



Review

Serotonin, the periaqueductal gray and panic

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Abstract

This article reviews experimental evidence and theoretical constructs that implicate serotonin (5-HT) modulation of defensive behavior within the midbrain periaqueductal gray in panic disorder (PD). Evidence with conflict tests in experimental animals indicates that 5-HT enhances anxiety, whereas results with aversive stimulation of the dorsal periaqueductal gray point to an anxiolytic role of 5-HT. To solve this contradiction, it has been suggested that the emotional states determined by the two types of animal model are different. Conflict tests would generate conditioned anxiety, whereas periaqueductal gray stimulation would produce unconditioned fear, as evoked by proximal threat. Clinically, the former would be related to generalized anxiety while the latter to PD. Thus, 5-HT is supposed to facilitate anxiety, but to inhibit panic. This hypothesis has been tested in the animal model of anxiety and panic named the elevated T-maze, in two procedures of human experimental anxiety applied to healthy volunteers or panic patients, and in CO₂-induced panic attacks. Overall, the obtained results have shown that drugs that enhance 5-HT function increase different indexes of anxiety, but decrease indexes of panic. Drugs that impair 5-HT function have the opposite effects. Thus, so far the predictions derived from the above hypothesis have been fulfilled.

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1. Introduction

The present interest on serotonin (5-HT) and anxiety is mainly due to the widespread use of selective serotonin uptake inhibitors (SSRIs) in the treatment of anxiety disorders. Nevertheless, earlier hypotheses relating 5-HT

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and anxiety originated in behavioral experiments carried out in laboratory animals during the late 1960s and early 1970s.

Based on clinical observation, Freud [36] was able to distinguish chronic anxiety from anxiety (panic) attacks, and in the early 1960s, Klein [84] observed that panic attacks were reduced by chronic administration of the monoamine reuptake inhibitor imipramine. This laid the ground for the establishment of panic disorder (PD) as a nosological entity, in the DSM III classification [3].

PD is characterized by recurrent panic attacks, either unexpected or associated with particular situations. These are sudden surges of intense fear or terror, desire of fleeing and feeling of imminent death, going crazy or losing control. These subjective symptoms are accompanied by major neurovegetative changes, such as palpitation, hypertension, difficulty in deep breathing, sweating, urge to void the bladder and increased peristalsis. This leads to worry about the next attack or anticipatory anxiety, and avoidance of places where a panic attack would be embarrassing. Ultimately, generalized avoidance or agoraphobia may ensue.

During the last three decades, developments in pre-clinical and clinical research converged to establish a relationship between 5-HT and PD. The meeting point was the periaqueductal gray matter (PAG), a neural structure placed in the midbrain that, among other functions, integrates defensive behavior.

The aim of the present review is to follow the conceptual developments that resulted in theoretical constructs implicating 5-HT modulation of defense within the PAG in the therapeutic response and pathophysiology of PD.

2. Punished behavior

In the 1960s, studies of behavioral pharmacology had shown that punishment or conflict tests were the best predictors of the clinical anxiolytic action of drugs. In this experimental paradigm, deprived animals—mostly rats and pigeons—were trained to operate a device to deliver food or water reward. Once a baseline of regular responding was established, a concurrent contingency was introduced: the same response that gave access to food or water also resulted in the presentation of an aversive stimulus, generally mild electric shock. These contingencies generated an approach-avoidance conflict and resulted in the suppression of responding, the extent of which was dependent on the degree of deprivation and the severity of shock. In addition to the resemblance between experimental conflict and clinical situations that generate anxiety, pharmacological evidence provided empirical support for conflict tests to be considered reliable animal models of anxiety. In this regard, several reported results have shown that anxiolytic drugs, such as meprobamate, barbiturates and benzodiazepines reduced the response suppression

determined by punishment. In addition, there was good correlation between the potency of these drugs in the conflict test and the average dosage used for alleviating clinical anxiety [49].

Despite the above-mentioned evidence pointing to the heterogeneity of anxiety disorders, in the early 1970s most psychiatrists viewed anxiety as a single emotion that ranged in intensity from normal to pathological or neurotic and that was reduced by anxiolytic drugs. This conceptual background influenced earlier ideas relating 5-HT and anxiety. Only a brief account of this topic will be given here, since it has been the object of a recent review [47], where original references may be found.

The first theoretical model on 5-HT and anxiety stemmed from experimental findings showing that inhibition of 5-HT synthesis in rats with *para*-chlorophenylalanine (PCPA) or blockade of 5-HT receptors in pigeons with methysergide or bromolysergic acid (BOL) released punished-suppressed responding, and that benzodiazepines decreased the turnover of brain 5-HT. Based on these results, Stein and co-workers [140] figured out a conceptual model in which noradrenergic pathways originating in the brain stem and innervating the forebrain directed the animal toward reward, thus constituting a brain approach system. In opposition, serotonergic neurons located in the midbrain raphe nuclei that send nerve fibers to forebrain limbic structures and to the PAG led the animal away from the source of punishment. The anti-conflict and, by inference, the anxiolytic actions of benzodiazepines would be due to the drug-induced impairment of such 5-HT punishment system. Therefore, this theoretical model ascribes to 5-HT an anxiogenic role, by acting both in forebrain limbic structures and in the PAG.

Several reported results support the suggestion that 5-HT exerts an anxiogenic action in the forebrain. For instance, selective destruction of ascending 5-HT pathways by microinjecting the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) into the midbrain tegmentum of the rat prevented the acquisition of response suppression by foot-shock punishment. Also, the anti-punishment effect of systemically administered lysergic acid diethylamide (LSD-25) and mescaline in the rat was attributed to decreased activity of ascending serotonergic neurons. In the opposite direction, electrical stimulation of the median raphe nucleus (MRN), which contains 5-HT neuron cell bodies that send fibers to forebrain limbic structures induced behavioral inhibition together with neurovegetative changes—raised fur, defecation, urination and teeth clattering—that are characteristic of conditioned fear. The effect of MRN electrical stimulation is likely to be mediated by 5-HT since it was reduced by pre-treatment with PCPA. As the 5-HT neurons of the MRN project mainly into the dorsal hippocampus, these results agree with Gray's [58] fundamental hypothesis that the septo-hippocampal system is the seat of the brain inhibition system (BIS), its activation being equivalent to anxiety.

There are also experimental results indicating that 5-HT increases anxiety by acting on the amygdala, a brain structure that has been implicated in learning and expression of conditioned or anticipatory anxiety. In this regard, microinjection of 5-HT or the 5-HT_{1A} receptor agonist 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT) into the basolateral amygdala of the rat has been shown to enhance response suppression determined by punishment [70]. In addition, microinjection of the preferential 5-HT_{2A/2C}-receptor antagonist ketanserin into the same region of the amygdala releases responding suppressed by punishment [107]. These results indicate that 5-HT enhances anxiety in the amygdala by acting on 5-HT receptors of both types 1 and 2.

More recent evidence casts doubt on the anxiogenic role of the forebrain 5-HT_{1A} receptor. For instance, knockout mice lacking this receptor show increased anxiety-like behavior and restoration of 5-HT_{1A} receptors in the hippocampus and cortex, but not in the raphe nuclei, is sufficient to reverse this change [61]. Also, bilateral injection of 8-OH-DPAT into the amygdala had an anxiolytic-like effect in the elevated T-maze (ETM) [145], an animal model of anxiety and panic that will be thoroughly discussed in Section 4.6. In contrast, there is substantial evidence indicating that the 5-HT_{2C} (former 5-HT_{1C}) receptor subtype is critical for anti-anxiety drug action [79]. As to the cellular mechanisms involved in its anxiogenic role, experiments recently performed in a slice preparation of the rat basolateral amygdala have shown that 5-HT_{2C}-receptor stimulation enhances synaptic function mediated by the *n*-methyl-D-aspartate (NMDA) receptor. This promotes long-term potentiation (LTP), an electrophysiological process that is supposed to underlie learning and memory processes [24], including those of conditioned anxiety.

3. PAG aversive stimulation

In contrast to the above evidence supporting the hypothesis that 5-HT enhances anxiety by acting upon forebrain limbic structures such as the hippocampus and the amygdala, experimental testing of the additional hypothesis that 5-HT exerts a similar action in the PAG has yielded inconsistent results. Most of this experimental evidence has been obtained using aversive electrical stimulation of the PAG. As early as 1954, Delgado and co-workers [31] had shown that laboratory animals readily learn to operate a device that switches off electrical current delivered in the dorsal region of the PAG. Further experimental evidence qualifies the dorsal PAG (DPAG) as the main substrate of aversion in the brain [42]. Anxiety motivates behavior that reduces or terminates this emotional state. As a consequence, electrical stimulation of the PAG seemed a likely model of anxiety. If 5-HT exerts an anxiogenic action in the DPAG, drugs that increase 5-HT activity would be expected to facilitate switching off behavior, whereas the opposite

effect would occur following drugs that reduce 5-HT activity in the DPAG. However, pharmacological evidence obtained with either systemic or intra-cerebral drug injection gave results that do not comply with these predictions.

Starting with systemically injected drugs, it has been found that either the 5-HT synthesis inhibitor PCPA [80,82] or the 5-HT receptor blocker cyproheptadine [114] facilitated escape from DPAG electrical stimulation, thus having an anxiogenic action. In the opposite direction, the synthesis precursor 5-hydroxytryptophan and the 5-HT reuptake inhibitor chlorimipramine [81] reduced switch-off responding. Increasing the release of 5-HT in the DPAG by electrically stimulating the dorsal raphe nucleus (DRN) had the same effect [83]. Overall, these results indicate that 5-HT reduces anxiety generated in the DPAG. Accordingly, the benzodiazepine anxiolytic chlordiazepoxide was found to decrease escape from DPAG electrical stimulation [115].

