

The Dual Role of Serotonin in Defense and the Mode of Action of Antidepressants on Generalized Anxiety and Panic Disorders

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Abstract: Antidepressants are widely used to treat several anxiety disorders, among which generalized anxiety disorder (GAD) and panic disorder (PD). Serotonin (5-HT) is believed to play a key role in the mode of action of these agents, a major question being which pathways and receptor subtypes are involved in each type of anxiety disorder. The dual role of 5-HT in defense hypothesis assumes that 5-HT facilitates defensive responses to potential threat, like inhibitory avoidance, related to anxiety, whereas it inhibits defensive responses to proximal danger, like one-way escape, related to panic. The former action would be exerted at the forebrain, chiefly the amygdala and medial prefrontal cortex (PFC), while the latter would be exerted at the dorsal periaqueductal gray (DPAG) matter of the midbrain. The present review is focused on studies designed to test this hypothesis, performed in animal models of anxiety and panic, as well as in human experimental anxiety tests. The reviewed results suggest that chronic, but not acute, administration of antidepressants suppress panic attacks by increasing the release of 5-HT and enhancing the responsiveness of post-synaptic 5-HT_{1A} and 5-HT_{2A} receptors in the DPAG. The attenuation of generalized anxiety, also caused by the same drug treatment, would be due to the desensitization of 5-HT_{2C} receptors and, less certainly, to increased stimulation of 5-HT_{1A} receptors in forebrain structures. This action would result in less activation of the amygdala, medial PFC and insula by warning signals, as shown by the reviewed results obtained with functional neuroimaging in healthy volunteers and patients with anxiety disorders.

Keywords: Antidepressants, serotonin, anxiety disorders, animal models, human experimental anxiety, neuroimaging.

INTRODUCTION

Generalized anxiety disorder (GAD) and panic disorder (PD) clearly differ in their clinical symptoms. While GAD is mainly characterized by persistent, often fluctuating worry about everyday events, the hallmark of PD is the repeated occurrence of the panic attack (PA), which is a sudden and unexpected rise in fear, of dying, losing control or going crazy, which is accompanied by marked neurovegetative symptoms, such as palpitation, tremor, dizziness, shortness of breath, chest pain, nausea or abdominal distress, chills and paresthesias. The discomfort reaches a peak within about 10 minutes, and usually subsides within 30 minutes. Initially, the patient is otherwise normal, but with time anticipatory anxiety about having another PA develops, together with avoidance of places where having a PA is embarrassing. At the extreme, the patient becomes unable to leave home alone, a condition known as agoraphobia [1]. These clinical differences add to genetic and biological specificities, such as the sensitivity to lactate and other panicogenic agents in PD, to indicate that each condition has its own pathophysiology.

A pivotal clinical finding linking 5-HT and anxiety was the observation that chronic treatment with imipramine has beneficial effect in panic patients [2]. This led to the pharma-

cological demarcation between PD and GAD that ultimately resulted in the DSM III classification of these disorders as distinct nosological entities. Nevertheless, further research has shown that imipramine is also effective in GAD [3]. Since imipramine is able to block the reuptake of both noradrenaline (NA) and 5-HT, the involvement of the latter neurotransmitter in its therapeutic effects was not immediately recognized. Only in the late 1980s, with the introduction of fluoxetine, the link between 5-HT and clinical anxiolytic effects became clear. The latter is a selective serotonin re-uptake inhibitor (SSRI), being the prototype of a large group of compounds that nowadays are considered as first-line pharmacological treatment for several anxiety disorders, including GAD, PD, social anxiety disorder (SAD) and post-traumatic stress disorder (PTSD) [4].

The fact that all these conditions improve after chronic administration of SSRIs raises the question of whether these drugs are acting on a core dysfunction that is common to them or, alternatively, affect specific neural processes that are particular to each disorder. Because the neural basis of SAD and PTSD is less well understood than that of GAD and PD, this review focuses on preclinical and human experimental research that sheds light on how antidepressants act to attenuate the symptoms of the last two disorders.

