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REVIEW

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On serotonin and experimental anxiety

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Abstract *Background:* This review describes the development of a research line on the role of serotonin (5-HT) in experimental anxiety that was initiated in 1969, in the laboratory founded by P.B. Dews, W.H. Morse and R.T. Kelleher at the Harvard Medical School, and has evolved until this date. *Results:* Initially, it was found that two non-selective 5-HT receptor antagonists released punished responding in pigeons with a magnitude comparable to that of benzodiazepine anxiolytics. This result was one of the key evidences that led to the concept that 5-HT enhanced anxiety by acting both in the forebrain and in the periaqueductal gray matter (PAG). Further evidence supported this hypothesis regarding the forebrain, but results with electrical stimulation and intracerebral drug injection into the PAG indicated that 5-HT inhibited aversive behavior evoked from this area. As a result, it has been suggested that 5-HT has a dual role in the regulation of defense, namely enhancing learned responses to potential or distal threat through actions in the forebrain while inhibiting unconditioned responses to proximal threat by acting on the PAG. The former would be related to generalized anxiety and the latter to panic disorder. To test this hypothesis, a new animal model, named the elevated T-maze, has been designed. It consists of one arm enclosed by walls that is perpendicular to two open arms elevated from the floor. The same rat performs two tasks, namely inhibitory avoidance of the elevated open arms, representing conditioned anxiety and one-way escape from one of the open arms, representative of unconditioned fear. *Conclusion:* The differential effects of drugs acting on 5-HT observed in the two tasks of the ETM generally support the hypothesis under scrutiny.

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Introduction

This review describes the development of a research line on the role of serotonin (5-HT) in experimental anxiety that was initiated in the laboratory founded by P.B. Dews, W.H. Morse and R.T. Kelleher at the Harvard Medical School and has evolved until this date.

While working as postdoctoral fellows under the supervision of P.B. Dews, R.I. Schoenfeld and I unexpectedly found that methysergide markedly released responding suppressed by punishment, a property characteristic of anxiolytic drugs (Graeff and Schoenfeld 1970). Since methysergide was known to block 5-HT receptors, a relationship between 5-HT and punishment was established. Soon after, the participation of 5-HT in the mode of action of benzodiazepine anxiolytics has been suggested (Wise et al. 1972).

However, the interest on the role of 5-HT in anxiety faded considerably during the late 1970s and early 1980s, because of the impact of the evidence implicating benzodiazepine receptors and γ-aminobutyric acid (GABA) in the anxiolytic action of benzodiazepines (Costa and Guidotti 1979). Nevertheless, the focus on 5-HT and anxiety has been re-established from the mid-1980s on, remaining until this day. The main reasons for this revival were the introduction in clinical practice of the non-benzodiazepine anxiolytic buspirone (Eison and Temple 1989) and, chiefly, the widespread use of antidepressants, in particular the selective serotonin reuptake inhibitors, for treating anxiety disorders (Argyropoulos et al. 2000).

The first section of this review summarizes experimental evidence indicating that 5-HT mediates punished behavior by acting in forebrain structures such as the septo-hippocampal system and the amygdala. The second section describes a series of results showing that 5-HT inhibits behavior controlled by aversive electrical

or chemical stimulation of the dorsal periaqueductal gray. A hypothesis proposing that 5-HT enhances anxiety by acting on the forebrain, but inhibits panic through actions in the dorsal periaqueductal gray, is outlined in the third section. The fourth section presents pharmacological results obtained with an animal model of anxiety designed to test the above hypothesis. The last section summarizes the reviewed evidence and states the conclusions.

5-HT and punished behavior

Although no explicit relation between 5-HT and punishment was suggested, the results of an early study by Robichaud and Sledge (1969) had shown that the selective inhibitor of 5-HT synthesis para-chlorophenylalanine (PCPA) increased bar pressing rates maintained by sweetened milk presentation, but simultaneously suppressed by response-contingent foot-shock in rats. In this conflict paradigm, originally described by Geller and Seifter (1960), there is a non-punished component in which bar-pressing responses are maintained at a regular rate by presentation of reinforcement at a variable-interval (VI) schedule and a punished component in which every response is simultaneously reinforced and punished.

Shortly thereafter, Graeff and Schoenfeld (1970), working with pigeons, found that methysergide dose-dependently increased key-pecking rates maintained by a fixed-interval 5 min (FI-5) schedule of food presentation and suppressed by response-contingent electric shocks. The shocks were delivered through golden electrodes implanted around the pubis bones at a fixed-ratio of ten responses (FR10) schedule. To avoid interference with feeding behavior, only non-reinforced responses were followed by shock. The pigeons had participated in an extensive study with several benzodiazepine anxiolytics (Wuttke and Kelleher 1970). As a result, the magnitude of the effect of methysergide could be compared to that of the classical anxiolytics, and it was observed that methysergide was as effective as the most potent benzodiazepines in releasing punished responding. Because methysergide had been shown to block 5-HT receptors in vitro, the involvement of such receptors in the anti-punishment effect of the drug was tested by the administration of another 5-HT receptor antagonist, bromolysergic acid (BOL) and a receptor agonist, α-methyltryptamine. The obtained results showed that BOL released punished behavior in the same way as methysergide, although to a lesser extent. In contrast, α-methyltryptamine further decreased the already low rates of punished responding. On the basis of these results and those reported by Robichaud and Sledge (1969), it was suggested that tryptaminergic mechanisms in the brain mediate the response suppression determined by punishment.

To these behavioral results, Wise et al. (1972) added in vitro neurochemical evidence supporting an involvement of 5-HT in anxiety. Their results have shown that the benzodiazepine anxiolytic oxazepam decreased 5-HT turnover in the rat midbrain, at the same dose that released punished responding in the Geller-Seifter procedure. Both the median raphe nucleus (MRN) and the dorsal raphe nucleus (DRN) are localized in the midbrain and contain cell bodies of 5-HT neurons that project to the forebrain and brain stem (Azmitia and Segal 1978). Wise et al. (1972) proposed that while noradrenaline would mediate reward, ascending 5-HT pathways would facilitate the effects of punishment by acting on structures localized in both the forebrain and midbrain, the function of which is to suppress ongoing behavior. One critical structure of the midbrain is the periaqueductal gray matter (PAG), which belongs to a longitudinally organized brain system that controls aversive behavior (see below). It was further postulated that benzodiazepine anxiolytics reduce anxiety by decreasing 5-HT release in these brain areas. Therefore, in this theoretical model, 5-HT is supposed to enhance anxiety by acting both in forebrain structures and in the PAG.