Such opposite effects of a benzodiazepine anxiolytic and a 5-HT antagonist contrast with the same anti-punishment effect of the two classes of drugs verified in conflict tests [49]. This raises the question of whether the difference is due to the task or the type of punishment. To answer this question, Morato de Carvalho et al. [99] used electrical stimulation of the DPAG as a punishing stimulus. The obtained results showed that the anxiolytics chlordiazepoxide and pentobarbital caused dose-dependent increases in responding suppressed by DPAG electrical stimulation. In contrast, cyproheptadine did not facilitate the same responding at doses that had been shown to markedly release behavior punished by foot-shock. Another 5-HT receptor antagonist, methysergide, was also ineffective. Therefore, the neural substrate of punishment delivered in the DPAG seems to be different from that of peripherally applied punishment.

This last view is supported by the results of a comparative study performed by Graeff and Rawlins [48] in Gray's laboratory, at the Department of Experimental Psychology of the University of Oxford. In this study, rats were trained to lever press to get food reward. After that, the animals were divided into two groups and a punishment contingency was added. In the first group every response was punished by foot-shock delivery while in the second group brief electrical stimulation of the DPAG followed each lever press. In both groups punishment reduced response rates to nearly the same level, that was less than 10% of pre-punishment rates. Then, electrolytic lateral septal lesion was performed in all rats. This 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 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 the BIS, that includes the septum-hippocampus [58] and a brain aversive system, that includes the DPAG [42] act together to suppress responding maintained by food presentation. Anxiolytic drugs would impair 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 is supposed to intensify behavioral inhibition. In the PAG, 5-HT is likely to inhibit aversion and, as a consequence, decreasing its activity would result in more, rather than less, response suppression.

A series of experiments exploring the effect of systemically injected drugs that affect 5-HT on escape from DPAG electrical stimulation has been conducted by Jenck and co-workers using a procedure in which rats were trained to jump over a ridge separating the two compartments of a shuttle box. In agreement with the results reported by Schenberg and Graeff [114], Jenck et al. [74] found that the non-selective 5-HT receptor antagonists, metergoline and mianserin facilitated escape from DPAG electrical stimulation. The selective 5-HT₂-receptor antagonist ketanserin had the opposite effect. These results indicate that 5-HT₂ receptors facilitate aversion in the DPAG. However, results with intra-cerebral drug injection led to the opposite conclusion, as described below. In the same study, Jenck et al. [74] reported that injection of the selective 5-HT_{1A}-receptor agonist, 8-OH-DPAT enhanced escape from DPAG electrical stimulation. This result is suggestive of a pro-aversive role of 5-HT_{1A} receptors. Again this conclusion is inconsistent with results obtained with drugs directly injected into the DPAG (see below). A further study by Jenck et al. [75] evidenced that the two SSRIs, namely fluvoxamine and sertraline reduced DPAG-induced escape in the shuttle box, in agreement with the aforementioned results with chlorimipramine reported by Kiser et al. [81].

The above studies used single administration of anti-depressant drugs, a regimen that differs from clinical treatment, where the therapeutic effect starts after repeated drug administration for several weeks [8]. Therefore, it is particularly interesting that daily administration of chlorimipramine for 21 days has been shown to decrease the occurrence of behavioral items like running and jumping, elicited by electrical stimulation of the DPAG in rats placed inside an open arena [130]. The implications of these results for PD will be discussed later.

The effects of systemically injected drugs on escape from DPAG stimulation are summarized in Table 1.

To explore the mode of action of 5-HT on the DPAG more directly, a series of experiments has been conducted, in which intra-cerebral drug administration was combined with electrical stimulation through the use of a permanent chemitrode with the tip localized inside the DPAG. For determining the aversive threshold, rats were placed inside one compartment of a shuttle box and electrical current was

Table 1

Effect of systemically injected drugs on escape from dorsal periaqueductal gray stimulation

Procedure	Drug	Escape	Reference
Lever pressing	PCPA ^a	+	[80,82]
	Cyproheptadine ^b	+	[114]
	5-Hydroxytryptophan ^c	–	[81]
	Chlorimipramine ^d	–	[81]
	Chlordiazepoxide ^e	–	[115]
Barrier jumping	Metergoline ^b	+	[74]
	Mianserin ^b	+	[74]
	Ketanserin ^f	–	[74]
	8-OH-DPAT ^g	+	[74]
	Fluvoxamine ^d	–	[75]
	Sertraline ^d	–	[75]
Elicited running	DLH		
	8-OH-DPAT ^g	+	[11]
	Chronic chlorimipramine ^d	–	[130]

Electrical stimulation of the DPAG was used, unless otherwise specified. DLH: excitatory amino acid injection. +, Facilitation; –, impairment.

^a 5-HT synthesis inhibitor.

^b Non-selective 5-HT-receptor antagonist.

^c Precursor of 5-HT synthesis.

^d 5-HT reuptake inhibitor.

^e Benzodiazepine-receptor agonist.

^f 5-HT₂-receptor antagonist.

^g 5-HT_{1A}-receptor agonist.

applied to the DPAG with gradually increasing intensity until the rat ran towards the opposite compartment of the box. This response switched off the brain stimulation. The procedure was repeated three times to establish the threshold value. After that, a drug microinjection was made and 10 min later the aversive threshold was determined once more, as before. The difference between the post-drug and the basal threshold measured the drug effect.

The results of the first study of this kind showed that 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 in a dose-dependent way. Pre-treatment with the 5-HT receptor antagonists metergoline or ketanserin blocked the anti-aversive effect of 5-HT. Considering that ketanserin is a 5-HT₂-receptor antagonist, it was concluded that 5-HT acts on 5-HT₂ receptors in the DPAG to inhibit aversion [117].

In agreement, the results of a later study [103] revealed that microinjection of the 5-HT_{2A/2C}-agonist 2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) raised the aversive threshold. The preferential 5-HT_{2C}-agonist 1-(*m*-chlorophenyl) piperazine (*m*CPP) was ineffective. Thus, among the subtypes of 5-HT₂ receptors 5-HT_{2A} rather than 5-HT_{2C} receptors are likely to mediate the anti-aversive effect of 5-HT in the DPAG. In addition, the 5-HT_{1A} receptor seems to be involved, because the same study evidenced that the 5-HT_{1A} agonists 8-OH-DPAT and

ipsapirone raised the threshold of aversive electrical stimulation in a dose-dependent way. To make things more complex, the 5-HT_{1A}-receptor blocker NAN-190 and 5-HT_{2A}-receptor blocker spiperone antagonized either 8-OH-DPAT or DOI, leading to the conclusion that both 5-HT_{1A} and 5-HT_{2A} receptors have to be functional for the expression of each one's activation to occur.

In addition, it should be remembered that systemic administration of 8-OH-DPAT had a pro-aversive effect and administration of ketanserin had an anti-aversive effect [74]. Although there is no clear explanation for the contrasting results concerning the 5-HT₂ receptor, the results of experiments performed in Charles Marsden's laboratory provide a plausible explanation for the contradiction regarding the 5-HT_{1A} receptor. In these experiments, performed by Becket and Marsden [11], microinjection of the excitatory amino acid D,L-homocysteic acid (DLH) into DPAG was used to elicit running behavior recorded in an open arena. Systemic injection of 8-OH-DPAT increased running (as in Ref. [74]), whereas intra-PAG injection of the same drug decreased the same behavior. To reconcile these conflicting results, the authors have suggested that the systemically injected drug would stimulate autonomic 5-HT_{1A} receptors. Since these receptors are found in the cell bodies of DRN 5-HT neurons and inhibit neuronal firing, the ultimate effect would be a reduction of 5-HT activity on post-synaptic 5-HT_{1A} receptors in the innervated structures [1]. In the DPAG, 5-HT_{1A}-receptor stimulation would reduce flight behavior and aversion, as previously suggested by Nogueira and Graeff [103]. Additional results obtained by the same research group have shown that the selective 5-HT_{1A}-receptor blocker WAY 100635 antagonized the anti-aversive effect of 8-OH-DPAT, whereas the preferential 5-HT_{2C}-receptor agonist *m*CPP facilitated running induced by intra-DPAG injection of DLH [11,12]. The last result ascribes a pro-aversive role to 5-HT_{2C} receptors in the DPAG.

It is worth remarking that in the study by Schütz et al. [117] intra-DPAG administration of the SSRI zimelidine not only potentiated the anti-aversive effect of subsequently injected 5-HT, but also had an anti-aversive effect of its own. This implies 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 with isamoltane of pre-synaptic 5-HT_{1B} inhibitory receptors to increase the release of 5-HT raised the aversive threshold of DPAG electrical stimulation. Pre-treatment with the 5-HT₂-receptor blockers, ketanserin and ritanserin, antagonized this effect [102]. Accordingly, 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, a widely used animal model of anxiety [9]. A recent study has also shown that chronic treatment with imipramine (15 mg/kg IP, daily for 21 days) enhanced the effect intra-DPAG injection of 8-OH-DPAT

Table 2

Effect of intra-cerebrally injected drugs on escape from dorsal periaqueductal gray stimulation

Procedure	Drug	Escape	Reference
Shuttle box			
	5-HT	–	[117]
	5-MeODMT ^a	–	[117]
	8-OH-DPAT ^b	–	[72,103]
	Ipsapirone ^b	–	[103]
	DOI ^c	–	[72,103]
	<i>m</i> CPP ^d	0	[103]
	Zimelidine ^e	–	[117]
	Isamoltane ^f	–	[102]
Elicited running			
DLH	8-OH-DPAT ^b	–	[11,12]
DLH	<i>m</i> CPP ^d	+	[12]

Electrical stimulation of the DPAG was used, unless otherwise specified. DLH: excitatory amino acid injection. +, Facilitation; –, impairment.

