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Chronic imipramine enhances 5-HT_{1A} and 5-HT_2 receptors-mediated inhibition of panic-like behavior in the rat dorsal periaqueductal gray

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Abstract

Electrical stimulation of the dorsal periaqueductal gray (DPAG) has been used to induce panic-like behavior in rats. In the present study, we investigated the effect of chronic imipramine treatment on the sensitivity of different 5-HT receptor subtypes in inhibiting aversion induced by electrical stimulation of this brain area. For that, the effects of intra-DPAG administration of the endogenous agonist 5-HT (20 nmol), the 5-HT_{1A} receptor agonist 8-OH-DPAT (8 nmol) and the 5-$HT_{2A/2C}$ receptor agonist DOI (16 nmol) were measured in female Wistar rats given either chronic injection of imipramine (15 mg/kg, 3 weeks, ip) or saline. The results showed that the three receptor agonists raised the threshold of aversive electrical stimulation in both groups of animals, but this antiaversive effect was significantly higher in rats treated with imipramine. Treatment with imipramine did not change the basal threshold of aversive electrical stimulation measured before intra-DPAG injection of the 5-HT agonists. The results suggest that sensitization of both 5-HT_{1A} and 5-HT_2 receptors within the DPAG may be involved in the beneficial effect of imipramine in panic disorder (PD).

Keywords: Aversive brain stimulation; 5-HT; Dorsal periaqueductal gray; Imipramine; 8-OH-DPAT; DOI; Panic disorder

1. Introduction

The dorsal periaqueductal gray matter (DPAG) integrates behavioral and autonomic expression of defensive reactions (for reviews, see Bandler and Shipley, 1994; Graeff, 1990, 1994). The electrical or chemical stimulation of this structure causes neurovegetative and behavioral changes suggestive that the experimental animal (Olds and Olds, 1962) or human patient (Nashold et al., 1974) is undergoing a markedly aversive experience. Given the striking similarities between the autonomic and behavioral effects of the DPAG stimulation and the symptoms of panic attacks, it has been suggested that the DPAG is involved in the genesis of panic disorder (PD) in human and that the DPAG stimulation can model panic attacks (Graeff, 1990; Jenck et al., 1995; Lovick, 2000).

There is abundant experimental evidence indicating that serotonin (5-HT) inhibits aversion generated in the DPAG. Early results have shown that lever-pressing behavior maintained by either reduction or termination of DPAG electrical stimulation in rat is facilitated by *para*-chlorophenylalanine, a 5-HT synthesis inhibitor (Kiser and Lebovitz, 1975), or by the 5-HT receptor blockers methisergide and cyproheptadine (Schenberg and Graeff, 1978) and inhibited by the precursor of 5-HT synthesis 5-hydroxytryptophan (5-HTP), or by the presynaptic 5-HT uptake inhibitor clomipramine (Kiser et al., 1978).

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In a series of experiments, rats with a cannula-electrode (chemitrode) chronically implanted into the DPAG were placed inside a shuttle-box and electrically stimulated with increasing current intensities until an escape response occurred. It was shown that intra-DPAG injection of drugs that mimic or facilitate 5-HT activity caused an antiaversive effect (Schütz et al., 1985; Audi et al., 1988; Nogueira and Graeff, 1991). Using this method, Nogueira and Graeff (1995) additionally showed that 5-HT_{1A} and 5-HT_2 receptors in the DPAG might mediate the antiaversive action of 5-HT. Thus, the 5-HT_{1A} receptor agonists 8-OH-DPAT and BAY-R-1530 raised the threshold of DPAG electrical stimulation for inducing escape response. The effect of 8-OH-DPAT was antagonized by the 5-HT_{1A} receptor blocker NAN-190. Similarly, microinjection of the 5-$HT_{2A/2C}$ agonist DOI causes an antiaversive effect that was antagonized by the 5-HT_{2A} receptor blocker spiperone. Moreover, it was shown that whereas spiperone also counteracted the effect of 8-OH-DPAT, NAN-190 antagonized the effect of DOI, raising the possibility that both receptors have to be functional for the expression of each one's activation to occur.