The suggestion that 5-HT increases the effects of punishment by acting on the forebrain (Wise et al. 1972) got strong support from a study by Tye et al. (1977) with the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), which selectively destroys 5-HT neurons. The results of this study show that microinjection of 5,7-DHT into the ventromedial tegmentum of the rat midbrain caused 70% depletion of cortical 5-HT, indicating that a major part of the ascending 5-HT projections had been destroyed. This treatment prevented the acquisition of response suppression induced by foot-shock in a modified Geller-Seifter procedure. As complementary evidence, Graeff and Silveira Filho (1978) found that electrical stimulation of the MRN inhibited ongoing lever-pressing behavior maintained by a VI schedule of water presentation as well as induced defecation, urination, piloerection, teeth clattering and exophthalmos. Thus, electrical stimulation of the MRN mimicked the effect of a conditioned aversive stimulus, as in the classical conditioned suppression and conditioned emotional response procedures (Estes and Skinner 1944; Millenson and Leslie 1974). In addition, pretreatment with PCPA reduced the effect of MRN electrical stimulation, suggesting a 5-HT mediation of the response suppression. Since MRN 5-HT neurons project mainly to the dorsal hippocampus (Azmitia and Segal 1978), the latter results are concordant with Gray's (1982) proposal that anxiety is due to activation of the septo-hippocampal system. In the same vein, Schoenfeld (1976) observed that the hallucinogens LSD and mescaline released punished licking in the rat and suggested that this effect may be due to decreased activity of ascending serotonergic neurons.

In addition to the septo-hippocampal system, 5-HT may also act in the amygdala to mediate the effects of punishment. In this regard, microinjection of 5-HT antagonists into the basolateral amygdala was shown to release water licking suppressed by electric shock punishment (Petersen and Scheel-Krüger 1984). As a counterpart, microinjection of 8-hydroxy-2(di-n-propyl-

amino)tetralin (8-OH-DPAT) into the same area of the amygdala has been reported to further decrease punished lever pressing in a modified Geller-Seifter procedure (Hodges et al. 1987). These results add to a large body of evidence implicating the amygdala in the learning and expression of conditioned fear and anxiety (Davis 1992; LeDoux 1993).

The evidence reviewed so far is in agreement with the suggestion by Wise et al. (1972) that 5-HT enhances anxiety by acting in forebrain structures. However, the additional proposal that 5-HT actions in the PAG similarly enhance anxiety has not been supported by experimental evidence, as discussed in the following session.

5-HT and the PAG

Electrical stimulation of the dorsal PAG (dPAG) in laboratory animals induces defensive reactions, such as vigorous flight or defensive aggression (Hunsperger 1956). These defense strategies are expressed in natural conditions when a predator is very close to or in direct contact with the prey (Blanchard and Blanchard 1988; Blanchard et al. 2001). Fight or flight reactions are also induced by cutaneous nociceptive stimuli or by suffocation (Deakin and Graeff 1991), and a low suffocation threshold has been implicated in the genesis of panic attacks that occur in panic disorder (PD) patients (Klein 1993). In this regard, electrical stimulation of the PAG has been reported to induce panic-like symptoms (DSM IV; American Psychiatric Association 1994) in neurosurgical patients. These were palpitation, blushing of face and neck and respiratory arrest or hyperventilation, feelings of terror or impending death, and desire to flee (Nashold et al. 1974; Amano et al. 1978). Such evidence, among others, led to the suggestion that the dPAG may be involved in PD (Gentil 1988; Graeff 1988, 1990, 1991; Deakin and Graeff 1991, Lovick 2000; Gray and McNaughton 2000).

Laboratory animals easily learn to switch off electrical stimulation of the dPAG (Delgado et al. 1954). According to the theoretical model on 5-HT and anxiety elaborated by Wise et al. (1972), 5-HT is expected to facilitate this escape behavior. However, reported results with a procedure in which rats are trained to lever press in order to decrease the intensity of electrical current applied to the dPAG by 5% of the original current suggest the opposite action. Indeed, using this decremental escape procedure, Kiser and Lebovitz (1975) have shown that the 5-HT-depleting drug PCPA markedly increased bar-pressing rates, a result confirmed in a later study performed in the same laboratory (Kiser et al. 1978b). A similar investigation has additionally shown that administration of the precursor 5-hydroxytryptophan (5-HTP) dose-dependently reduced decremental bar pressing, and that the 5-HT reuptake inhibitor clomipramine had the same effect (Kiser et al. 1978a). Finally, electrical stimulation of the DRN, which sends 5-HT nerve fibers to the PAG, has also been reported to depress decremental escape from dPAG electrical stimulation (Kiser et al. 1980). Therefore different ways of increasing 5-HT activity in the dPAG had an anti-aversive effect, whereas 5-HT depletion resulted in facilitation of escape from dPAG electrical stimulation.

A series of experiments performed at the Department of Pharmacology of the Medical School of Ribeirão Preto, University of São Paulo further substantiate this conclusion. In the first study, rats were trained to lever-press to switch off electric stimuli applied to the dPAG and adjoining tectum of the mesencephalon. The 5-HT receptor antagonist cyproheptadine decreased the average latencies of switch-off responses in six of eight rats and methysergide did the same in one out of three rats. In turn, doses of chlordiazepoxide that caused little sedation or ataxia produced dose-dependent increases in escape latencies (Schenberg and Graeff 1978). Such opposite effects of a benzodiazepine anxiolytic and the 5-HT antagonist contrast with the similar anti-punishment effects of the two classes of drugs verified in conflict tests (see preceding section). To explore this issue further, Morato de Carvalho et al. (1981) carried out an experiment using electrical stimulation of the dPAG as a punishing stimulus. Lever-pressing behavior was maintained by water reinforcement and punished by dPAG stimulation. A multiple schedule with VI-2 non-punished component and a punished component, in which every response (FR1) was both rewarded and punished, was used. The results demonstrated that the anxiolytics chlordiazepoxide and pentobarbital caused dose-dependent increases in dPAG punished responding. In contrast, cyproheptadine did not increase punished responding at doses that had been shown to markedly release behavior punished by foot-shock (Graeff 1974); methysergide was also ineffective. Therefore the neural substrate of dPAG-delivered punishment seems to be different from that of peripherally applied punishment.