^a Non-selective 5-HT-receptor agonist.

^b 5-HT_{1A}-receptor agonist.

^c 5-HT_{2A}-receptor agonist.

^d Preferential-HT_{2C}-receptor agonist.

^e 5-HT reuptake inhibitor.

^f 5-HT_{1B}-receptor antagonist.

(8 nmol) and DOI (16 nmol), indicating sensitization of post-synaptic 5-HT_{1A} and 5-HT_{2A/2C} receptors [72]. This action might be related to the anti-panic effect of this drug regimen, as it will be discussed latter.

Another interesting issue is that intra-DPAG administration of 5-HT receptor antagonists alone has no effect on the aversive threshold measured in the shuttle box [102, 117]. This finding contrasts with the major aversive effects caused by compounds that block γ -amino-butyric acid type A (GABA_A) receptors in the DPAG [116]. 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. It has been further suggested that the modulatory influence of 5-HT in the DPAG would be manifested only under conditions that engage 5-HT systems, the most important of which is stress [28].

The effect of intra-cerebrally injected drugs on escape from DPAG stimulation are summarized in Table 2.

4. Dual action of 5-HT on defense

Summarizing the evidence reviewed so far, it may be said that the first tenet of Stein and co-worker's model [140], namely that 5-HT is anxiogenic in the limbic forebrain has so far withstood experimental testing. In contrast, the second tenet—that 5-HT is similarly anxiogenic in the PAG—failed to meet the challenge. On the contrary, the experiments performed so far to test this hypothesis provided solid evidence indicating that 5-HT

reduces anxiety-like behavior generated in the DPAG. Nevertheless, it should be kept in mind that the two lines of evidence are based on different animal models of anxiety, the punishment or conflict test and escape from DPAG stimulation, respectively. Therefore, the paradox—that 5-HT both increases and decreases anxiety—remains only if it is supposed that both models generate the same emotional state. As a consequence, the contradiction may be solved by assuming that anxiety disorders constitute a heterogeneous group of (related) nosological entities, and that different animal models represent distinct disorders. In the present case, a correlation of conflict tests with generalized anxiety disorder (GAD) and of escape from DPAG stimulation with PD has been suggested. However, this notion was achieved only after important changes in both clinical and pre-clinical theoretical frameworks had taken place.

4.1. *The brain defense system*

In addition to maintaining switch-off responding, electrical stimulation of the DPAG elicits a defense reaction characterized by vigorous flight, threatening postures or affective aggression. Threat and aggression are prevalent in species endowed with natural weapons, like sharp teeth or claws, and less evident in not so harmful species, such as the rat or the marmoset [45]. In addition, the choice between these strategies is highly dependent on the environment. For instance, the animal is more likely to flee if an escape route is available; otherwise, defensive aggression occurs. Fernandez de Molina and Hunsperger [34] systematically explored the brain with stereotaxically implanted electrodes and revealed that the defense reaction of the cat could be elicited from brain regions other than the DPAG, such as the medial hypothalamus (MH) and the amygdala. However, they found that the response to amygdala stimulation was delayed and lasted for some time after termination of the electrical stimulation. In contrast, the response obtained from either the MH or the DPAG was stimulus-bound. Using two GABA_A-receptor antagonists and one inhibitor of GABA synthesis, Pierre Schmitt and co-workers [116] have further shown that the escape response of the rat had different characteristics when elicited from the MH as compared to the PAG. In the former structure, the animals showed escape behavior oriented by the environment, as if looking for an outlet, and they effectively escaped from an open arena by jumping above the wall. In contrast, rats injected inside the DPAG showed bursts of vertical, aimless jumps, alternating with periods of tense immobility or freezing.

Since anatomical studies revealed that the amygdala, the MH and the DPAG are heavily inter-connected by nerve fibers [22], the concept of a longitudinal, hierarchically organized brain defense system (BDS) emerged. As a consequence Fanselow [33] suggested that the amygdala would synthesize the stimulus input from the environment

and then signal to the PAG the degree of threat that they represent to the organism. The latter would be in charge of selecting, organizing and executing the appropriate behavioral and neurovegetative defensive reactions.

4.2. *Levels of predatory defense*

A concurrent development was happening in the field of the etho-experimental analysis of behavior, due mainly to the systematic study of defensive strategies displayed by wild rats in response to different kinds of predatory threat, which has been carried out by Caroline and Robert Blanchard [16], in Hawaii. The obtained results led to the conclusion that these defensive strategies could be subdivided into three levels that were determined chiefly by the presence or absence of actual danger and by the distance between predator and prey. The first level of defense would occur when the animal enters either a novel situation or an environment where an actual threat had been met in the past, but is no longer present. As a response to this potential or uncertain danger, the rat displays a characteristic behavior pattern named risk-assessment, which is characterized by cautious exploration with stretched body and the abdomen kept close to the floor, or nose poking out of wall openings followed by quick retreat. At the second level, the predator is present and has been detected by the prey, but is placed at a safe distance and did not start an attack. For this reason, this level of defense has been named distal threat. In that case, the animal freezes, thus decreasing the probability of detection by the predator and preparing for active defense. The third level—proximal threat—takes place when the predator approaches the prey over a critical distance, or actually strikes its body (therefore named ‘circa-strike’ defense by Fanselow [33]). Comparative studies led to the conclusion that there are homologous types of defense strategies in animal species other than the rat, including non-mammalian animals, testifying to their widespread adaptive value [17]. Finally, the results of one investigation carried out in college students evaluating their answers to a questionnaire containing different threat scenarios indicate that similar defensive strategies may well be present in human beings [18].

4.3. *Behavioral–brain correlation*

The next conceptual advance was an attempt to correlate brain and behavioral data. Concerning potential threat, the Blanchards [16] themselves have remarked that their first level of defense—potential threat—was very close to Gray’s concept of the BIS. As a consequence, the key underlying structure suggested by Gray—the septo-hippocampal system—would be a major candidate for the neural substrate of risk-assessment behavior. For the second level of defense, the brain–behavior correlation is not so simple. Yet, some clues are provided by the work of Davis [25] with the animal model of anxiety called fear-potentiated startle. In this test, the magnitude of flinch and jumping caused by

foot-shock is amplified when that rat is in the presence of an exteroceptive stimulus or environmental context that had been previously associated with pain, as in the CER paradigm. Before startling, the rat is immobile and tense, and the amount of this freezing behavior is proportional to the magnitude of startle potentiation [88]. The neural substrate of potentiated startle has been carefully studied and the conclusion drawn was that the central nucleus of the amygdala and the ventral PAG (VPAG) are essential for the display of freezing determined by the warning stimulus or context [26]. As a consequence, the same brain structures acquire the status of serious candidates for being the neural basis of Blanchard's second level of defense. Finally, regarding the third level of defense, what has been discussed so far about the DPAG places this structure as the most likely option for organizing fight or flight behavior evoked by proximal threat.

A few additional elements may be added to the picture originally drawn by the Blanchard's. Concerning the first level of defense, Gray and McNaughton [59] have pointed out that behavioral inhibition and risk-assessment only occur when the animal is driven to approach the source of danger, thus characterizing an approach-avoidance conflict. Otherwise, the animal will simply go away from the threatening object or situation, and will learn to avoid it in the future. This concept is critical for an operational distinction between anxiety and fear. Anxiety occurs whenever an approach-avoidance conflict is present; when there is only the escape-avoidance component, fear ensues. Learned escape-avoidance would be integrated in the amygdala while unconditioned fear would be dealt with in the MH, where Schmitt et al. [116] obtained environmentally oriented escape by injecting anti-GABAergic drugs.

Another point refers to the likely existence of functionally different types of immobility or freezing, despite their overt similarity. In this regard, recently reported work has clearly shown that freezing behavior elicited by electrical stimulation of the DPAG is not context-dependent, in contrast to conditioned freezing, in which the temporal association between environment and the unconditioned aversive stimulus is readily acquired [134,135]. The latter, but not the former was abolished by lesion of the VPAG [136]. Thus, conditioned and unconditioned freezing seem to have a different neural substrate, which may be related to anticipatory anxiety and panic, respectively (see below).

4.4. Defense strategies and anxiety disorders

The final step was to correlate the above levels of predatory defense to anxiety-related emotions, normal as well as pathological. For this, it is necessary to take into account the developments that were taking place in the classification of anxiety disorders. A major shift in opinion occurred in 1980, when the 3rd Edition of the American Psychiatric Classification (DSM III) was released [3]. Replacing the preceding view that merged anxiety disorders

in the undifferentiated pool of psychoneurosis, the DSM III classification delineated distinct nosological entities, namely PD, agoraphobia, simple phobias, social phobia, post-traumatic stress disorder, obsessive–compulsive disorder, and GAD. Except for a few changes in diagnostic criteria, the same theoretical stand remained in the revised version of DSM III [4] and in the DSM IV [5] classification, being also adopted by the ICD-10 classification of the World Health Organization [142]. Although these classifications are mainly based on overt symptoms and therapeutic response, it is implicit that different manifestations are likely to be due to distinct neural substrates. This motivated basic researchers in several fields to look for specific animal models and neural correlates for each disorder [143].