On the basis of clinical and preclinical evidences, such as the ones mentioned above, it has been suggested that 5-HT mechanisms regulating aversion in the DPAG are involved in PD and in the mode of action of antipanic drugs (Deakin and Graeff, 1991; Graeff, 1991, 1993; Graeff et al.,1993). Moreover, the results indicating that both 5-HT_{1A} and 5-HT_{2A} receptors inhibit aversion in the DPAG (Schütz et al., 1985; Audi et al., 1988; Nogueira and Graeff, 1995) suggest the participation of these receptors in the therapeutic effect of antipanic drugs.

Concerning the role of DPAG 5-HT_{1A} receptors on the effects of antipanic drugs, Mongeau and Marsden (1997) have shown that long-term treatment with the classical antipanic agent imipramine increased the inhibitory effect of intra-DPAG administration of 8-OH-DPAT on escape and subsequent freezing behavior induced by D,L-homocysteic acid (DLH). Therefore, imipramine seems to cause a sensitization of 5-HT_{1A} receptors in the DPAG. Interestingly, long-term treatment with tricyclic antidepressants also results in sensitization of the rat hippocampal and forebrain neurons to 5-HT or 8-OH-DPAT (de Montigny and Aghajanian, 1978; Blier et al., 1987; Bijak and Tokarski, 1994; Bijak et al.,1996; Chaput et al., 1991).

Although evidence described to date implicates 5-HT_{1A} receptors of the DPAG in the antipanic effect of imipramine, other studies indirectly suggest that 5-HT_2 receptors in the DPAG may also contribute to this effect. In this study, we further explore the role of the DPAG 5-HT_{1A} and investigate the participation of 5-HT_2 receptors in the antipanic effect of the imipramine. For that, we investigated the effects of intra-DPAG injection of 5-HT, 8-OH-DPAT and DOI on the DPAG threshold of electrical stimulation-inducing escape behavior in rats treated chronically with imipramine.

2. Materials and method

2.1. Animals

Female albino Wistar rats, weighing 199–237 g, were housed in groups of six, with free access to food and water, under a 12L:12D cycle (lights on at 06:45 h). Room temperature was maintained at 23 ± 1 °C. The experiments were performed in compliance with the recommendations of SBNeC (Brazilian Society of Neuroscience and Behavior), which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Apparatus

The behavioral responses induced by DPAG electrical stimulation were evaluated in a $25 \times 20 \times 20$-cm box placed inside an insulating chest provided with 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 (Marseillan, 1977). 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.

2.3. Drugs

The following drugs were used: imipramine hydrochloride (Sigma, USA), 5-hydroxytryptamine creatinine sulfate (5-HT; Sigma), (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT; Sigma) and (±)-1-(2,5-dimethoxy-4-iodophenyl)piperazine dihydrochloride (DOI; RBI, USA). All drugs were dissolved in sterile saline.

2.4. Surgery

Sixteen days after the beginning of daily treatment with imipramine, rats were anaesthetized with tribromoethanol (250 mg/kg, ip) and operated in a stereotaxic instrument (David Kopf, USA) for the implant of a chemitrode in the DPAG. The chemitrode was made of stainless steel guide cannula (outside diameter 0.6 mm, 12.5 mm long) glued to a brain electrode made of stainless steel wire (diameter 250 μm), enamel insulated except at the cross-section of the tip, reaching 1 mm below the lower end of the cannula. The electrode wire was connected to a male pin, parallel to the outer end of the cannula, that could be plugged into an amphenol socket at the end of a flexible electrical cable and used for brain stimulation. Holding the incisor bar 2.4 mm below the interaural line, the chemitrode was introduced 1.9 mm lateral to lambda, at an angle of 22° with the sagittal plane, until the electrode tip was 5.2 mm below the

surface of the skull. The chemitrode was attached to the skull by means of acrylic resin and two stainless steel screws. A stylet with the same length of the guide cannula was introduced inside it to prevent obstruction.

2.5. Procedure

2.5.1. Imipramine treatment

Rats were injected daily with imipramine (15 mg/kg, ip) during the 24 days of the experiment. Control animals were injected with saline.