This last view is supported by the results of a comparative study performed at the Department of Experimental Psychology of the University of Oxford (Graeff and Rawlins 1980). Two groups of rats were trained to lever press on an FR1 schedule of food presentation. In one group, every response was subsequently punished by foot-shock delivery; in the other, by brief electrical stimulation of the dPAG. In both groups, response rates were reduced to less than 10% of pre-punishment rates, but responding was not completely suppressed. Also, response rates did not significantly differ between the two groups, either before or after the introduction of punishment. Lateral septal lesion significantly increased responding in the animals punished by foot-shock, but did not affect responding suppressed by dPAG stimulation. Injection of chlordiazepoxide significantly increased punished responding in both groups of rats, before as well as after the septal lesion. Before the septal lesion was made, responding suppressed by foot-shock was significantly more released by chlordiazepoxide than responding punished by dPAG stimulation. These results indicate that in punishment tests using foot-shock, both a behavioral in-

hibitory system (BIS), including the septo-hippocampal structures (Gray 1982) and a brain aversive system (BAS) that includes the dPAG (Graeff 1981), act together to produce response suppression. Anxiolytic drugs would depress both these systems in order to cause their anti-punishment effect. Within this perspective, it may be suggested that 5-HT drugs that release punished behavior act through the BIS alone, in which 5-HT would enhance behavioral inhibition (Graeff and Silveira Filho 1978). In the BAS, 5-HT is likely to inhibit aversion and, as a consequence, decreasing its activity would result in more, rather than less, response suppression (Graeff and Rawlins 1980).

The concept of 5-HT inhibiting aversion in the dPAG is supported by a series of experiments using intracerebral drug administration associated with electrical stimulation made through chemitrodes chronically implanted into the dPAG. For the test, rats bearing chemitrodes were placed inside a shuttle box and the intensity of a sinusoidal current was gradually increased until the rat ran towards the opposite compartment of the shuttle box. This response switched off the brain stimulation. The same procedure was repeated three times to determine the basal aversive threshold. Soon after, a drug microinjection was made and 10 min later the aversive threshold was re-determined. The difference between the magnitude of the post-drug and that of the basal threshold measures the drug effect on aversion. In the first study, microinjection of 5-HT as well as of the 5-HT receptor agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) into the dPAG raised the aversive threshold dose-dependently. Local pre-treatment with the 5-HT receptor antagonists metergoline or ketanserin blocked the anti-aversive effect of 5-HT, whereas pre-treatment with the 5-HT reuptake inhibitor zimelidine potentiated the same effect. Moreover, zimelidine raised the aversive threshold when given alone. Since ketanserin is a relatively selective 5-HT_2-receptor antagonist, these results suggest that 5-HT inhibits aversion in the dPAG by stimulating this type of 5-HT receptor (Schütz et al. 1985).

The role of 5-HT receptor subtypes in the regulation of aversion in the dPAG was further explored by measuring the effect of 5-HT_{1A} and 5-$HT_{2A/2C}$ receptor agonists microinjected into the dPAG. The 5-HT_{1A} agonists 8-OH-DPAT and ipsapirone raised the threshold of aversive electrical stimulation in a dose-dependent way. Similarly, microinjection of the 5-$HT_{2A/2C}$ agonist 2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) increased the aversive threshold. The 5-HT_{2C} preferential agonist 1-(m-chlorophenyl) piperazine (mCPP) was ineffective. Previous intra-DPAG administration of the non-selective 5-HT_{1A}-receptor blocker NAN-190 antagonized the anti-aversive effect of 8-OH-DPAT, whereas pretreatment with the 5-HT_{2A}-receptor blocker spiperone antagonized the effect of DOI. Spiperone also counteracted the effect of 8-OH-DPAT and NAN-190 counteracted the effect of DOI. These results indicate that activation of 5-HT_{1A} and 5-HT_{2A} receptors inhibits aversion in the dPAG and that both receptors have to be functional for the expression of each one's activation to occur (Nogueira and Graeff 1995). Work performed in another laboratory also points to an inhibitory role of 5-HT_{1A} on defense reactions elicited from the PAG. In these results, microinjection of 5-HT_{1A} agonists into the PAG attenuated running behavior induced by microinjection of an excitatory amino acid inside the same brain region. The selective 5-HT_{1A}-receptor blocker WAY 100635 antagonized this effect, whereas the preferential 5-HT_{2C}-receptor agonist mCPP facilitated running (Beckett et al. 1992; Beckett and Marsden 1997).

However, Jenck et al. (1989) arrived at an opposite view, namely that stimulation of 5-HT_{1A} receptors was pro-aversive. Since these researchers used systemic injection of drugs and a procedure in which rats were trained to jump over a ridge separating the two compartments of a shuttle box the interpretation of these results in terms of PAG mechanisms is not as ease as with intracerebral injection. For instance, systemically injected drugs may be acting mainly on pre-synaptic receptors located outside the PAG. In this regard, Beckett and Marsden (1997) have shown that peripheral injection of 8-OH-DPAT had a pro-aversive effect (like in the results by Jenck et al. 1989), whereas intra-PAG injection of the same drug had an anti-aversive effect on chemically induced defense. A likely explanation for this difference suggests that the peripherally injected 8-OH-DPAT stimulates autosomic 5-HT_{1A} receptors localized in the cell bodies of DRN 5-HT neurons, which inhibit neuronal firing (Aghajanian 1992). There is also some controversy concerning the role of 5-HT_2 receptors in aversion. In agreement with previously reported results (Schenberg and Graeff 1978), Jenck et al. (1989) found that the non-selective 5-HT receptor antagonists metergoline and mianserin decreased the threshold of escape from electrical stimulation of the dPAG. However, the selective 5-HT_2-receptor antagonist ketanserin had the opposite effect. The last result indicates that 5-HT_2 receptors facilitate aversion, contrary to the above suggestion based on results with intracerebral drug injection.

The anti-aversive effect of single systemic administration of clomipramine, originally described by Kiser et al. (1978a), has been generalized to the more selective 5-HT reuptake inhibitors fluvoxamine and sertraline (Jenck et al. 1990). In a recent study, the behavioral changes caused by dPAG electrical stimulation have been systematically observed in rats placed inside an arena (Vargas and Schenberg 2001). Under these experimental conditions, chronic treatment with clomipramine for 21 days attenuated behavioral items, like running and jumping, that are characteristic of the rat's defense reaction to proximal danger (Blanchard and Blanchard 1988; Blanchard et al. 2001). Since this regimen of drug administration is effective in the clinical treatment of PD (Gentil et al. 1993), the experimental paradigm used by Vargas and Schenberg (2001) may be viewed as a putative animal model of PD.

The anti-aversive effect of the 5-HT reuptake inhibitor zimelidine (Schütz et al. 1985) mentioned above im-

plies the existence of 5-HT nerve fibers in the dPAG that are regulating aversion. This view is strengthened by further experimental evidence showing that blockade of pre-synaptic 5-HT_{1B} receptors that inhibit 5-HT release with isamoltane, microinjected into the dPAG, raised the aversive threshold of dPAG electrical stimulation. Local pre-treatment with the 5-HT_2-receptor blockers, ketanserin and ritanserin antagonized this effect (Nogueira and Graeff 1991). Microinjection of the non-selective 5-HT_{1B} receptor antagonist (and β-adrenergic antagonist), propranolol into the dPAG had an anxiolytic effect blocked by ritanserin in rats exposed to the elevated plus-maze, an animal model of anxiety that will be further discussed below (Audi et al. 1991).