ACTIONS OF ANTIDEPRESSANTS ON THE 5-HT NEUROTRANSMISSION

Single administration of antidepressants, such as imipramine and fluoxetine, inhibits the reuptake in a matter of

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minutes. However, the therapeutic effects of these compounds need at least two-three weeks to appear. Among the proposals that have been made to explain this delay the most accepted is based on electrophysiological studies. Their results indicate that repeated administration of SSRIs desensitizes inhibitory 5-HT_{1A} autonomic receptors, without affecting 5-HT_{1A} postsynaptic function [5]. Although imipramine does not share this effect with SSRIs, it sensitizes 5-HT_{1A} postsynaptic receptors located in areas such as the hippocampus and amygdala [6]. In both cases 5-HT-mediated neurotransmission in projection areas would be increased. Stimulation of post-synaptic 5-HT_{1A} receptors in limbic structures has been proposed to mediate at least part of the anxiolytic effects of these drugs [6, 7]. In addition, 5-HT₂ receptors are also likely to be involved, since long-term administration of several classes of antidepressants, including SSRIs desensitizes 5-HT_{2C} receptors in limbic areas, and these receptors have been proposed to increase anxiety. Since 5-HT_{2C} receptors are thought to be constitutively active, whereas postsynaptic 5-HT_{1A} receptors require spontaneous release of 5-HT, an increase in 5-HT mediated neurotransmission would shift the balance towards 5-HT_{1A}-mediated anxiolytic effects [8]. In synthesis, antidepressants would cause anxiolytic effects by down-regulating 5-HT_{2C} receptors and by enhancing effects mediated by post-synaptic 5-HT_{1A} receptors in limbic structures that are related to anxiety (see below).

A compelling evidence relating 5-HT to the anxiolytic effects of SSRIs came from clinical studies using tryptophan depletion to decrease brain 5-HT concentration. The reported results have shown that the therapeutic effect of SSRIs is reversed by 5-HT depletion in PD and SAD [9, 10]. Therefore, the therapeutic effect of SSRIs seems to need 5-HT release. Supporting this view, further reported results have shown that administration of the 5-HT precursor 5-hydroxytryptophan (5-HTP) was able to attenuate panic symptoms induced by intravenous injection of CCK4 or CO₂ inhalation in PD patients [11, 12], whereas either tryptophan depletion or blockade of 5-HT receptors with metergoline facilitated these symptoms [13, 14]. In a similar way, patients with PTSD fully recovered on SSRIs have been shown to become more sensitive to a trauma-related challenge after tryptophan depletion [15]. However, in OCD only mood, but not OCD symptoms were sensitive to tryptophan depletion. This suggests that the therapeutic effect of SSRIs on OCD symptoms is independent of short-time availability of 5-HT [16]. Also in GAD patients, tryptophan depletion does not seem to affect the therapeutic response to SSRIs, since an experimental study has shown that in patients with GAD who showed sustained clinical improvement with SSRI treatment 7.5% CO₂ inhalation significantly increased rating scale scores related to GAD, compared with air inhalation [17]. Therefore, the mechanism of action of SSRIs in OCD and GAD seems to be different to that in PD, SAD and PTSD, and may involve long-term 5-HT receptor changes or alterations downstream of 5-HT.

In addition to the primary action on 5-HT neurotransmission, long-term treatment with SSRIs has been shown to induce several plastic changes, among them increased neurogenesis in the dentate gyrus of the hippocampus, probably through activation of post-synaptic 5-HT_{1A} receptors [18].

This action may be relevant to the therapeutic effect, since hippocampal neurogenesis inhibition has been reported to prevent anxiolytic effects of these drugs in mice [18]. In addition, hippocampal levels of the brain-derived neurotrophin factor (BDNF) have been related to stress-induced behavioral changes, and some behavioral effects of chronically administered SSRIs have been shown to depend on BDNF signaling [19]. Despite these suggestive findings, the acute reappearance of anxiety symptoms in SSRIs treated-patients after tryptophan depletion discussed above indicates that caution must be exerted when considering these neuroplastic changes as unitary explanations for the long-term effects of SSRIs.