Starting with GAD, in both Gray's BIS and Blanchard's first level of defense the septo-hippocampal system is supposed to be the main brain structure underlying anxiety. More recently, Gray and McNaughton [59] have made an important addition, by assimilating the contribution of several North American research groups, among which stand those led by LeDoux [89] and Davis [26], who established the critical role played by the amygdala in the learning and expression of the so-called conditioned fear. In this paradigm, although no explicit conflict exists, the animal is placed inside an experimental box from which escape is impossible. Therefore, the escape tendency is contained by behavioral inhibition, as in the case of conflict. In favor of this interpretation, reported evidence has shown that the pharmacological profile of conditioned fear is close to that of conflict tests; in contrast, one-way escape and avoidance tasks, which would be appropriate animal models of fear, are resistant to anxiolytic drugs [49]. Due to the predictive, face and construct validity of conflict tests for GAD, it is reasonable to suppose that the same brain mechanisms are called upon in both instances. Thus, CER would be related to anticipatory anxiety, which in clinical conditions is also reduced by anxiolytics. Conciliating the two lines of evidence, Gray and MacNaughton [59] have argued that the septo-hippocampal system, now conceived as a conflict detector, would contribute with the cognitive component (worry), whereas the amygdala would impart the affective tone to anxiety. In regard to fear, conditioned escape and avoidance as well as unconditioned escape, seemingly organized by the amygdala and the MH, respectively, and being resistant to anxiolytics, have been related to the innate and learned components of specific phobias [28]. Arriving now at the main concern of this review, a more detailed account will be given on the association between the DPAG and panic.

Early arguments relating 5-HT, the BDS (also called the brain aversive system [42], and panic were based on the experimental evidence with intra-cerebral injection of drugs in the MH and DPAG of rats: "Because the experimental evidence...indicates that GABAergic neurotransmission tonically inhibits the brain aversive system, this deficiency could be related to the persistent character of GAD. In

contrast, the panic attack is a form of episodic anxiety of sudden onset, usually accompanied by marked autonomic changes. ...The symptoms of the panic attack are similar to the behavioral and neurovegetative effects of electrical stimulation of brain aversive areas. Furthermore, 5-HT uptake inhibitors which attenuate the behavioral effects of CG (*central gray or PAG*), probably because they enhance the inhibitory function of neuronally released 5-HT, have been reported to decrease the frequency of panic attacks... Therefore, it is conceivable that a deficiency of 5-HT modulation of the brain aversive system may be involved in the triggering of panic attacks. Their episodic occurrence, as opposed to the persistency of GAD, may be related to the phasic nature of 5-HT inhibition of the brain aversive system, as suggested by the aforementioned lack of effect of intra-CG administered 5-HT receptor blockers" [43].

The above hypothesis considered the action of 5-HT in the BDS as a whole. However, the contrasting effects of anti-GABAergic drugs in the MH and the DPAG [116] and of serotonergic drugs injected in the amygdala and in the PAG led to the dual hypothesis of 5-HT action in the BDS, and to the relation of panic with the DPAG and anxiety with the amygdala. Considering reported results showing that patients with low levels of the 5-HT metabolite 5-hydroxy-indolacetic acid (5-HIAA) in the cerebrospinal fluid are associated with high somatic or unconditioned anxiety, meaning panic attacks with autonomic manifestations and with low anticipatory or psychic anxiety [113], it has been argued that "This dual concept of anxiety should be kept in mind, since it might put together the seemingly contradictory hypotheses about the role of 5-HT in anxiety... Thus, it may be the case that 5-HT in the septo-hippocampal system mediates anticipatory or psychic anxiety, whereas 5-HT in the BAS (BDS) inhibits unconditioned anxiety or panic. ...there is still the possibility that 5-HT facilitates aversion in the AM (*amygdala*) whereas exerting an anti-aversive action in the MH and the DCG (*DPAG*)" [44]. The latter statement was based on the reported anti-conflict effect of 5-HT antagonists [70,107] and the pro-conflict effect of 5-HT [70] following microinjection into the basolateral amygdala.

The similarity between the effects of PAG electrical stimulation in neurosurgical patients reported by Nashold et al. [101]—palpitation, blushing of face and neck and respiratory arrest or hyperventilation, feelings of terror or impending death, and desire to flee—and the symptoms of panic attack in the DSM III [3], led the Brazilian psychiatrist Gentil [37,38] to suggest a participation of the PAG in the pathophysiology panic attacks. Commenting on the rat DPAG aversive stimulation, Gentil [39] remarked: "I believe that (*this animal*) model is particularly useful for the understanding of the pathophysiology of panic attacks, especially the 'spontaneous' attacks. ... (*Bearing in mind that*) the panic attack is a very primitive behavior. ... the isomorphic validity of the central gray's (*DPAG*) poorly organized responses to γ -aminobutyric acid (GABA-A)

antagonists and electrical stimulation to the maladaptive flight behavior of full-blown panic seems high".


4.5. The Deakin–Graeff model

In an attempt to reconcile the prevailing '5-HT-excess' hypothesis of anxiety with the also widely held '5-HT-deficiency' hypothesis of depression—two conditions that often occur together in the same patient—Deakin [27] suggested that stimulation of 5-HT₂ receptors in the forebrain, most likely in the amygdala and the frontal cortex, increases the sensitivity to aversive stimuli (acute stress), thus enhancing anxiety. At the same time, stimulation of 5-HT_{1A} receptors in the hippocampus would improve resilience to chronic stress. Failure of the latter process would result in depression. An opposing balance between these two types of 5-HT receptors was also postulated. Two ascending 5-HT pathways were involved in Deakin's model, since the amygdala and the frontal cortex are mainly innervated by the DRN, whereas the (dorsal) hippocampus gets its 5-HT terminals from the MRN [10]. This hypothesis predicts that blockade of 5-HT₂ receptors would decrease anxiety. Accordingly, a double-blind placebo-controlled study carried out by Ceulemans and co-workers [23] in patients with GAD showed that ritanserin was as effective as lorazepam in improving this condition. In contrast, a similar study comparing ritanserin with fluvoxamine in patients with PD evidenced that if anything, ritanserin tended to aggravate panic [32]. Deakin and co-workers [29] also reported significant worsening of PD by ritanserin. The conclusion drawn was that GAD and PD were modulated in opposing direction by 5-HT, correlating with the facilitatory and inhibitory actions of 5-HT in the forebrain limbic structures and the in the PAG, respectively [46].

This suggestion has been incorporated into a more encompassing model of the role played by three 5-HT pathways in PD, GAD and depression, respectively [28]. The first is the DRN-periventricular pathway that projects onto the MH and the PAG. In these core structures of the BDS, 5-HT would inhibit fight or flight behavior elicited by acute unconditioned stimuli such as pain, suffocation or proximal threat. These defensive reactions may be related to PD. The second pathway goes from the DRN to the amygdala and the frontal cortex, running through the medial forebrain bundle. It is supposed to facilitate anticipatory anxiety determined by acute stimuli that predict some noxious or aversive consequence. This type of anxiety would be related to GAD. The third pathway originated in the MRN impinges mainly on the hippocampus. In this structure, 5-HT would enhance neural processes that allow the animal to adapt to chronic stressful stimuli or situation. Failure of this resilience process would result in depression. The portion of this theoretical model that deals with acute aversive stimuli is summarized in Table 3.

Among the empirical evidence that originally supported

Table 3
Levels of defense, related emotions and their neural substrate

Distance (auditory, visual, olfactory)		Contact (skin)	Internal (visceral)
Cues (signals)	Contexts (places)		
<hr/>			
Conditioned			
CER, conflict, social	Place aversion		
Innate			
Predators (calls, sight, smell)	Plus-maze, open space,	Vibrissae	Asphyxia (pCO ₂)
Dominance and territorial displays	lack cover and escape	Touch	
	foreign territory	Pain	
Analyzers			
Basolateral circuit, amygdala	Parahippocampal gyrus hippocampus	Barrel fields Dorsal horn	Chemosensitive Zone
Effectors			
	AMYGDALA	HYPOTHALAMUS	PAG
Behavior			
Stop, freeze, vigilance	Directed escape	Fight, freeze, undirected flight	
Facilitated by 5-HT		Inhibited by 5-HT	
Psychopathology			
Anticipatory anxiety and GAD	Innate phobias	Spontaneous panic	
Learned phobias	Situational panic		

CER, conditioned emotional response; PAG, periaqueductal gray; GAD, generalized anxiety disorder. Modified from Ref. [28].

the Deakin–Graeff model were reported results from studies with two experimental paradigms that induce anxiety in human subjects, namely aversive Conditioning of Skin Conductance Responses (CSCR) to tones and Simulated Public Speaking (SPS).

The CSCR procedure was developed by Vila and Beech [137] and later modified by Wang [139]. It measures the amplitude of SCRs to a tone presented 10 times before (habituation) and 10 times after (extinction) its pairing with an aversive white noise (one-trial acquisition). The effect of tone–noise pairing is to reinstate responding to further presentation of the tone, now a conditioned stimulus (CS). This appears to involve an associative mechanism, since there was no sustained reinstatement of responding when the 11th tone was omitted and the loud noise occurred in temporal isolation from the tones [64]. Therefore, it is assumed that this model generates anticipatory anxiety, related to GAD.

In the SPS test, elaborated by McNair et al. [97], the subject is requested to prepare a speech and then deliver it in front of a video camera, the performance being recorded in

videotape. Anxiety and other subjective states are assessed at different phases of the experimental session through self-rating scales, the most used being the Visual Analog Mood Scale (VAMS) [104]. Fear of speaking in public is the most common social fear found in epidemiological studies [125], being rather constant across gender, race and age [108]. In addition, the SPS test has been shown to provoke anxiety in healthy volunteers, irrespective of trait anxiety level, while another procedure, the Stroop Color Test, was anxiogenic only in persons with high trait anxiety [105]. As a consequence, unconditioned fear mechanisms are supposed to be mobilized by SPS.