It is worth remarking that intra-dPAG administration of 5-HT receptor antagonists alone has no effect on the aversive threshold (Schütz et al. 1985; Nogueira et al. 1991). This finding contrasts with the major aversive effects caused by compounds like bicuculline, which block $GABA_A$ receptors in the dPAG (Brandão et al. 1982). It may be concluded that while GABAergic terminals tonically inhibit the neurons of the dPAG that control defensive behavior, serotonergic fibers seem to exert a phasic inhibition of aversion. Therefore, their modulatory influence would be manifested only under conditions that engage 5-HT systems, such as stress (Deakin and Graeff 1991).

Dual role of 5-HT in defense

Overall, the results reviewed so far indicate that 5-HT facilitates anxiety by acting on forebrain structures such as the septo-hippocampal system and the amygdala, but inhibits aversion through actions in the dPAG.

A wealth of experimental evidence reviewed elsewhere (Graeff 1990) has led to the conclusion that the amygdala, the medial hypothalamus and the PAG constitute a set of interrelated structures, the aforementioned BAS, which controls defensive strategies and elaborates the accompanying emotional and motivational states. Although the three components of the BAS would work together to generate defensive behaviors, these structures are likely to exert different functions. In this regard, Fanselow (1991) has suggested that the amygdala synthesizes the stimulus input from the environment and then signals to the PAG the degree of threat that they represent to the organism. The PAG would be in charge of selecting, organizing and executing the appropriate behavioral and neurovegetative defensive reactions.

R.J. Blanchard and D.C. Blanchard, at the Department of Psychology of Hawaii University, have undertaken the systematic study of the defensive strategies adopted by wild rats facing different types of predatory threat. The obtained results led to the concept of three levels of defense, namely potential (uncertain), distal and proximal threat, each evoking a different type of defense reaction. For instance, rats perform cautious exploration aimed at risk-assessment when danger is uncertain, like in novel environments. When the predator is perceived at distance, tense and attentive immobility (freezing) ensues. Finally, when the predator is near or in actual contact with the rat, the animal flees whenever possible or otherwise threatens back or even attacks the predator defensively (Blanchard and Blanchard 1988). Comparative studies led to the conclusion that homologous types of defense strategies can be found in other animals, including non-mammalian species (Blanchard et al. 2001). Although the present knowledge about the neural substrate of these defense strategies is incomplete, there have been attempts to relate each level of defense with brain structures thought to be critical for the expression of the corresponding defense reaction. In addition, it has been suggested that a particular emotion would be associated with a given level of defense. Shortly, the septo-hippocampal system and the amygdala would be the key structures for risk-assessment behavior in response to potential threat, the related emotion being anxiety (Graeff 1994). The septo-hippocampal system would provide the cognitive component of anxiety while the affective component would be integrated in the amygdala (Gray and MacNaughton 2000). As mentioned before, the vigorous, undirected flight elicited by proximal danger would be related to panic, the critical structure being the dPAG. At the intermediate level of distal threat, well-directed escape related to fear would be elaborated by the medial hypothalamus (Graeff 1994). A further step concerns psychopathology. In this regard, the first level of defense has been related to generalized anxiety disorder (GAD) and the third with PD, the second being implicated in specific phobias (Deakin and Graeff 1991; Gray and MacNaughton 2000).

Both the amygdala and the PAG receive serotonergic input mainly from the DRN. The axons that project onto the amygdala follow the DRN-forebrain tract, while those that go to the PAG run through the DRN-periventricular tract (Azmitia and Segal 1978). Most of the nerve fibers that originate in the DRN are thin and have small varicosities that make preferential contact with 5-HT_2 receptors (Mamounas et al. 1991).

Given this background, the dual 5-HT-defense hypothesis proposes that activation of the DRN results in facilitation of the defense strategies that are mainly integrated at the amygdala. At the same time, the defense reactions organized in the dPAG are inhibited. The adaptive function of this disposition would be to inhibit extreme defense patterns like flight or fight in situations where the predatory threat is uncertain or distant from the prey. In these circumstances, such behaviors are inappropriate, because they enhance the probability of detection by the predator. Instead, more flexible, largely learned, responses are likely to lead to successful escape or avoidance.

Testing the dual 5-HT-defense hypothesis

An experimental model that produces two types of defensive behavior in the rat has been developed (Graeff et al. 1993; Viana et al. 1994) to test the dual 5-HT hy-

pothesis. The model has been named the elevated-T maze because it derives from the elevated X- (Handley and Mithani 1984) or plus- (Pellow et al. 1985) maze, a widely used animal model of anxiety. Both models are based on the innate fear rats have of being on an elevated, non-protected alley (Montgomery 1955), probably because they are unable to perform thygmotaxis with their vibrissae (Treit et al. 1993). The elevated plus-maze consists of two opposed arms enclosed by walls except at the central end, the closed arms. These are perpendicular to two open arms of equal dimensions, which are devoid of any wall. The whole apparatus is elevated above the floor. The elevated T-maze (ETM) was obtained by closing the entry to one of the open arms of the elevated plus-maze. The rationale for the development of this model is explained in the following.

The above anti-punishment effect of non-selective 5-HT receptor antagonists, such as methysergide, cyproheptadine, cinanserin and metergoline, has been consistently reported in rats and pigeons (reviewed in Graeff 1981). However, the effect on classical conflict tests of more selective compounds, such as 5-HT_{1A} partial agonists and 5-HT_2 and 5-HT_3 receptor antagonists has been inconsistent (Griebel 1995). An exception is key-pecking punishment in pigeons, with which dose-dependent release of suppressed responding has been consistently observed (Barrett and Gleason 1991). To overcome this difficulty, new models based on ethology have been developed to reliably detect the anxiolytic-like action of these compounds in the rat, among which the elevated plus-maze. However, the reported results on 5-HT acting anxiolytics with this model have been equally variable (Griebel 1995). A plausible explanation for this variability is that the elevated plus-maze is a mixed model, in the sense that multiple defense reactions are displayed while the rat freely explores the apparatus. Since distinct 5-HT pathways may influence these defense strategies in different, even opposite ways (see preceding section), the effect of 5-HT-acting drugs would vary as a function of the predominance of one or the other of such defense reactions (Handley et al. 1993). For instance, the anxiogenic-like effect of 8-OH-DPAT in the elevated X-maze turned into anxiolytic-like when the brightness of the light on the apparatus was increased (McBlane and Handley 1994). Analyzing this problem, we noticed that two types of threats and defense strategies occur when the rat is exploring the elevated plus-maze. The first one is when the rat is leaving a closed arm and confronts with the open arms. This would result in inhibitory avoidance learning. The second is when the animal is exploring an open arm. In this situation, the rat is exposed to an innate fear stimulus and performs one-way escape to seek safety inside one of the closed arms. When the rat is freely exploring the maze, these conditions happen in a variable way. To increase experimental control, these defense strategies were separated, becoming two independent tasks carried out in the ETM.