To understand how the changes induced by antidepressants in the functioning of 5-HT neurotransmission result in improvement of anxiety disorders' symptoms the role of 5-HT in anxiety is to be considered.

THE ROLE OF 5-HT IN ANXIETY

The 5-HT system is highly complex, and this increases the difficulty in understanding its role in anxiety. There are more than 14 receptor subtypes, located either pre- or postsynaptically and, in some cases, in both locations, and their activation sometimes produces opposite effects [20]. In addition, the 5-HT system has an intricate neuroanatomy. The widespread supply of 5-HT fibers that innervate the fore-brain originates from the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN) of the midbrain. These nuclei project to partly distinct sets of brain structures [21], and have a high degree of neuronal heterogeneity [22]. For example, the projections to cortical and limbic structures have been shown to arise from neurons located in a division of the caudal DRN, which are selectively activated by stressful stimuli (for review, see [23]).

Several preclinical studies have been performed to investigate the role of 5-HT in anxiety (for comprehensive reviews, see, e. g., [24, 25]). A substantial amount of the reviewed evidence supports the hypothesis that 5-HT enhances anxiety by acting upon forebrain limbic structures. However, contrary results have been obtained in the midbrain periaqueductal gray matter. Electrical stimulation of the dorsal periaqueductal gray (DPAG) in laboratory animals induces defensive reactions, such as vigorous flight or defensive aggression [26], that are expressed in natural conditions when a predator is very close to or in direct contact with the prey [27, 28]. Testifying to the aversive character of such stimulation, laboratory animals easily learn to switch off electrical stimulation of the DPAG [29]. If 5-HT would only increase anxiety, such escape behavior should be facilitated by manipulations that enhance 5-HT action.

However, reported results with a procedure in which rats are trained to lever press in order to decrease the intensity of DPAG electrical stimulation suggest the opposite action of 5-HT. Thus, using this decremental escape procedure, Kiser and coworkers have reported that the inhibitor of 5-HT synthesis PCPA markedly increases escape responding. Further results have shown that the precursor of 5-HT synthesis 5-hydroxytryptophan (5-HTP) reduces the same bar pressing, and that the 5-HT reuptake inhibitor clomipramine has the same effect. Finally, electrical stimulation of the DRN,

which sends 5-HT nerve fibers to the DPAG, has also been reported to depress responding. Altogether, these results show that manipulations that increase 5-HT activity in the DPAG have an anti-aversive effect, whereas 5-HT depletion results in facilitation of escape from DPAG electrical stimulation. Results obtained in our laboratory further support an inhibitory action of 5-HT on aversion generated in the DPAG. They have shown that the 5-HT receptor antagonists, cyproheptadine and methysergide, decrease responding that switches-off electrical stimulation of the DPAG. Furthermore, using electrical stimulation of the DPAG as a punishing stimulus, the anxiolytics chlordiazepoxide and pentobarbital released responding maintained by water reinforcement, suppressed by the response-contingent delivery of electrical stimuli in the DPAG. In contrast, the 5-HT receptor antagonist cyproheptadine did not increase punished responding at doses that had been shown to markedly release behavior punished by foot-shock, and methysergide was also ineffective. To explore the function of 5-HT in the PAG more directly, rats bearing a chemitrode – allowing microinjection of drugs associated with electrical stimulation – implanted into the DPAG 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 was made to switch off the brain stimulation. The same procedure was repeated ten times to determine the basal aversive threshold. Soon after, a drug microinjection was made and 10 min later the aversive threshold was determined again. The difference between the magnitude of the post-drug and that of the basal threshold measures the drug effect on aversion. Using this method, we have shown 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 pretreatment with the 5-HT receptor antagonists metergoline or ketanserin blocked the anti-aversive effect of 5-HT. Since ketanserin is a relatively selective 5-HT_{2A}-receptor antagonist, these results suggest that 5-HT inhibits aversion in the DPAG by stimulating this 5-HT receptor subtype. Such view has been further supported by results showing that the preferential 5-HT_{2A} agonist 2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) increases the aversive threshold. Additional results have shown that the 5-HT_{1A} agonists 8-OH-DPAT and ipsapirone have a similar effect. Therefore stimulation of either the 5-HT_{1A} or the 5-HT_{2A} receptor in the DPAG seems to decrease aversion generated in the DPAG. Since the obtained results have further shown that microinjection of a selective inhibitor of 5-HT reuptake into the DPAG not only potentiate 5-HT, but also have an anti-aversive effect of its own, the suggestion has been made that 5-HT nerve fibers in the DPAG are likely to physiologically regulate aversive behavior. Interestingly, since administration of 5-HT receptor antagonists alone into the DPAG has no effect on the aversive threshold, serotonergic fibers seem to exert a phasic inhibition on aversion, implying that the modulatory action of 5-HT would only appear under conditions, such as stressful situations, that engage 5-HT systems. For a comprehensive review of the evidence on 5-HT and DPAG-induced aversion, see [30].