A critical difference was found in the response of the above tests to ritanserin. The drug selectively decreased the amplitude of skin conductance responses to the tone during extinction [67] whereas the same drug treatment prolonged the rise in anxiety determined by SPS [62]. These findings indicate that ritanserin attenuates anticipatory anxiety, but facilitates unconditioned fear. The opposite effects of ritanserin in the two experimental anxiety tests were correlated with the results of the mentioned clinical trials

showing that the drug improved GAD [23], but tended to aggravate PD [29,32].

Another finding that has been related to PD is the increase in SPS-induced anxiety determined by acute administration of chlorimipramine [63]. Chlorimipramine is effective in treating PD. However, the therapeutic action only appears following several weeks of repeated drug administration. Actually, the symptoms of PD are aggravated during the initial phase of treatment [40,76]. As a result, the pro-anxiety effect of a single dose of chlorimipramine observed in the SPS test has been correlated with this initial aggravation of PD [63]. Reported neurochemical evidence has shown that acute administration of 5-HT reuptake inhibitors markedly increases the extraneuronal concentration of 5-HT at the raphe nuclei where the soma of serotonergic neurons is localized. As a result, autosomic 5-HT_{1A} receptors that inhibit neuronal firing are stimulated, thus decreasing 5-HT neurotransmission [1,109]. Therefore, the pro-anxiogenic effect of chlorimipramine may be due to lack of 5-HT inhibition of brain mechanisms that underlie SPS anxiety.

From the tenets of the Deakin–Graeff model several predictions can be made that are amenable to experimental testing, some of which have been explored. The results so far obtained in the rat as well as in human subjects are summarized in the following sections.

4.6. Testing the dual 5-HT-defense hypothesis in experimental animals

Regarding animal models of anxiety, the following predictions can be made: (1) increasing the activity of 5-HT in forebrain limbic structures should increase behavioral indexes of anxiety in tasks that involve approach-avoidance conflict or risk-assessment, whereas 5-HT decrease should have anxiolytic effects; and (2) increasing 5-HT in the DPAG should reduce indexes of fear in tasks that involve escape from actual danger, the opposite being produced by 5-HT decrease.

The most critical tests of these predictions have been made by interfering with drugs or lesions in the 5-HT pathways that arise in the DRN and innervate the amygdala and the DPAG, respectively. To make the comparison between the two kinds of tasks more stringent, an animal model of anxiety has been developed to allow the same rat to undergo the two tasks in succession, within the same experimental session, by performing the same motor pattern. This experimental model has been named the ETM [52].

The idea of the ETM came from observation of the behavior of the rat while exploring the elevated X- or plus-maze, a widely used animal model of anxiety [65,106]. 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. Since rats have innate fear of elevated open places, they enter less and stay for a shorter time in the open arms as compared to the enclosed arms when allowed to freely explore the elevated plus-maze. Typically, benzodiazepine anxiolytics increase the number of entries into and the time spent on the open arms. Nevertheless, non-benzodiazepine anxiolytics, such as buspirone that primarily affect 5-HT had inconsistent effects in this test [60]. Following a line of reasoning very similar to that of the dual 5-HT-defense hypothesis Handley [66] has convincingly argued that such inconsistencies may be explained by the fact that the elevated plus-maze is a mixed test, in the sense that the rat displays different strategies of defense while exploring the maze, which could be influenced in opposite directions by 5-HT. At least two of them are clearly noticed if one observes a rat exploring this maze, namely avoidance of open arms when the rat is in an enclosed arm, and escape from an open arm towards an enclosed arm. The ETM was designed to separate these tasks by shutting the entrance of one of the enclosed arms of the plus-maze. For the inhibitory avoidance task, the rat is placed at the end of the remaining enclosed arm 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. Pre-exposure to the open arm for 30 min, 24 h before the test has been found to decrease the first withdrawal latency from the open arm and increase the drug sensitivity of the escape task [128,146]. The resemblance of the motor performance in both tasks 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.

Behavioral and pharmacological validation of the ETM [50,143] has shown that the increase in avoidance latency is a function of the aversive character of the open arm, and that the pharmacological profile of the avoidance task is close to that of GAD, in contrast to that of the escape task. The latter is insensitive to doses of benzodiazepines that have an anxiolytic effect (decrease of withdrawal latency) in the avoidance task, and is impaired by chronic, but not acute administration of imipramine [128], chlorimipramine and fluoxetine [110], reminding the drug response of PD.

Given this background, the following predictions can be made from the dual 5-HT-defense hypothesis about

the effects of manipulations of the DRN 5-HT pathways: (1) increase in 5-HT activity should enhance inhibitory avoidance in the ETM by acting on the limbic forebrain, but impair one-way escape by acting on the DPAG; and (2) decrease in 5-HT activity should do the opposite, that is impair inhibitory avoidance and facilitate escape.

One experimental test of the first prediction made use of systemic administration of *D*-fenfluramine. This drug selectively releases 5-HT from the terminals of serotonergic thin fibers that arise mainly from the DRN nucleus [133] and, as a consequence, is a convenient tool for testing the hypothesis. As expected, the obtained results have shown that IP injection of *D*-fenfluramine facilitated inhibitory escape while impairing escape in the ETM [51].

A more direct approach has been the microinjection of drugs inside the DRN. In one study with this method [119], the 5-HT_{1A}-receptor agonist 8-OH-DPAT impaired inhibitory avoidance while facilitating one-way escape. The interpretation of these results has been made on the basis of the drug's action on autosomic 5-HT_{1A} receptors of the DRN. As pointed out above, stimulation of these receptors is supposed to decrease 5-HT neuron firing rate and, consequently, decrease 5-HT activity on post-synaptic 5-HT receptors. In the present case, less 5-HT would be available in the limbic forebrain and in the DPAG, resulting in impairment of inhibitory avoidance and facilitation of escape, as shown by the obtained results. Following the same argument, destruction of 5-HT neurons in the DRN with 5,7-DHT should mimic the effect of neuronal inhibition with 8-OH-DPAT, and that was also shown by the results reported by Sena et al. [119].

The 5-HT neurons of the DRN are tonically inhibited by a GABAergic input [1]. Therefore, another way of influencing their activity is to either increase or decrease this GABAergic modulation. This first action was achieved by injecting the direct GABA_A-receptor agonist muscimol into the DRN. As expected, muscimol had the same effects on inhibitory avoidance and one-way escape in the ETM as the administration of 8-OH-DPAT or the 5,7-DHT lesion. In contrast, the benzodiazepine-receptor inverse agonist FG 7142 had contrary effects, that is, enhanced avoidance and impaired escape [119]. This is likely to be due to a decrease in GABAergic tone on 5-HT neurons, resulting from the removal of benzodiazepine amplification of GABA action on GABA_A receptors [77].

A direct stimulation of 5-HT neurons (among others) has been made by injecting the excitatory amino acid kainate into the DRN. As expected, inhibitory avoidance was facilitated and one-way escape was impaired [51]. This result is particularly important for the testing of the dual 5-HT-defense hypothesis, because microdialysis experiments have shown that the same drug treatment increased extra-neuronal levels of 5-HT both in the amygdala and in the PAG of wakeful rats [132].

Pointing to the same direction, a recent study by Zangrossi and Pobbe [144] showed that intra-DRN injection

of the 5-HT_{1A}-receptor antagonist WAY-100635 (0.37 nmol) facilitated inhibitory avoidance while impairing one-way escape. As in the former study [51], the same effects were observed after intra-DRN injection of kainic acid (60 pmol). More important, pre-administration of WAY-100635 (0.37 nmol) into the DPAG counteracted the effect induced by intra-DRN injection of either kainate or WAY-100635 on one-way escape, but not on inhibitory avoidance. These results suggest that stimulation of serotonergic neurons of the DRN inhibits one-way escape behavior by the activation of 5-HT_{1A} receptors in the DPAG, while the effects on inhibitory avoidance are exerted elsewhere, probably on forebrain limbic structures.

The contrasting effects of either stimulating or blocking 5-HT_{1A} receptors in the DRN on the two tasks performed in the ETM are shown in Fig. 1.

The role of the DRN in defense has also been explored in a series of studies conducted by Maier and co-workers. These workers have extended the dual 5-HT-defense hypothesis to encompass the learned helplessness model of depression [93]. In this experimental paradigm, pre-exposure to unavoidable electric shocks impairs learning of escape from controllable shock. In addition, pre-exposed animals learn conditioned freezing more readily. Viewing the escape deficit as a decrease of innate fear, Meier et al. suggested that enhanced fear conditioning and the innate fear reduction would be due to sensitization of DRN 5-HT neurons projecting to the amygdala and the DPAG, respectively. The results of an experiment designed to test this hypothesis have shown that electrolytic lesion of either the amygdala or the DRN facilitated fear conditioning, but only the DRN lesion prevented the escape deficit caused by pre-exposure to inescapable shock, most likely because the inhibitory 5-HT input to the DPAG was removed [96]. In the same direction, enhancement of GABAergic inhibition of 5-HT neurons by microinjection of chlordiazepoxide into the DRN, applied either before the administration of inescapable shocks or before the test, blocked both enhanced fear conditioning and escape deficit [95]. Similar effects were caused by directly stimulating 5-HT_{1A} autoreceptors in the DRN by local microinjection of 8-OH-DPAT [94]. These results fulfill the predictions derived from the hypothesis under scrutiny.