In the inhibitory avoidance task, the rat is placed at the end of the enclosed arm of the ETM and the latency to withdraw from this arm with the four paws is recorded in three successive trials made at 30-s intervals. Learning is indicated by the increase in withdrawal latency along the trials. For the escape task, which initiates 30 s after the completion of the avoidance training, the rat is placed at the end of one of the open arms and the withdrawal latency from this arm is similarly recorded. In the studies performed so far, the number of trials of this task has varied from one to three. For reasons that will be further discussed, pre-exposure to the open arm for 30 min, 24 h before the experimental has been used in more recent studies.

It is worth remarking that the motor performance required by both tasks of the ETM is similar. This serves as a control for non-specific drug effects on motor activity, particularly when the latencies to withdraw from the enclosed arm and from the open arm are changed to opposite directions by the treatment. However, whenever the latencies are similarly increased or decreased, there is need for independent assessment of motor effects. Measuring motor activity inside an arena fulfils this requirement, albeit adding more complexity to the test.

A series of experiments has been performed to validate the ETM behaviorally (Zangrossi Jr and Graeff 1997). The obtained results have shown that restraining the animals at the end of the enclosed arm for 30 s with a partition before the first trial did not change the baseline withdrawal latency. This indicates that rats are not escaping from the experimenter's hand. In addition, rats placed in a modified T-maze having the three arms enclosed by walls did not show the usual increase in withdrawal latency along the three consecutive trials. Therefore, habituation of exploratory activity does not seem to contribute significantly to the increase in latency along trials that happens when the transversal arms of the maze are open. Such increase is likely to represent learning of inhibitory avoidance. Another experiment has further shown that the latency to leave the open arm did not undergo habituation over five consecutive trials, evidencing that this behavior is maintained by aversive motivation, constituting a genuine escape response.

The pharmacological validation of the ETM has been reviewed in detail elsewhere (Graeff et al. 1996c, 1998). Table 1 summarizes the effect of drugs administered systemically measured in the ETM.

Overall, the results with anxiolytic and anxiogenic drugs endow the inhibitory avoidance task in the ETM with a high predictive value in regard to the pharmacology of GAD. This comes as no surprise, since this task may be viewed as a modification of the classical punishment tests, from which elements that posed restrictions to their validity or feasibility have been eliminated. These are food or water deprivation and pain that add confounding motivational variables to the test, and prolonged training that is required by most kinds of punishment tests.

In addition, the observed anxiolytic-like effect of the 5-$HT_{2B/2C}$ antagonists SB 200646A and SER 082, and the 5-HT_{2A} antagonist SR 46349B are compatible with

Table 1 Effect of drugs administered systemically on the two tasks performed in the elevated T-maze. + Facilitation, – impairment and 0 no change with respect to control ($P<0.05$)

Drug class	Compound (doses in mg/kg)	Avoidance	Escape
Anxiolytic			
	Diazepam (0.5–4)	–	0
	Buspirone (0.3–3)	–	0
	Ipsapirone (0.25–2)	–	0
	Ritanserin (0.3–3)	–	0
	SB 200646 (3–30)	–	0
	SER 082 (0.1–1)	–	0
	SR 46349B (1–10)	–	0
	RP 62203 (0.25–4)	0	0
Anxiogenic			
	Yohimbine (0.3–3)	+	0
	MCPP (0.1–0.8)	+	–
	TFMPP (0.1–0.8)	+	–
Antidepressant			
	Moclobemide (3–30)	0	0
	Clomipramine (3–30)	+	0
	Imipramine (5–15)	+	–[b,c]
	Imipramine[a] (5–15 daily)	–	–[b,c]
5-HT releaser			
	d-Fenfluramine (0.03–0.3)	+	–

[a] Chronic administration
[b] Pre-exposure to the open arm
[c] Two trials

the view that 5-HT facilitates inhibitory avoidance or enhances the suppressive effect of punishment, as previously discussed.

The results illustrated in Table 1 also show that the pharmacological profile of one-way escape from the open arm of the ETM is different from that of inhibitory avoidance. None of the anxiolytic drugs that impaired inhibitory avoidance significantly changed escape. Among the anxiogenic drugs tested, yohimbine was ineffective, but the two $5\text{-HT}_{2C/2B}$ agonists TFMPP and mCPP moderately attenuated escape, in contrast to their facilitating effect on inhibitory avoidance. Chronic imipramine and *d*-fenfluramine impaired one-way escape, although clomipramine had no effect on the same task.

Yohimbine, TFMPP and mCPP are usually classified as panicogenic agents. Therefore, the ineffectiveness of yohimbine and the anti-escape effect of the two latter drugs in the ETM lend no support to the proposal that this task is related to panic. Nevertheless, it has been argued that in contrast to lactate, or CO_2 inhalation, these agents do not produce true panic attacks, but rather enhance anticipatory anxiety (Bourin et al. 1998). The above results showing that these drugs enhance inhibitory avoidance in the ETM, but decrease escape are compatible with this view.

The results with *d*-fenfluramine and chronic imipramine deserve further comment due to their relevance for the testing of the dual 5-HT-defense hypothesis. Neurochemical studies have shown that *d*-fenfluramine selectively releases 5-HT from thin nerve terminals from the DRN (Series et al. 1994; Viana et al. 1996). Since these nerve endings project to both the amygdala and the dPAG (Azmitia and Segal 1978), 5-HT release is expected to facilitate inhibitory avoidance in the ETM by acting on the amygdala, as well as to impair open arm escape by acting on the dPAG. Both predictions have been fulfilled by *d*-fenfluramine (Graeff et al. 1996a, 1996b).

Fenfluramine has been reported to induce attacks in panic patients (Targum and Marshall 1989). Yet, the drug enhanced inhibitory avoidance in the ETM, which is supposed to correlate with GAD, whereas markedly impairing one-way avoidance, which may be related to PD. Nevertheless, this inconsistency may be only apparent, since Targun and Marshall (1989) themselves have pointed out that *d*,*l*-fenfluramine induced a slow wave of anxiety, more like an enhancement of anticipatory anxiety, and different from the sudden surge characteristic of panic attacks. Indeed, the results of a recent study (Mortimore and Anderson 2000) have shown that pretreatment with *d*-fenfluramine enhanced anticipatory anxiety in PD patients following CO_2 inhalation. In contrast, the intensity of the CO_2-induced panic attack was decreased by the same drug treatment. In agreement, an open study with PD patients resistant to conventional drug treatment (Solyom 1994) as well as a case history (Hetem 1995) indicates that fenfluramine improves this disorder. Therefore, the results obtained with *d*-fenfluramine in the ETM suggest that one-way escape may have predictive value in regard to drug effects on PD.