2.5.2. Intracerebral injection

For drug injection into the DPAG, a needle (outside diameter 0.3 mm) was introduced through the guide cannula until its tip was 1 mm below the cannula end. A volume of 0.25 μl was injected for 60 s using a 10-μl microsyringe (Hamilton, USA), its embolus being pushed by a micrometer. The displacement of an air bubble inside the polyethylene catheter connecting the syringe needle to the intracerebral needle was used to monitor the microinjection. The intracerebral needle was introduced 60 s before the beginning of the injection and was removed 60 s after the injection was finished. Each animal received four microinjections in counterbalanced order, according to previous randomization. An interval of 24 h was followed between injections. Saline, 5-HT (20 nmol), 8-OH-DPAT (8 nmol) or DOI (16 nmol) were administered 3 h after the last imipramine injection (21–24 days). These doses were selected on the basis of previous results showing their inhibitory effects on DPAG-induced escape responses (Schütz et al., 1985; Nogueira and Graeff, 1995).

2.5.3. Aversive thresholds determination

Five days after the surgery, the animals were placed in the box and the aversive threshold was determined through an electrical stimuli (AC, 60 Hz, 15 s) presented through the implanted chemitrode. The interstimulus interval was 15 s. The current intensity started at the level of 20 μA and was increased by steps of 8 μA until the rat presented running or jumping reactions, characterizing the escape behavior. The basal aversive threshold was defined as the lowest current intensity that produced these behaviors in three successive trials of electrical stimulation. Through this method, the determination of the aversive thresholds took nearly 5 min. Animals with basal thresholds above 200 μA were discarded. Following basal threshold determination, rats received the intracerebral injection, and the aversive threshold was redetermined either 10 (5-HT and 8-OH-DPAT) or 20 (DOI) min after the injection. Previous study has shown that on using a similar test protocol, there is no difference between animals tested 10 and 20 min after intra-DPAG injection of saline (Nogueira and Graeff, 1995). Therefore, all control animals were tested 10 min after intra-DPAG injection of saline.

2.6. Histology

After the experiment, animals were sacrificed under deep anesthesia with urethane. The brain was perfused through the heart with saline solution (0.9%) followed by 10% formalin solution, before being removed and fixed in 10% formalin. Frozen sections of 55 μm were cut using a microtome in order to localize the positions of the electrode tips according to the atlas of Paxinos and Watson (1986). Only the data from rats having electrode tips inside the DPAG were included in the statistical analysis.

2.7. Statistical analysis

The effect of long-term imipramine treatment on basal aversive threshold determined in four consecutive days (predrug evaluations) was analysed by a 2×4 analysis of variance. One of the factors was related to the intraperitoneal imipramine treatment and was analysed as a between-subject group with two levels (saline or imipramine). The other factor was related to the four predrug evaluation trials and was analysed as a within-subject group with four levels. The effects of intra-DPAG injection of drugs were also analysed by a 2×4 analysis of variance. Again, one of the factors was related to the intraperitoneal imipramine treatment. The other factor was related to the intra-DPAG treatment (saline, 5-HT, 8-OH-DPAT or DOI) and was analysed as a within-subject group with four levels. When appropriated, post hoc comparisons were performed by the Newman–Keuls test.

3. Results

3.1. Behavioral effects of the DPAG electrical stimulation

Electrical stimulation of the DPAG caused the following sequence of behavioral changes as the intensity of electrical

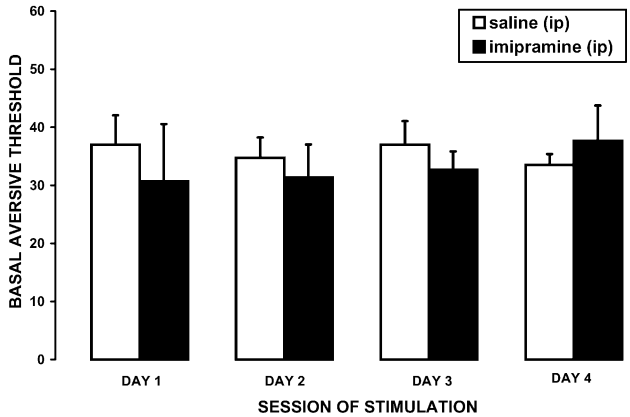

Fig. 1. Lack of imipramine (15 mg/kg, 21–24 days) effect on the basal aversive threshold inducing escape behavior after DPAG electrical stimulation. The aversive thresholds were determined in four consecutive days. Columns represent the mean and bars represent the S.E.M. of six to eight rats.