Interference in the MRN has also been made to test the dual 5-HT-defense hypothesis in the ETM. Like the DRN, the MRN sends ascending 5-HT-containing nerve fibers to the forebrain. However, the territory innervated by the two nuclei is not always superimposed. For instance, the amygdala is mainly innervated by the DRN while the dorsal hippocampus receives 5-HT terminals chiefly from the MRN [10]. This arrangement implicates the MRN in the functioning of Gray's BIS [58]. Therefore, a deficit in the functioning of the MRN-hippocampal pathway would be expected to impair inhibitory avoidance in the ETM, leaving the escape task unaffected. Accordingly, experimental results have shown that stimulation of autosomic 5-HT_{1A}

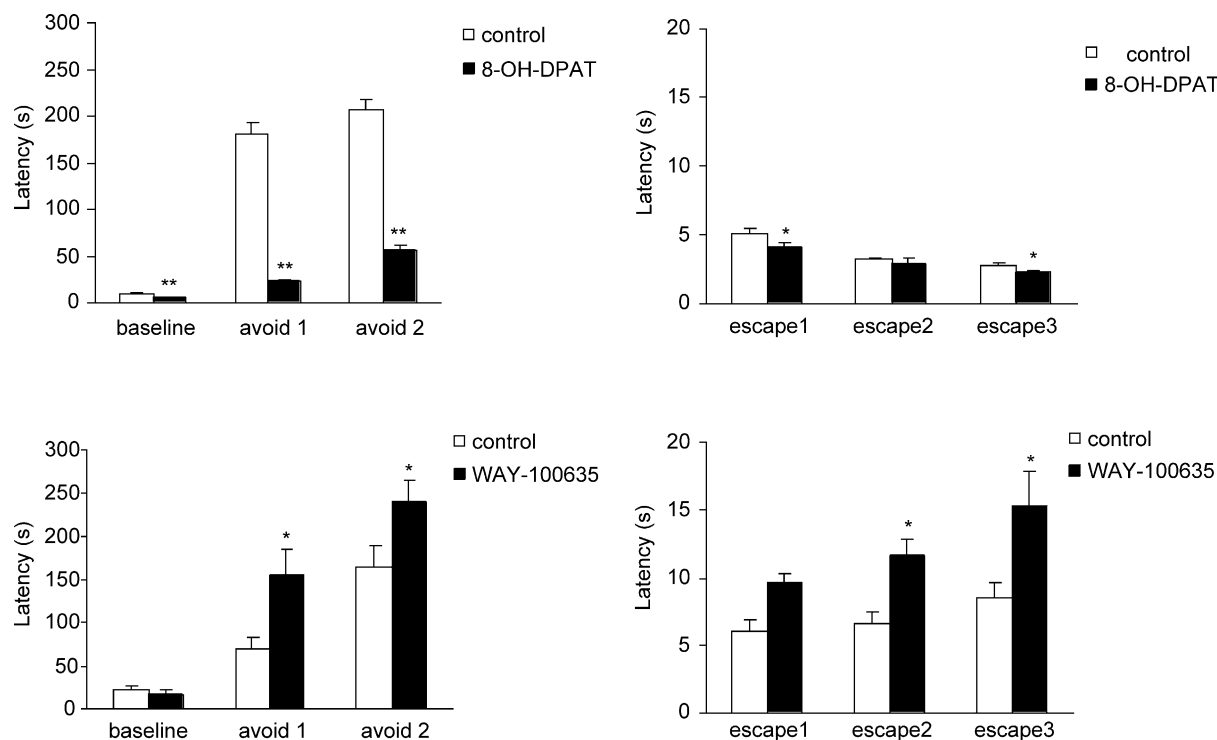


Fig. 1. Contrasting effects of microinjection into the dorsal raphe nucleus of the 5-HT_{1A}-receptor agonist 8-OH-DPAT (8 nmol) and of the 5-HT_{1A} antagonist WAY 100635 (0.37 nmol) on inhibitory avoidance and one-way escape tasks measured in the elevated T-maze. Columns represent mean; vertical bars the SEM. The latency to leave the enclosed arm (baseline, avoid 1 and 2) and one of the open arms (escape 1–3) were measured sequentially at 30-s intervals beginning 10 min after the last drug injection. The day before, all animals were exposed to one of the open arms for 30 min. * $P < 0.05$, ** $P < 0.01$ (Duncan's test); $N = 13–14$.

receptors with 8-OH-DPAT injected in to the MRN impaired avoidance without affecting escape in the ETM [6]. Neurotoxic lesion of the MRN with 5,7-DHT had the same effect [7].

The results so far obtained with drugs injected into the DPAG have drawn a less clear picture. A recent study [146] evidenced that the neurotransmitter 5-HT, the 5-HT_{1A} agonist 8-OH-DPAT, the preferential 5-HT_{2A} receptor agonist DOI and the preferential 5-HT_{2C} agonist *m*CPP all impaired one-way escape. However, although as expected DOI was ineffective on inhibitory avoidance, the remaining three drugs impaired the performance of this task. The results with 8-OH-DPAT, DOI and 5-HT on escape are concordant with the reported anti-aversive effects with either electrical [117,103] or chemical (DLH) stimulation [11,12] of the DPAG. However, the inhibition of escape by *m*CPP contrasts with the facilitation of chemically induced escape [12] and the ineffectiveness on escape induced by electrical stimulation of the DPAG [103]. Nevertheless, systemic administration of the same drug has been shown to impair switch-off responding [75] and escape in the ETM [50], though it is uncertain whether the effect of *m*CPP is localized in the DPAG or elsewhere in the brain.

In any case, the impairments of escape caused by *m*CPP, administered either systemically or intra-DPAG, are disturbing in terms of clinical correlation, since this drug is generally classified as a panicogenic, rather than

a panicolytic agent. Nevertheless, it has been argued that in contrast to lactate injection or CO₂ inhalation, *m*CPP does not bring about a true panic attack, but rather enhances anticipatory anxiety [19]. The result showing that *m*CPP enhances inhibitory avoidance in the ETM after systemic administration [50] is compatible with this view.

According to the Deakin–Graeff model, the DPAG would control escape, while inhibitory avoidance would be dependent on the amygdala. For that reason, the changes in inhibitory avoidance verified after local administration of drugs (5-HT, 8-OH-DPAT and *m*CPP) into the DPAG [146] pose another difficulty for the model. In addition, recently obtained results (Zangrossi and Strauss, unpublished) have shown impairment of one-way escape by microinjection into the medial amygdala of both muscimol (0.022 nmol in 0.2 μ l; escape 1 latency: vehicle = 7.4 ± 1.2 s, muscimol = 15.5 ± 1.9 s, *t* test $P < 0.01$) and midazolam (0.2 nmol in 0.2 μ l; escape 1 latency: vehicle = 9.1 ± 0.8 s, midazolam = 15.6 ± 1.7 s, *t* test $P < 0.01$), without any change in inhibitory avoidance acquisition. This effect was not due to a motor deficit, as neither drug has affected locomotion in the open-field. Intra-medial amygdala injection of muscimol or midazolam also failed to change the anxiety indexes measured by the light/dark transition test, and animal model related to GAD [49]. Therefore, GABA_A receptors in the medial amygdala seem to participate in the mediation of panic-related, but not GAD-related responses.

In contrast, bilateral injection of midazolam (or of 8-OH-DPAT) into the basolateral amygdala impaired avoidance without affecting escape in the ETM [145].

The above results with interference in the PAG and in the amygdala question the rostro-caudal hierarchy of the BDS suggested by Deakin and Graeff [28]. Instead, they point to parallel, longitudinally organized systems controlling anxiety-related and panic-related defense, respectively. In this regard, a molecular study by Silveira et al. [122] showed that Fos-like immunoreactivity was increased in nearly the same set of brain structures after performance of either inhibitory avoidance or one-way escape in the ETM. Nevertheless there were differences in the relative magnitude of activation among these structures: the avoidance task increased Fos-like immunoreactivity more than the escape task in the medial nucleus of the amygdala (contrary to the preceding evidence), paraventricular nucleus of the thalamus, anterior hypothalamic nucleus and MRN, whereas performance of one way-escape enhanced Fos-like immunoreactivity relatively more in the DPAG. Mongeau et al. [98] have conducted a similar study in mice using ultra-sounds as innate fear stimulus. Two types of defensive reactions were observed: flight during the stimulus and freezing immediately afterwards. The degree of freezing was increased by a novel environment or inevitable foot shocks given 24 h before in a different environment (sensitization), and was attenuated by alprazolam. As a consequence, it was related with anxiety, in contrast to the panic-like flight response. There was a negative correlation between freezing and flight, reminding of the proposal that anticipatory anxiety inhibits panic (see Section 5). Finally, quantitative *c-fos in situ* hybridization revealed that sensitized mice displaying predominantly freezing behavior had preferential neural activity in the lateral septal ventral (reminding of Gray's BIS [58]) and several medial and periventricular nuclei, whereas mice predominantly displaying flight had more activity in cortical, amygdalar and striatal motor areas, the dorsolateral posterior zone of the hypothalamus, and the vertical limb of the diagonal band. Several brain structures, including the PAG were similarly activated during the two types of defense reactions. The inter-relation among the components of the BDS in animal defense and anxiety-related disorders has been thoroughly discussed by Gray and McNaughton [59], Canteras et al. [22] and Sewards and Sewards [120], but it is an issue that requires more investigation.

In spite of these shortcomings, the reviewed evidence with experimental manipulation of the DRN and the MRN clearly indicates that there is an opposed regulation by 5-HT of anxiety-related and panic-related defense, which is exerted in the forebrain and in the midbrain, respectively.

Table 4 summarizes the effect of drugs injected intracerebrally on the two tasks performed in the ETM.

4.7. Experimental tests in healthy volunteers

Part of the evidence on 5-HT-acting drugs and human experimental anxiety has been discussed above, since it belongs to the foundations of the Deakin–Graeff model. Nevertheless, critical tests of the hypothesis have been made later using the 5-HT-releasing and reuptake inhibiting drug D-fenfluramine, before its withdrawal from clinical use due to untoward effects on the cardiovascular system. As remarked before, there is neurochemical evidence showing that D-fenfluramine selectively releases 5-HT from serotonergic fibers that arise in the DRN [133] and, fulfilling the predictions derived from the dual 5-HT-defense hypothesis, the drug enhanced avoidance, but inhibited escape in the EPM [51]. Moreover, it has been argued that the CSCR test generates anticipatory anxiety related to GAD and that the SPS test evokes unconditioned fear, related to PD. As a consequence D-fenfluramine should facilitate CSCR and reduce SPS-induced anxiety.