To investigate this question further, experiments have been performed with imipramine, which was the first drug found effective on PD when administered repeatedly for several weeks (Klein and Flink 1962). The results obtained have shown that daily, IP administration for 21 days of imipramine prolonged escape latency from the open arm of the ETM (Teixeira et al. 2000). This finding correlates with the above clinical observation, further supporting the hypothesis that ETM escape is related to PD. Interestingly, the same drug treatment impaired the acquisition of inhibitory avoidance. This effect also has a clinical correlate, since there are reported results showing that prolonged administration of imipramine is as effective as a benzodiazepine anxiolytic to improve GAD (Kahn et al. 1986).

The study by Teixeira et al. (2000) also led to a procedural change in the ETM test, which is pre-exposure for 30 min to the open arms of the maze 24 h before the test and repeated measurement of the escape latency. Only after these modifications did the anti-escape effect of chronic imipramine become statistically significant. Since pre-exposure shortens the first withdrawal latency, it is likely that this procedure fosters habituation to the open arms, minimizing exploratory activity in the day of the test, which may interfere with one-way escape. It should be kept in mind, however, that a different emotional state might be generated by re-exposure to the open arms, as it has been shown in the elevated plus-maze (File et al. 1993).

Using this modified procedure, it has recently been shown that chronic administration (5–15 m/kg, IP, daily for 21 days) of fluoxetine, a drug that is effective on PD (Michelson et al. 1999), selectively impaired escape, without affecting inhibitory avoidance (S. Poltronieri, H. Zangrossi Jr and M.B. Viana, unpublished). In the opposite direction, the cholecystokinin derivative CCK-8 (0.01–0.1 mg/kg, IP) has been shown to decrease escape latencies while not affecting avoidance latencies in the ETM (J.M. Zanoveli, C. Ferreira Netto, F.S. Guimarães and H. Zangrossi Jr, unpublished data). Accordingly, the similarly acting peptide CCK-4 has been shown to induce panic attacks in panic patients as well as in healthy volunteers following intra-venous administration (Bradwejn et al. 1990). Although the last findings are encouraging, other drugs that clinically improve or aggravate PD should be tested to further validate one-way escape as an animal model of PD.

In agreement with the assumption that the avoidance and escape tasks in the ETM are processed differently in the brain the results of a recent c-Fos study have shown that performance of the avoidance task in the ETM increased Fos-like immunoreactivity preferentially in the medial nucleus of the amygdala, paraventricular nucleus of the thalamus, anterior hypothalamic nucleus and MRN. In contrast, performance of escape from the open arm enhanced Fos-like immunoreactivity particularly in the dPAG. Both behavioral tasks increased Fos-like immunoreactivity in the dorsomedial hypothalamus. Withdrawal from an enclosed arm of a modified T-maze, having the three arms enclosed by walls, was used as control. Therefore, inhibitory avoidance and one-way escape seem to activate different sets of brain structures (Silveira et al. 2001).

Experiments using intra-cerebral drug injection and brain lesion in rats performing in the elevated T maze have also been carried out to test the dual 5-HT hypothesis (for a recent review, see Zangrossi et al. 2001). For the same purpose, several drugs acting on 5-HT neurotransmission have been assayed in healthy volunteers under two experimental models supposed to generated different types of anxiety. For a recent review and critical discussion of these results, see Graeff et al. (2001).

Conclusion

The line of research reviewed here started by the observation of the anti-conflict effect of the 5-HT receptor antagonist methysergide. This effect is compatible with the hypothesis that 5-HT is anxiogenic. However, an opposite conclusion stemmed from the observed effects of 5-HT drugs on aversive behavior elicited by electrical or chemical stimulation of the dPAG. To conciliate these contradictory views, a dual 5-HT-defense hypothesis was proposed, suggesting that 5-HT enhance conditioned anxiety by acting in the forebrain whereas inhibiting unconditioned fear through actions in the dPAG. The pharmacological results so far obtained with an animal model of anxiety that is intended to generate both types of anxiety in the same rat, the elevated T-maze, generally support this hypothesis. The comparison between these results with reported clinical evidence indicates that the inhibitory avoidance task in the elevated T-maze may be related to GAD while the escape task may relate to PD.

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References

Aghajanian GK (1992) Central 5-HT receptor subtypes: physiological responses and signal transduction mechanisms. In: Marsden CA, Heal DJ (eds) Central serotonin receptors and psychotropic drugs. Blackwell, Oxford, pp 39–55

Amano K, Tanikawa T, Iseki H, Notani M, Kawamura H, Kitamura K (1978) Single neuron analysis of the human midbrain tegmentum. Appl Neurophysiol 41:66–78

American Psychiatry Association (1994) Diagnostic and statistical manual of mental disorders, 4th edn. APA Press, Washington D.C.

Argyropoulos SV, Sandford JJ, Nutt DJ (2000) The psychobiology of anxiolytic drug. Part 2: pharmacological treatments of anxiety. Pharmacol Ther 88:213–227

Audi EA, de Oliveira RMW, Graeff FG (1991) Microinjection of propranolol into the dorsal periaqueductal gray causes an anxiolytic effect in the elevated plus-maze antagonized by ritanserin. Psychopharmacology 105:553–557

Azmitia EC, Segal M (1978) An autodiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J Comp Neurol 179:641–688

Barrett JE, Gleeson S (1991) Anxiolytic effects of 5-HT_{1A} agonists, 5-HT3 antagonists and benzodiazepines: conflict and drug discrimination studies. In: Rodgers RJ, Cooper SJ (eds) 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines: their comparative behavioural pharmacology. Wiley, Chichester, pp 59–105

Beckett S, Marsden CA (1997) The effect of central and systemic injection of the 5-HT_{1A} receptor agonist 8-OHDPAT and the 5-HT_{1A} receptors antagonist WAY 100635 on periaqueductal grey-induced defence behaviour. J Psychopharmacol 11:35–40

Beckett SRG, Lawrence AJ, Marsden CA, Marshal PW (1992) Attenuation of chemically induced defence response by 5-HT_1 receptor agonists administered into the periaqueductal gray. Psychopharmacology 108:110–114

Blanchard DC, Blanchard RJ (1988) Ethoexperimental approaches to the biology of emotion. Annu Rev Psychol 39:43–68

Blanchard DC, Griebel G, Blanchard RJ (2001) Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic. Neurosci Biobehav Rev 25:205–218