current increased: first alert, sometimes freezing, and finally running, which stopped as soon as the brain stimulation was switched off. The mean basal aversive threshold along the 4 days of experiment was 34.76 ± 1.69 μA (peak to peak).

3.2. Effects of long-term imipramine treatment on the basal aversive threshold

As shown in Fig. 1, long-term treatment with imipramine did not change the basal aversive threshold along the 4 days of experiment. Analysis of variance revealed a nonsignificant effect of treatment [$F(1,12) = 0.20$, $P = .67$], trial [$F(3,36) = 0.18$, $P = .91$] or Treatment × Trial interaction [$F(3,36) = 0.76$, $P = .52$].

3.3. Effects of long-term imipramine treatment on the aversive threshold measured after intra-DPAG injection of the serotonergic agonists

As illustrated in Fig. 2, microinjection of 5-HT, 8-OH-DPAT or DOI into the DPAG raised the aversive threshold in rats chronically treated with both saline or imipramine [effect intraperitoneal treatment—$F(1,10) = 15.36$, $P = .003$]. Repeated comparisons with saline intra-DPAG by means of Newman–Keuls test evidenced significant effects for each of the three 5-HT agonists used in both saline (5-HT, $P = .013$; 8-OH-DPAT, $P = .049$; DOI, $P = .009$) and imipramine (5-HT, $P = .0017$; 8-OH-DPAT, $P = .0014$; DOI, $P = .0027$) groups.

Analysis of variance also revealed a significant effect of intra-DPAG drug treatment [$F(3,30) = 20.81$, $P < .001$] and an interaction between the two factors analysed [$F(3,30) = 4.08$, $P = .015$]. Post hoc comparison indicated that the effect of the intra-DPAG injection of the 5-HT agonists, but not of saline, was higher in the group chronically treated with imipramine (saline, $P = .7$; 5-HT, $P = .006$; 8-OH-DPAT, $P = .0003$; DOI, $P = .012$).

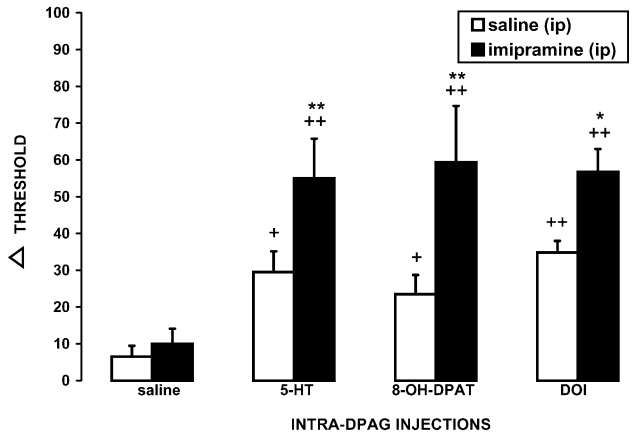

Fig. 2. Effect of imipramine treatment (15 mg/kg, 21–24 days) on the antiaversive effect caused by intra-DPAG microinjection of 5-HT (20 nmol), 8-OH-DPAT (8 nmol) and DOI (16 nmol). The change in threshold (Δ) was the difference between threshold values obtained post- and pre-DPAG injection of the drugs in the same animal. Columns represent the mean and bars the S.E.M. ($N = 6–8$). * $P < .05$ and ** $P < .01$, compared with animals injected intraperitoneally with saline. $^{+}P < .05$ and $^{+}P < .01$, compared with the respective saline intra-DPAG injected group.