Overall, the results reviewed so far indicate that 5-HT facilitates inhibitory avoidance by acting on forebrain struc-

tures, such as the septo-hippocampal system and the amygdala, whereas inhibiting escape in the DPAG. The amygdala and the PAG, plus the medial hypothalamus, make up a set of interrelated structures, often referred to as the brain defense system (BDS), which controls defensive strategies and elaborates the accompanying emotional and motivational states. Although the three components of the BDS would work together to generate different kinds of defensive behavior, each structure is likely to exert specific functions. In this regard, Fanselow [28] 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, whereas the PAG would be in charge of selecting, organizing and executing the appropriate behavioral and neurovegetative defensive reactions. On the basis of the systematic study of antipredator defense in wild rats, Caroline and Robert Blanchard [27] have formulated the concept of defense strategies. Three such strategies have been identified as a function of the threat being potential (uncertain), distal or proximal. Thus, when danger is uncertain, like in novel environments, rats perform cautious exploration aimed at risk-assessment; when the predator is perceived at safe distance, tense and attentive immobility (freezing) ensues, and when the predator is near or in actual contact with the rat, the animal rapidly flees, whenever possible or otherwise threatens back or even attacks the predator defensively. Comparative studies have led to the conclusion that these basic defense strategies can be found in virtually all animal species, including human beings [31].

Attempts to relate each defense strategy with critical brain structures and to particular emotions led to the suggestion that the prefrontal cortex (PFC), the septo-hippocampal system and the amygdala would be the key structures for risk-assessment behavior and inhibitory avoidance, the related emotion being anxiety. The PFC and the septo-hippocampal system would provide the cognitive component of anxiety while the affective component would be integrated in the amygdala. At the other extreme, 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. Concerning psychopathology, the first defense strategy has been related to GAD and the third with PD, the second being implicated in specific phobias (Table 1). For a review, see [32].

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 [33]. Most of the nerve fibers that originate in the DRN are thin and have small varicosities that make preferential contact with 5-HT₂ receptors [34]. Given this background, a working hypothesis has been conceived to reconcile the experimental evidence on the role of 5-HT in defense reviewed above (Graeff 1991). According to this view, activation of the DRN results in facilitation of defense strategies that are mainly integrated at the amygdala and other forebrain areas, such as risk assessment and inhibitory avoidance. At the same time, proximal defense reactions that are chiefly organized in the DPAG, such as escape and fight, are inhibited. The adaptive function of this disposition would be to inhibit

Table 1. Antipredator Defense, Related Emotions and Anxiety Disorders

Threat	Defense Reaction	Critical Brain Structure	Emotion	Disorder	Drug Treatment
Uncertain Conflict	Risk assessment Behavioral inhibition	PFC Septum-hippocampus Amygdala	Anxiety	GAD	Anxiolytics Antidepressants
Anticipated (CS)	Freezing (No exit)	Amygdala VPAG	Anxiety	Anticipatory anxiety	
	Avoidance	Amygdala	Conditioned fear	Specific phobias	None
Distal (US)	Escape	Medial hypothalamus	Unconditioned fear	Specific phobias	None
Proximal (US)	Flight or Immobility	DPAG	Panic	PD SAD PTSD	Antidepressants