Bourin M, Baker GB, Bradwejn J (1998) Neurobiology of panic disorder. J Psychosom Res 44:163–180

Bradwejn J, Koszycki D, Meterissian G (1990) Chlecystokinin-tetrapeptide induces panic attacks in patients with panic disorder. Can J Psychiatry 35:83–85

Brandão ML, de Aguiar JC, Graeff FG (1982) GABA mediation of the anti-aversive action of minor tranquilizers. Pharmacol Biochem Behav 16:397–402

Costa E, Guidotti A (1979) Molecular mechanisms in the receptor action of benzodiazepines. Annu Rev Pharmacol Toxicol 19:531–545

Davis M (1992) The role of the amygdala in fear and anxiety. Annu Rev Neurosci 15:353–375
Deakin JFW, Graeff FG (1991) 5-HT and mechanisms of defence. J Psychopharmacol 5:305–315
Delgado JMR, Roberts WW, Miller NE (1954) Learning motivated by electrical stimulation of the brain. Am J Physiol 179:587–593
Eison AS, Temple DL (1989) Buspirone: review of its pharmacology and current perspectives on the mechanism of action. Am J Med 80:1–9
Estes WK, Skinner FB (1941) Some quantitative properties of anxiety. J Exp Psychol 29:390–400
Fanselow MS (1991) The midbrain periaqueductal gray as a coordinator of action in response to fear and anxiety. In: Depaulis A, Bandler R (eds) The midbrain periaqueductal gray matter: functional, anatomical and immunohistochemical organization. Plenum, New York, pp 151–173
File SR, Zangrossi H Jr, Viana MB, Graeff FG (1993) Trial 2 in the elevated plus-maze: a different form of fear? Psychopharmacology 111:491–494
Geller I, Seifter J (1960) The effects of meprobamate, barbiturates, *d*-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia 1:482–492
Gentil V (1988) The aversive system, 5HT and panic attacks. In: Simon P, Soubrié P, Willocher D (eds) Animal models of psychiatric disorders, vol 1. Karger, Basel, pp 142–145
Gentil V, Lotufo-Neto F, Andrade L, Cordás T, Bernik M, Ramos R, Maciel L, Miyakawa E, Gorenstein C (1993) Clomipramine, a better reference drug for panic/agoraphobia. I. Effectiveness comparison with imipramine. J Psychopharmacol 7:316–324
Graeff FG (1974) Tryptamine antagonists and punished behavior. J Pharmacol Exp Ther 189:344–350
Graeff FG (1981) Minor tranquilizers and brain defense systems. Braz J Med Biol Res 14:239–265
Graeff FG (1988) Animal model of aversion. In: Simon P, Soubrié P, Willocher D (eds) Animal models of psychiatric disorders, vol 1. Karger, Basel, pp 115–141
Graeff FG (1990) Brain defense systems and anxiety. In: Roth M, Burrows GD, Noyes R (eds) Handbook of anxiety, vol 3. The neurobiology of anxiety. Elsevier, Amsterdam, pp 307–354
Graeff FG (1994) Neuroanatomy and neurotransmitter regulation of defensive behaviors and related emotions in mammals. Brazil J Med Biol Res 27:811–829
Graeff FG, Rawlins JNP (1980) Dorsal periaqueductal gray punishment, septal lesions and the mode of action of minor tranquilizers. Pharmacol Biochem Behav 12:41–45
Graeff FG, Schoenfeld RI (1970) Tryptaminergic mechanisms in punished and nonpunished behavior. J Pharmacol Exp Ther 173:277–283
Graeff FG, Silveira Filho NG (1978) Behavioral inhibition induced by electrical stimulation of the median raphe nucleus of the rat. Physiol Behav 21:477–484
Graeff FG (1991) Neurotransmitters in the dorsal periaqueductal gray and animal models of panic anxiety. In: Briley M, File SE (eds) New concepts in anxiety. MacMillan, London, pp 288–312
Graeff FG, Viana MB, Tomaz C (1993) The elevated T maze, a new experimental model of anxiety and memory: effect of diazepam. Brazil J Med Biol Res 26:67–70
Graeff FG, Viana MB, Mora PO (1996a) Opposed regulation by dorsal raphe nucleus 5-HT pathways of two types of fear in the elevated T-maze. Pharmacol Biochem Behav 53:171–177
Graeff FG, Viana MB, Mora PO (1996b) Dual role of 5-HT in defense and anxiety. Neurosci Biobehav Rev 54:129–141
Graeff FG, Guimarães FS, de Andrade TGCS, Deakin JFW (1996c) Role of 5-HT in stress, anxiety and depression. Pharmacol Biochem Behav 54:129–141
Graeff FG, Ferreira Netto C, Zangrossi H Jr (1998) The elevated T-maze as an experimental model of anxiety. Neurosci Biobehav Rev 23:237–246
Graeff FG, Silva M, Del-Ben CM, Zuardi AW, Hetem LA, Guimarães FSG (2001) Comparison between two models of experimental anxiety in healthy volunteers and panic disorder patients. Neurosci Biobehav Rev 25:753–759
Gray JA (1982) The neuropsychology of anxiety. Oxford University Press, New York
Gray JA, McNaughton N (2000) The neuropsychology of anxiety. Oxford University Press, Oxford
Griebel G (1995) 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol Ther 65:319–395
Handley SL, Mithani S (1984) Effects of alpha-adrenoceptor agonists in a maze-exploration model of "fear"-motivated behaviour. Naunyn-Schmiedeberg's Arch Pharmacol 327:1–5
Handley SL, McBlane JW, Critchley MAE, Njung'e K (1993) Multiple serotonin mechanisms in animal models of anxiety: environmental, emotional and cognitive factors. Behav Brain Res 58:203–210
Hetem LAB (1995) Addition of *d*-fenfluramine to benzodiazepines produced a marked improvement in refractory panic disorder – a case report. J Clin Psychopharmacol 16:77–78
Hodges H, Green S, Glenn B (1987) Evidence that the amygdala is involved in benzodiazepine and serotonergic effects on punished responding but not in discrimination. Psychopharmacology 92:491–504
Hunsperger RW (1956) Affektreaktionen und elektrische Reizung im Hirnstam der Katze. Helvet Physiol Pharmacol Acta 14:70–92
Jenck F, Broekkamp CLE, van Delft AML (1989) Opposite control mediated by central $5HT_{1A}$ and non-$5HT_{1A}$ ($5HT_{1B}$ or $5H_{1C}$) receptors on periaqueductal gray aversion. Eur J Pharmacol 161:219–221
Jenck F, Broekkamp CLE, van Delft AML (1990) The effect of antidepressants on aversive PAG stimulation. Eur J Pharmacol 177:201–204
Kahn RJ, MacNair DM, Lipman RS, Covi L, Rickels K, Downing R, Fischer S, Grankenthaler LM (1986) Imipramine and chlordiazepoxide in depressive and anxiety disorders. II. Efficacy in anxious outpatients. Arch Gen Psychiatry 43:79–85
Kiser RS Jr, Lebovitz RM (1975) Monoaminergic mechanisms in aversive brain stimulation. Physiol Behav 15:47–53
Kiser RS, German DC, Lebovitz RM (1978a) Serotonergic reduction of dorsal central gray area stimulation-produced aversion. Pharmacol Biochem Behav 9:27–31
Kiser RS Jr, Lebovitz RM, German DC (1978b) Anatomic and pharmacologic differences between two types of aversive midbrain stimulation. Brain Res 155:331–342
Kiser RS, Brown CA, Sanghera MK, German DC (1980) Dorsal raphe nucleus stimulation reduces centrally-elicited fearlike behavior. Brain Res 191:265–72
Klein DF (1993) False suffocation alarms, spontaneous panics, and related conditions. Arch Gen Psychiatry 50:306–317
Klein DF, Flink M (1962) Psychiatric reaction patterns to imipramine. J Psychiatry 119:432–438
LeDoux JE (1993) Emotional memory systems in the brain. Behav Brain Res 58:69–79
Lovick TA (2000) Panic disorder – a malfunction of multiple transmitter control systems within the midbrain periaqueductal gray matter? Neuroscientist 6:48–59
Mamounas LA, Mullen CA, O'Hearn E, Molliver ME (1991) Dual serotonergic projections to forebrain in the rat: morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. J Comp Neurol 314:558–586
McBlane JW, Handley SL (1994) Effects of two stressors on behavior in the elevated X-maze: preliminary investigation of their interaction with 8-OH-DPAT. Psychopharmacology 116:173–182
Michelson D, Pollack M, Lydiard RB, Tamura R, Tepner R, Tollefson G (1999) Continuing treatment of panic disorder after acute response: randomised, placebo-controlled trial with fluoxetine. The Fluoxetine Panic Disorder Study Group. Br J Psychiatry 174:213–218