4. Discussion

The present results show that the intra-DPAG injection of 5-HT, the 5-HT_{1A} receptor agonist 8-OH-DPAT and 5-$HT_{2A/2C}$ receptor agonist DOI enhanced the threshold of electrical stimulation necessary to induce escape behavior from the DPAG. This antiaversive effect was more pronounced in rats chronically treated with imipramine.

These results confirm previously reported observations that in drug-naive animals, direct application of compounds that mimic or facilitate 5-HT activity in DPAG decreases the aversive effects of the stimulation of this brain area. More specifically, it has been shown that intra-DPAG administration of the three agonists used in the present study inhibits the expression of escape responses induced by the electrical stimulation of the DPAG (Schütz et al., 1985; Nogueira and Graeff, 1995). Furthermore, 8-OH-DPAT also decreases aversion generated by the chemical stimulation of the DPAG by the amino acid DLH (Beckett et al., 1992; Mongeau and Marsden, 1997).

It is noteworthy that, as in our study, Mongeau and Marsden (1997) did not detect any significant effect of chronic imipramine treatment on the baseline level of escape generated by DPAG stimulation. In our conditions, lack of imipramine effect was observed along four successive trials (21–24 days of chronic drug injection). Contrasting with these results, it has been recently shown that 21-day administration of the clinically effective antipanic drugs, clomipramine and fluoxetine, attenuated aversion induced by the electrical stimulation of the DPAG (Vargas and Schenberg, 2001). In the latter study, a reliable drug effect was revealed when flight behavior was split into single responses as galloping, trotting and jumping. Under this condition of analysis, the former behavior was shown to be particularly sensitive to both drugs. Therefore, it remains to be explored whether in the present study the use of an ethogram-based analysis of DPAG-induced escape behavior would have favored the observation of imipramine antiaversive effect.

With regard to the effects of imipramine, our results are the first demonstration that 5-HT_2 receptor subtypes within the DPAG, allied with 5-HT_{1A} receptors, are sensitized after chronic treatment with this panicolytic drug.

The observed potentiation of the 8-OH-DPAT antiaversive effect by imipramine is in full agreement with the results obtained by Mongeau and Marsden (1997) in male rats with the chemical, instead of the electrical, stimulation of the DPAG. These results are also supported by electrophysiological evidences showing that in different brain areas, such as the hippocampus (de Montigny and Aghajanian, 1978; Blier et al., 1987; Bijak and Tokarski, 1994; Bijak et al.,1996; Chaput et al., 1991), the amygdala (Wang

and Aghajanian, 1980), the suprachiasmatic nucleus (Mason and Meyer, 1982) and the somatosensory cerebral cortex (Jones, 1980), chronic tricyclic antidepressant treatment increases neuronal responsiveness to 5-HT and 5-HT_{1A} receptor agonists.

The present results also indicated that chronic treatment with imipramine increased the antiaversive effect of 5-$HT_{2A/2C}$ receptor agonist DOI when microinjected into the DPAG. Conversely, it has been reported that long-term treatment with imipramine decreased the sensitivity of forebrain 5-HT_2 receptors (Willner, 1985) and reduced head-shake response induced by the 5-HT_2 agonists quipazine and DOB (Eison et al., 1989; Wieland et al., 1993). A possible explanation for these divergent results might be related to different mechanisms of antidepressant action on the PAG in relation to forebrain structures. In fact, Graeff (1991, 1993) suggested that such down-regulation of forebrain 5-HT_2 receptors would result in the protracted anxiolytic action of antidepressants. However, this mechanism is unlikely to explain the antipanic activity of antidepressants, since 5-HT has an antiaversive effect in the DPAG probably through 5-HT_2 receptors (Schütz et al, 1985; Nogueira and Graeff, 1995). Moreover, there are clinical reports indicating that the 5-HT_2 antagonist ritanserin is ineffective or aggravates PD (Deakin et al., 1990; Den Boer and Westenberg, 1990). Therefore, Graeff (1991, 1993) has hypothesized that the effect of antidepressants on PD is mediated by the 5-HT projection of the dorsal raphe to the PAG, where 5-HT decreases aversion through stimulation of 5-HT_2 receptors. Shutting down the firing rate of 5-HT raphe neurons, antidepressants would first decrease 5-HT_2 receptor stimulation, leading to the initial aggravation of PD. With prolonged administration, as subsensitization of somatodendritic autoreceptors develops and the efficacy of 5-HT neurotransmission in the PAG increases, inhibition of the PAG neurones commanding aversive behavior occurs, resulting in the antipanic effect. Thus, contrary to the down-regulation of forebrain 5-HT_2 receptors caused by chronically administered antidepressants in the forebrain, but in accordance with Graeff's hypotheses, 5-HT_2 receptors in the PAG would be expected to be up-regulated or at least remain unaffected. The present results are consistent with this prediction.

