evoked by stimulation in the midbrain of man. J Neurosurg 1969;30:14–24.
[36] Nashold Jr BS, Wilson WP, Slaughter DG. The midbrain and pain. In: Bonica JJ, editor. Advances in neurology, vol. 4. New York: Raven Press; 1974. p. 191–6.
[37] Owens MJ, Nemeroff CB. Physiology and pharmacology of corticotropin-releasing factor. Pharmacol Rev 1991;43:425–73.
[38] Pandossio JE, Molina VV, Brandão ML. Prior electrical stimulation of the inferior colliculus sensitizes rats to the stress of the elevated plus-maze test. Behav Brain Res 2000;109:19–25.
[39] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.
[40] Poltronieri SC, Zangrossi Jr H, Viana MB. Antipanic-like effect of serotonin reuptake inhibitors in the elevated T-maze. Behav Brain Res 2003;147:185–92.
[41] Post RM. Transduction of psychosocial stress into the neurobiology of recurrent affective disorder. Am J Psychiatry 1992;49:999–1010.
[42] Redgrave P, Dean P. Does the PAG learn about emergencies from the superior colliculus? In: Depaulis A, Bandler R, editors. The midbrain periaqueductal gray matter: functional, anatomical, and neurochemical organization. New York: Plenum Press; 1991. p. 199–210.
[43] Rivier C, Rivier J, Vale W. Inhibition of adrenocorticotropic hormone secretion in the rat by immunoneutralization of corticotropin-releasing factor. Science 1982;218:377–9.
[44] Rosen JB, Schulkin J. From normal fear to pathological anxiety. Psychol Rev 1998;105:325–50.
[45] Schenberg LC, Povoa RM, Costa AL, Caldellas AV, Tufik S, Bittencourt AS. Functional specializations within the tectum defense systems of the rat. Neurosci Biobehav Rev 2005;29:1279–98.
[46] Sharp FR, Sagar SM, Hicks K, Lowenstein D, Hisanaga K. c-fos mRNA, Fos, and Fos-related antigen induction by hypertonic saline and stress. J Neurosci 1991;11:2231–321.
[47] Sudré EC, de Barros MR, Sudré GN, Schenberg LC. Thresholds of electrically induced defence reaction of the rat: short- and long-term adaptation mechanisms. Behav Brain Res 1993;58:141–54.
[48] Teixeira RC, Zangrossi Jr H, Graeff FG. Behavioral effects of acute and chronic imipramine in the elevated T-maze model of anxiety. Pharmacol Biochem Behav 2000;65:571–6.
[49] Titze-de-Almeida R, Lino de Oliveira C, Shida HW, Guimarães FS, Del Bel EA. Midazolam and the *N*-methyl-D-aspartate (NMDA) receptor antagonist 2-amino-7-phosphono-heptanoic acid (AP-7) attenuate stress-induced expression of c-fos mRNA in the dentate gyrus. Cell Mol Neurobiol 1994;14:373–80.
[50] van der Kolk BA. Psychobiology and psychopharmacology of PTSD. Hum Psychopharmacol 2001;6:S49–64.
[51] Vargas LC, Silva SR, Reis AM, Tufik S, Carobrez AP, Schenberg LC. Pharmacological and endocrinological properties of intracranially-induced panic-like behaviors. Soc Neurosci Abstr 2001;27:858.11.

[52] Viana MB, Tomaz C, Graeff FG. The elevated T-maze: a new animal model of anxiety and memory. Pharmacol Biochem Behav 1994;49:549–54.

[53] Yang L, Long C, Faingold CL. Neurons in the deep layers of superior colliculus are a requisite component of the neuronal network for seizures during ethanol withdrawal. Brain Res 2001;30(920):134–41.

[54] Yang L, Long C, Randall ME, Faingold CL. Neurons in the periaqueductal gray are critically involved in the neuronal network for audiogenic seizures during ethanol withdrawal. Neuropharmacology 2003;44:275–81.

[55] Zangrossi Jr H, File SE. Behavioral consequences in animal tests of anxiety and exploration of exposure to cat odor. Brain Res Bull 1992;29:381–8.

[56] Zanoveli JM, Nogueira RL, Zangrossi Jr H. Chronic imipramine treatment sensitizes 5-HT1A and 5-HT2A receptors in the dorsal periaqueductal gray matter: evidence from the elevated T-maze test of anxiety. Behav Pharmacol 2005;16:543–52.