Millenson JR, Leslie J (1974) The conditioned emotional response (CER) as a baseline for the study of anti-anxiety drugs. Neuropharmacology 13:1–9

Montgomery KC (1955) The relationship between fear induced by novel stimulation and exploratory behaviour. J Comp Physiol Psychol 48:254–260

Morato de Carvalho S, de Aguiar JC, Graeff FG (1981) Effect of minor tranquilizers, tryptamine antagonists and amphetamine on behavior punished by brain stimulation. Pharmacol Biochem Behav 15:351–356

Mortimore C, Anderson IM (2000) *d*-Fenfluramine in panic disorder: a dual role for 5-hydroxytryptamine. Psychopharmacology 149:251–258

Nashold BS Jr, Wilson NP, Slaughter GS (1974) The midbrain and pain. In: Bonica JJ (ed) Advances in neurology, vol 4: international symposium on pain. Raven Press, New York, pp 191–196

Nogueira RL, Graeff FG (1991) Mediation of the antiaversive effect of isamoltane injected into the dorsal periaqueductal grey. Behav Pharmacol 2:73–77

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

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open:closed arm entries in the elevated plus-maze as a measure of anxiety in the rat. J Neurosci Meth 14:149–167

Petersen EN, Scheel-Krüger J (1984) Anticonflict effects of 5-HT antagonists by intraamygdaloid injection. Abstracts of the 14th CINP Congress, p 654

Robichaud RC, Sledge KL (1969) The effects of *p*-chlorophenylalanine on experimentally induced conflict in the rat. Life Sci 8:965–969

Schenberg LC, Graeff FG (1978) Role of the periaqueductal gray substance in the antianxiety action of benzodiazepines. Pharmacol Biochem Behav 9:287–295

Schoenfeld RI (1976) Lysergic acid diethylamide- and mescaline-induced attenuation of the effect of punishment in the rat. Science 192:801–803

Schütz MTB, de Aguiar JC, Graeff FG (1985) Anti-aversive role of serotonin on the dorsal periaqueductal grey matter. Psychopharmacology 85:340–345

Series HG, Cowen PJ, Sharp T (1994) *p*-Chloroamphetamine (PCA), 3,4-methylenedioxy-methamphetamine (MDMA) and *d*-fenfluramine pretreatment attenuates *d*-fenfluramine-evoked release of 5-HT in vivo. Psychopharmacology 116:508–514

Silveira MCL, Zangrossi H Jr, Viana MB, Silveira R, Graeff FG (2001) Differential expression of Fos protein in the rat brain induced by performance of avoidance or escape in the elevated T-maze. Behav Brain Res 126:13–21

Solyom L (1994) Controlling panic attacks with fenfluramine. Am J Psychiatry 151:621–622

Targum SD, Marshall LE (1989) Fenfluramine provocation of anxiety in patients with panic disorder. Psychiatry Res 28:295–306

Teixeira RC, Zangrossi Junior H, Graeff FG (2000) Behavioral effects of acute and chronic imipramine in the elevated T-maze model of anxiety. Pharmacol Biochem Behav 65:571–576

Treit D, Menard J, Royan C (1993) Anxiogenic stimuli in the elevated plus-maze. Pharmacol Biochem Behav 44:463–469

Tye NC, Everitt BJ, Iversen SD (1977) 5-Hydroxytryptamine and punishment. Nature 268:741–742

Vargas LC, Schenberg LC (2001) Long-term effects of clomipramine and fluoxetine on dorsal periaqueductal grey-evoked innate defensive behaviours of the rat. Psychopharmacology 155:260–268

Viana MB, Tomaz C, Graeff FG (1994) The elevated T maze: a new animal model of anxiety and memory. Pharmacol Biochem Behav 49:549–554

Viana MB, Silveira R, Graeff FG (1996) D-fenfluramine releases 5-HT from terminals of the dorsal raphe nucleus. Brazil J Med Biol Res 29:639–642

Wise CD, Berger BD, Stein L (1972) Benzodiazepines: Anxiety-reducing activity by reduction of serotonin turnover in the brain. Science 177:180–183

Wuttke W, Kelleher RT (1970) Effects of some benzodiazepines on punished and unpunished behavior in the pigeon. J Pharmacol Exp Ther 172:397–405

Zangrossi H Jr, Graeff FG (1997) Behavioral validation of the elevated T-maze, a new animal model of anxiety. Brain Res Bull 44:1–5

Zangrossi H Jr, Viana MB, Zanoveli J, Bueno C, Nogueira RL, Graeff FG (2001) Serotonergic regulation of inhibitory avoidance and one-way escape in the elevated T-maze. Neurosci Biobehav Rev 25:637–645