In terms of the enhanced response to DOI, indirect evidences suggest the 5-HT_{2A} receptor population in the DPAG is mainly affected by imipramine administration. Thus, in a previous study, we have shown that the effect of DOI in inhibiting DPAG-induced escape response was blocked by the preferential 5-HT_{2A} receptor antagonist spiperone (Nogueira and Graeff, 1995). Additionally, it has been reported that the intra-DPAG administration of the preferential 5-HT_{2C} receptor agonist mCPP either enhanced (Beckett et al., 1992) or did not alter (Nogueira and Graeff 1995) the defense response induced by DPAG stimulation.

Altogether, the experimental evidences reported to date and the results of the present study strongly indicate that the activation of both 5-HT_{1A} and 5-HT_{2A} receptors mediates the antiaversive effect of 5-HT in DPAG. Besides, the present study supports the involvement of these two subtypes of 5-HT receptors within the DPAG in the antipanic effect exerted by imipramine. Further studies are necessary in order to verify if the present observations can be extended to other panicolytic drugs currently in use as the selective serotonin reuptake inhibitors and MAO inhibitors.

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References

Audi EA, Aguiar JC, Graeff FG. Mediation by serotonin of the antiaversive effect of zimelidine and propranolol injected into the dorsal midbrain central grey. J Psychopharmacol 1988;2:26–32.

Bandler R, Shipley MT. Columnar organization in the midbrain periaqueductal gray: modules for emotional expression? Trends Neurosci 1994; 17:379–89.

Beckett SRG, Lawrence AJ, Marsden CA, Marshall PW. Attenuation of chemically induced defence response by 5-HT_1 receptor agonists administered into the periaqueductal gray. Psychopharmacology 1992; 108:110–4.

Bijak M, Tokarski K. Prolonged administration of imipramine and (+)-oxaprotiline, but not citalopram, results in sensitization of the rat hippocampal CA1 neurons to serotonin en vivo. Pol J Pharmacol 1994; 46:163–7.

Bijak M, Tokarski K, Czyrak A, Mackowiak M, Wedzony K. Imipramine increases the 5-HT1A receptor-mediated inhibition of hippocampal neurons without changing the 5-HT1A receptor binding. Eur J Pharmacol 1996;305:79–85.

Blier P, Montigny C, Chaput Y. Modifications of the serotonin system by the antidepressant treatments: implications for the therapeutic response in major depression. J Clin Psychopharmacol 1987;7:24S–35S.

Chaput Y, de Montigny C, Blier P. Presynaptic and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments: an in vivo electrophysiology study in rat. Neuropsychopharmacology 1991;5:219–29.

Deakin JFW, Graeff FG. 5-HT and mechanisms of defense. J Psychopharmacol 1991;5:305–15.

Deakin JFW, Wang M, Guimarães FS. Early clinical experience with ritanserin in neurotic patients. A clinical profile of ritanserin. Chester: Adis International, 1990. p. 16–33.

de Montigny C, Aghajanian GK. Tricyclic antidepressants: long-term treatment increases responsivity of rat forebrain neurons to serotonin. Science 1978;202:1303–6.

Den Boer JA, Westenberg HGM. Serotonin function in panic disorder: a double blind placebo controlled study with fluvoxamine and ritanserin. Psychopharmacology 1990;102:85–94.

Eison AS, Eison MS, Yocca FD, Gianutsos G. Effects of imipramine and serotonin-2 agonists and antagonists on serotonin-2 and beta-adrenergic receptors following noradrenergic or serotonergic denervation. Life Sci 1989;44:1419–27.