A study conducted by Hetem and co-workers [69] tested these predictions. A group of 43 adult healthy volunteers were assigned to the SPS test and another group of 40 subjects to the CSCR test. As expected, oral administration of 30 mg of D-fenfluramine markedly decreased the rise in anxiety caused by public speaking, the dose of 15 mg of the drug having a lesser effect. However, in the CSCR model, the effect of D-fenfluramine was unclear: the lower dose of D-fenfluramine tended, non-significantly, to increase the amplitude of the skin conductance responses during the extinction phase, but the higher dose (30 mg) was ineffective. Therefore, as expected the drug seems to decrease innate fear, but the prediction concerning anticipatory anxiety was neither supported nor rejected by the obtained results.

Assuming that the SPS correlates with PD, the attenuation of SPS-induced anxiety found by Hetem et al. [69] seems quite puzzling, since Targum and Marshall [126] reported that fenfluramine induces panic attacks in patients with PD. Nevertheless, they pointed out that this drug causes a slow wave of anxiety that does not resemble the sudden surge that is characteristic of a true panic attack (for a critical discussion on panicogens, see Ref. [19]). The view that fenfluramine induces anxiety rather than panic is strengthened by the results of a critical experiment conducted by Mortimore and Anderson [100] using inhalation of 5% CO₂ in PD patients. The obtained results have shown that D-fenfluramine enhanced anticipatory anxiety, but markedly decreased the intensity of panic attacks induced by CO₂ inhalation. The last result correlates with the reducing effect of the drug on SPS anxiety, and is predicted by the dual 5-HT-defense hypothesis.

The preceding results with human subjects, together with the above evidence obtained with the ETM suggest that D-fenfluramine may ameliorate PD, instead of inducing panic. Accordingly, an open clinical trial conducted by

Table 4
Effect of intra-cerebral injections on the tasks performed in the elevated T-maze

Brain structure	Procedure	Avoidance	Escape	Reference
DRN	Kainic acid ^a	+	–	[51,144]
	FG 7142 ^b	+	–	[119]
	WAY-100635 ^c	+	–	[144]
	8-OH-DPAT ^d	–	+	[119]
	Muscimol ^e	–	+	[119]
	5,7-DHT lesion	–	+	[119]
MRN	8-OH-DPAT ^d	–	0	[6]
	5,7-DHT lesion	–	0	[7]
DPAG	5-HT	+(0)	–	[146]
	8-OH-DPAT ^d	–(0)	–	[146]
	DOI ^f	0	–	[146]
	mCPP ^g	+(0)	–(+) ^h	[146]
Amygdala Basolateral	8-OH-DPAT ^d	–	0	[145]
	Midazolam ⁱ	–	0	[145]
Medial	Muscimol ^e	0 (–)	–(0)	Zangrossi and Strauss, unpublished
	Midazolam ⁱ	0 (–)	–(0)	Zangrossi and Strauss, unpublished

DRN, dorsal raphe nucleus; MRN, median raphe nucleus; DPAG, dorsal periaqueductal gray. +, Facilitation; –, impairment; 0, no change. Whenever a discrepancy occurs, the expected result (Deakin–Graeff hypothesis) is given between brackets.

^a Excitatory amino acid.

^b Benzodiazepine-receptor inverse agonist.

^c 5-HT_{1A}-receptor antagonist.

^d 5-HT_{1A}-receptor agonist.

^e GABA_A-receptor agonist.

^f 5-HT_{2A}-receptor agonist.

^g Preferential 5-HT_{2C}-receptor agonist.

^h Assuming that mCPP is a true panicogen.

ⁱ Benzodiazepine-receptor agonist.

Solyom [124] evidenced that this drug reduced clinical symptoms in a group of panic patients that was resistant to conventional drug treatment and Hetem [68] reported that addition of D-fenfluramine to benzodiazepines produced a marked improvement in a PD patient.

Silva and co-workers [121] carried out an additional test of the dual 5-HT hypothesis using the anti-depressant agent nefazodone. This drug blocks 5-HT_{2A} receptors and inhibits 5-HT reuptake [127]. As argued before, in regard to chlorimipramine [63], it is believed that acute administration of 5-HT reuptake inhibitors results in over stimulation of 5-HT_{1A} autosomic receptors, reducing 5-HT action on post-synaptic 5-HT receptors. Therefore, nefazodone is expected to affect CSCR and SPS toward the opposite direction than D-fenfluramine. Twenty-nine adult healthy volunteers underwent the CSCR test and another 34 subjects performed the SPS task. In both cases, subjective states were evaluated through VAMS. In each experiment, subjects were randomly divided into three groups, which received placebo, 100 or 200 mg of nefazodone, respectively, under double-blind condition. As expected, nefazodone enhanced the rise in anxiety induced by SPS, but

attenuated the increase in subjective anxiety induced by the CSCR test. Although the amplitude of skin conductance responses to the tone (CS) was not significantly affected, nefazodone decreased the number of spontaneous fluctuations of skin conductance at both the habituation and extinction phases of the CSCR test [121].

The contrasting effects of D-fenfluramine and nefazodone on SPS-induced anxiety are shown in Fig. 2.

The pharmacological profile of CSCR and SPS has been analyzed in two recent review articles [54,56].

5. Studies in panic patients

The above pharmacological evidence indicates that enhancing 5-HT neurotransmission reduces unconditioned fear, as evaluated by the SPS model. On the contrary, impairing 5-HT neurotransmission intensifies this type of fear. Since unconditioned fear has been related to PD, panic patients are expected to behave differently from normal subjects in the SPS test. In turn, there is no reason to expect panic patients to be different from control in regard to

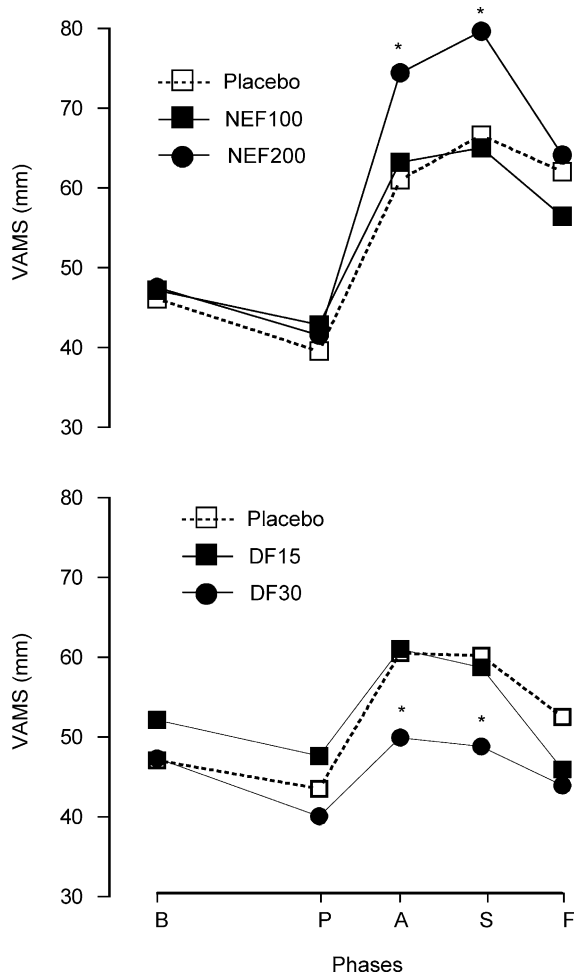


Fig. 2. Opposite effects of nefazodone (NEF, 100 and 200 mg) and D-fenfluramine (DF, 15 and 30 mg) on subjective anxiety induced by the Simulation of Public Speaking (SPS) test, measured by the Visual Analog Mood Scale (VAMS). Measures were taken at the beginning of the experimental session (B), at pre-stress just before the test (P), after the instructions (A), during speech (S) and 15 min after the end of the speech (F). Points in the figure represent the mean. Drugs were orally administered soon after B and either 1 h (NEF) or 2: 45 h (DF) before P. The asterisk indicates significant difference from placebo at $P < 0.05$ (Duncan's test); $N = 8-12$. Modified from Ref. [56].

aversive conditioning. To test the above predictions, Del-Ben et al. [30] have conducted a study in which 17 panic patients and matched controls performed both the SPS the CSCR tests. As in the preceding pharmacological studies, subjective states were evaluated through the VAMS. The obtained results have shown that panic patients had higher levels of anxiety along the whole experimental session than normal controls. In contrast, the SPS test increased anxiety in controls, but not in panic patients. This could be due to a ceiling effect, since anxiety before the test was already high. However, the degree of vigilance, measured by the VAMS Mental Sedation factor did not differ from controls as to the baseline level. In spite of this, vigilance did not increase during the SPS challenge in panic patients, as it occurred in

normal subjects. This led to the conclusion that panic patients are less sensitive to SPS than healthy subjects.

Consistent with the high baseline level of subjective anxiety described above, panic patients had more spontaneous fluctuations of skin conductance than controls during the CSCR test. Yet, the amplitude of skin conductance responses in panic patients did not differ from control subjects during conditioning. Therefore, anticipatory anxiety was normally learned in panic patients.