Graeff FG. Brain defense systems and anxiety. In: Roth M, Burrows GD,

Noyes R, editors. Handbook of anxiety. The neurobiology of anxiety, vol. 3. Amsterdam: Elsevier, 1990. p. 307–54.

Graeff FG. Neurotransmitters in the dorsal PAG and animal models of panic anxiety. In: Briley M, File SE, editors. New concepts in anxiety. London: Macmillan, 1991. p. 288–312.

Graeff FG. Role of 5-HT in defensive behavior and anxiety. Rev Neurosci 1993;4:181–211.

Graeff FG. Neuroanatomy and neurotransmitter regulation of defensive behaviors and related emotions in mammals. Braz J Med Biol Res 1994;27:811–29.

Graeff FG, Silveira MC, Nogueira RL, Audi EA, Oliveira RMW. Role of the amygdala and periaqueductal gray in anxiety and panic. Behav Brain Res 1993;58:123–31.

Jenck F, Moreau J-L, Martin JR. Dorsal periaqueductal gray-induced aversion as a simulation of panic anxiety: elements of face and predictive validity. Psychiatr Res 1995;57:181–91.

Jones RS. Long-term administration of atropine, imipramine and viloxazine alters responsiveness of rat cortical neurons to acetylcholine. Can J Physiol Pharmacol 1980;58:531–5.

Kiser RS, Lebovitz RM. Monoaminergic mechanisms in aversive brain stimulation. Physiol Behav 1975;15:47–53.

Kiser RS, Lebovitz RM, German DC. Anatomic and pharmacologic differences between two types of aversive midbrain stimulation. Brain Res 1978;155:331–42.

Lovick TA. Panic disorder—a malfunction of multiple transmitter control systems within the midbrain periaqueductal gray matter. Neuroscientist 2000;6:48–59.

Marseillan RF. A solid state sine-wave stimulator. Physiol Behav 1977;19: 339–40.

Mason R, Meyer JH. Enhanced responsiveness of rat suprachiasmatic nucleus (SCN) neurones to 5-hydroxytryptamine (5-HT) following chronic imipramine treatment. J Physiol (London) 1982;332:105P–6P.

Mongeau R, Marsden CA. Effect of imipramine treatments on the 5-HT_{1A}-receptor-mediated inhibition of panic-like behaviours. Psychopharmacology 1997;131:321–8.

Nashold 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.

Nogueira RL, Graeff FG. 5-HT mediation of the antiaversive effect of isamoltane injected into the dorsal periaqueductal grey. Behav Pharmacol 1991;2:73–7.

Nogueira RL, Graeff FG. Role of 5-HT receptor subtypes in the modulation of dorsal periaqueductal gray aversion. Pharmacol, Biochem Behav 1995;52:1–6.

Olds ME, Olds J. Approach–escape interactions in rat brain. Am J Physiol 1962;203:803–10.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press, 1986.

Schenberg LC, Graeff FG. Role of the periaqueductal gray substance in the antianxiety action of benzodiazepines. Pharmacol, Biochem Behav 1978;9:287–95.

Schütz MTB, Aguiar JC, Graeff FG. Anti-aversive role of serotonin in the dorsal periaqueductal grey matter. Psychopharmacology 1985;85: 340–5.

Vargas LC, Schenberg LC. Long-term effects of clomipramine and fluoxetine on dorsal periaqueductal grey-evoked innate defensive behaviours of the rat. Psychopharmacology 2001;155:260–8.

Wang RY, Aghajanian GK. Enhanced sensitivity of amygdala neurons to serotonin and norepinephrine after chronic antidepressant treatment. Commun Psychopharmacol 1980;4:83–90.

Wieland S, Fischette CT, Lucki I. Effect of chronic treatments with tandospirone and imipramine on serotonin-mediated behavioral responses and monoamine receptors. Neuropharmacology 1993;32: 561–73.

Willner P. Antidepressants and serotonergic neurotransmission: an integrative review. Psychopharmacology 1985;85:387–404.