According to the hypothesis under scrutiny, panic patients are supposed to lack 5-HT inhibition in brain networks responsible for the processing of unconditioned fear. Therefore, they should be more, not less sensitive to SPS, as found by Del-Ben et al. [30]. Nonetheless, this discrepancy may be explained by an additional hypothesis on the interaction between anxiety and panic. The high level of baseline anxiety shown by panic patients is generally attributed to anticipation of panic attacks [86]. Yet, an alternative view put forward by Deakin and Graeff [28] is that by keeping themselves anxious, panic patients may be inhibiting an abnormally susceptible unconditioned fear system. Many panic patients prefer to keep themselves in a state of busy readiness than relax, and relaxation therapy may precipitate panic attacks [2]. Furthermore, the frequency of panic attacks is higher at the beginning of agoraphobia, when there is little anticipatory anxiety, than in the later phase, when this anxiety has fully developed [85]. Therefore, ongoing anxiety seems to suppress panic attacks. As a consequence, the lack of response to SPS observed in panic patients could be due to restraint of their unconditioned fear system by persistently heightened anxiety.

The hypothesis that anticipatory anxiety reduces innate fear has been recently tested in experimental animals by Magierek et al. [92]. As expected, the obtained results have shown that rats exposed to contextual cues that had been previously associated with electrical foot shocks were less likely to display flight evoked by electrical stimulation of the DPAG. In the same vein, the results of Mongeau et al. [98] discussed in Section 4.6 evidenced a negative correlation between freezing and flight in the mouse, the former behavior being related to anticipatory anxiety and the latter to panic, respectively.

There is an important difference between anticipatory anxiety and panic in regard to the functioning of the hypothalamic-pituitary-adrenal (HPA) axis, since reported results show that plasma cortisol levels do not rise during either experimentally induced [90,123] or spontaneous [20, 141] panic attacks. Assuming that the emotional state induced by SPS is akin to panic attacks, this challenge should not activate the HPA axis. To test this prediction, Leal and co-workers [87] have assayed salivary cortisol in three groups of subjects before and after SPS. The first group consisted of 18 symptomatic panic patients, the second of 16 non-symptomatic patients under drug treatment and the third of 17 healthy controls. In parallel with

the magnitude of anxiety (VAMS anxiety factor), the level of cortisol was high at the beginning of the experimental session (anticipatory anxiety) and decreased after 70 min in the laboratory [$t(50) = 6.63$, $P < 0.001$]. Preparation and performance of SPS raised the anxiety score to the same extent than anticipatory anxiety, but failed to increase salivary cortisol in the three experimental groups during the 60-min period following SPS [$t(50) = 2.03$, $P > 0.05$, Bonferroni's correction]. The last result indicates that SPS does not activate the HPA axis. The described results are shown in Fig. 3.

In accordance with these results in human subjects, electrical stimulation of the DPAG in the rat, which is viewed as a model of the panic attack [115], did not increase plasma levels of either adrenocorticotrophic hormone (ACTH) or prolactin [131]. This may be a critical criterion for an animal test to be considered a true model of panic, since another candidate, escape and physiological changes induced by microinjection of biccuculine into the dorsomedial hypothalamic nucleus, increases ACTH and corticosterone secretion [78]. A neural pathway that originates in the PAG and innervates the posterior division of paraventricular thalamic nucleus, releasing cholecystokinin (CCK) there is likely to mediate the inhibition of the HPA axis by the PAG [15].

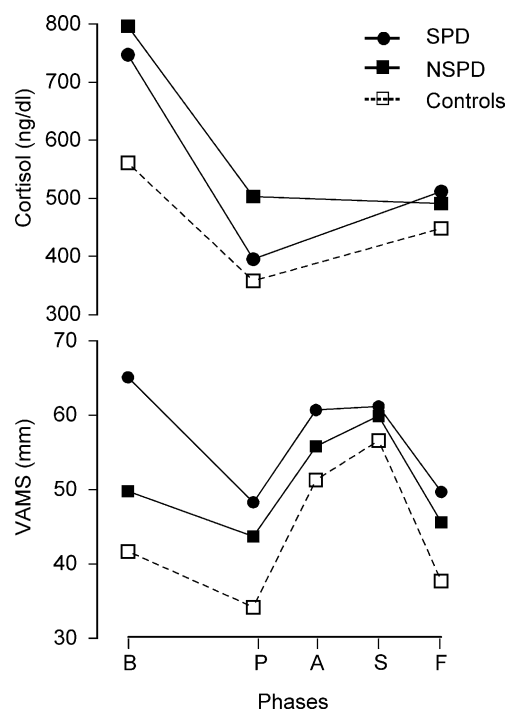


Fig. 3. Changes in salivary cortisol (upper panel) and in VAMS anxiety (lower panel) along the SPS test measured in symptomatic panic patients (SPD), non-symptomatic patients (NSPD) and healthy controls. The phases of the experimental session are specified in Fig. 2. Points in the figure represent the mean. The last value of salivary cortisol (F) is the highest level in four successive measures taken 0, 15, 30 and 60 min from end of the experimental session. $N = 16-18$.

As shown in the lower panel of Fig. 3, subjective anxiety along the experimental session tended to be higher and the response to SPS smaller in panic patients than in healthy controls, as previously reported [30]. Asymptomatic panic patients were positioned in-between. As pointed out by Del-Ben et al. [30], this profile of panic patients during the SPS test resembles the effect of the non-selective 5-HT-receptor blocker metergoline, a drug that has been shown to increase anxiety at pre- and post-stress, but not during speech preparation or performance [57]. In fact, one of the volunteers of the former study who had taken metergoline, reported symptoms suggestive of a panic attack during the night following the experimental session (unpublished observation). In addition, it has been reported that metergoline facilitates panic attacks induced by inhalation of 5% CO_2 [14]. This evidence supports the suggestion that lack of 5-HT inhibition of neurons responsible for unconditioned defense reactions may lead to PD, most likely in the DPAG [28,44,46,53,55,91].

Overall, the above results support the view that the CSCR and the SPS tests generate different emotional states. The conditioned anxiety induced by the former model seems to be enhanced by 5-HT and related to GAD, whereas SPS-induced anxiety is likely to be unconditioned, inhibited by 5-HT and related to PD. In addition, the low sensitivity of panic patients to the SPS indicates that they process unconditioned fear abnormally.

6. Neuroimaging studies in human subjects

From the above discussion, it is expected that anticipatory anxiety and panic attacks would activate different brain neural networks. Although more studies are needed, the evidence so far obtained from functional neuroimaging studies using positron emission tomography (PET) tends to support this view.

The first study of this kind showed activation of the whole BDS during lactate-induced panic attacks, including anterior temporal lobe, amygdala and an area subjacent to the superior colliculi, probably the PAG [111] and a similar investigation has shown that CCK-4-induced panic attacks activate the hypothalamus and PAG [73]. The latter study has further shown that the medial frontal cortex was not activated by the drug challenge, a finding also reported during a natural, unexpected panic attack [35]. In contrast, anticipatory anxiety has been reported to activate the anterior cingulate cortex [71]. Such evidence led Swards and Swards [120] to conclude that, in contrast to anticipatory anxiety, the “extreme fear experienced by these (*panic*) patients is not due to cortical activities, but is produced by neuronal activities in the hypothalamic and PAG representations”.

The last statement supports the present view, that the same neural network that commands innate defensive reactions elicited by proximal danger also orchestrates panic attacks.

In seeming contrast with this hypothesis, Gorman and co-workers [41] have emphasized the role of the fear-conditioning neuronal network in PD. However, conciliation between the two hypotheses is possible, considering that PD involves other manifestations in addition to panic attacks, such as anticipatory anxiety, avoidant behavior and even situation-bound panic attacks, where the conditioning model is appropriate. Also, cognitive aspects of the disorder are likely to involve neocortical structures, as also pointed out by Gorman et al. [41].

Another aspect to be considered is vulnerability to PD. In this regard, a PET study performed during the inter-critical phase in panic patients evidenced a positive correlation between susceptibility to lactate-induced panic attacks and lower left/right ratio of parahippocampal region activation [112]. In addition, two morphometric studies with nuclear magnetic resonance imaging (MRI) indicate abnormality in temporal lobe structures of PD patients as compared to normal controls [129,138].

7. Conclusion

The reviewed evidence indicates that anxiety and panic are different emotions, not only in terms of subjective experience, but also in behavioral and physiological manifestations, response to drugs and neural substrate. Anxiety is associated with defensive reactions to potential threat, attenuated by anxiolytic drugs, integrated in limbic forebrain structures such as the amygdala and the hippocampus, and activates the HPA hormonal axis. Panic is related to defense reactions elicited by proximal threat, resistant to anxiolytics, integrated in primitive structures of the hindbrain, such as the hypothalamus and PAG, and does not activate the HPA axis. The origins of this dichotomy may be traced back to Selye's 'general adaptation syndrome' [118] and Cannon's 'emergency reaction' [21], respectively.

Both emotions seem to be modulated by serotonergic fibers that come from the DRN, although toward opposite directions. Thus, 5-HT seems to enhance anxiety, but to inhibit panic. This knowledge has clinical implications for the pathophysiology and pharmacotherapy of PD. For instance, it has been suggested that a deficit in 5-HT inhibition at the level of the DPAG may underlie the susceptibility to panic attacks that characterizes PD. Conversely, intensification of 5-HT inhibition in the DPAG by anti-depressants may be a mechanism of the anti-panic action of these compounds. In this regard, the sensitization of 5-HT_{1A} and 5-HT₂ receptors following chronic administration of imipramine recently reported by Jacob et al. [72] is highly suggestive, since the anti-depressant action of this drug has been attributed to a similar action on hippocampal 5-HT_{1A} receptors [109]. Furthermore, 5-HT availability seems to be necessary to maintain the response to SSRIs, since tryptophan depletion has been

reported to allow the precipitation of panic attacks by flumazenil in panic patients that had recovered following chronic treatment with paroxetine [13].

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