CS=conditioned stimulus, US=unconditioned stimulus, PFC=prefrontal cortex, VPAG=ventral periaqueductal grey, DPAG=dorsal periaqueductal grey, GAD=generalized anxiety disorder, PD=panic disorder, SAD=social anxiety disorder, PTSD=post-traumatic stress disorder. Modified from [36]

extreme defense patterns, like flight or fight, in situations where predatory threat is uncertain or far from the prey. In these circumstances, such reactions 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. This theoretical construct, known as the dual role of 5-HT in defense hypothesis, has been extended to encompass pathological anxiety and depression [36].

EXPERIMENTAL TESTS OF THE DUAL ROLE OF 5-HT IN DEFENSE HYPOTHESIS: ANIMAL STUDIES

According to the clinical extension of the dual role of 5-HT in defense hypothesis [36], failure in 5-HT mechanisms controlling defensive behavior at the level of the DPAG would underlie the susceptibility to panic attacks. Therefore, antipanic drugs should compensate for this deficit. In the recent years, results obtained in rats, both in the above discussed DPAG electrical stimulation model of panic and in an animal model of anxiety and panic named the elevated T-maze (ETM), have given full support to this prediction.

The ETM consists of three arms of equal dimension elevated from the floor. One of these arms is enclosed by lateral walls and stands perpendicular to two opposite unprotected arms. One-way escape, which has been related to panic, is measured by recording the time taken by animals to withdraw from one of the open arms in three consecutive trials. Being on the open arms is an aversive experience. As a consequence, when the rat is placed at the end of this arm the animal quickly moves towards the closed arm, assumed as an escape response. The ETM also allows measurement of inhibitory avoidance which has been related in terms of psychopathology to GAD. In the test, inhibitory avoidance is inferred from the time taken for the rats to withdraw from the enclosed arm in three consecutive trials. When placed at the distal end of this arm for the first time, the rat can see the open arms only after its head reaches beyond the walls of the

closed arm. After experiencing the aversive stimuli offered by the open arms, when subsequently placed in the closed arm the animal will refrain from quickly leaving this arm. Inhibitory avoidance learning is indicated by the stepwise increase in withdrawal latency across trials [37-40]. By measuring both inhibitory avoidance and escape in the same animal, the ETM is allowing a more thorough investigation of the corollaries of the dual role of 5-HT in defense hypothesis

The pharmacological validation of this test has so far largely endorsed the view that escape relates to PD, and inhibitory avoidance to GAD. Accordingly, it has been shown that anxiolytic drugs such as diazepam, buspirone, ipsapirone and ritanserin impair inhibitory avoidance while leaving one-way escape unchanged. This selective effect on avoidance correlates with the clinical effectiveness of these drugs on GAD, as opposed to their inefficacy on PD. Tricyclic antidepressants usually aggravate anxiety earlier in treatment, the therapeutic effect appearing only following several days of repeated administration. This correlates with the anxiogenic-like effect of clomipramine and imipramine on inhibitory avoidance following single injection. More important, an anxiolytic effect of imipramine on avoidance developed after chronic administration, and escape from the open arm was simultaneously impaired, which correlates well with the clinical effectiveness of this drug regime on both GAD and PD. Other antidepressants have also been tested and the results showed that chronic administration of the SSRIs fluoxetine, escitalopram and sertraline inhibited escape expression, in agreement with their clinical panicolytic effect. The effect on inhibitory avoidance was dependent on the drug used: while fluoxetine and sertraline were ineffective, escitalopram caused anxiolysis both after acute and chronic treatment. In contrast to the effects of these compounds, acute injection of cholecystokinin agonists, which induce panic attacks in humans, facilitated escape expression, suggesting a panicogenic-like effect (for reviews and original references, see [39-42]).

A series of studies has shown that the intra-DPAG injection of 5-HT-acting drugs causes the same effect on ETM escape as that observed in the DPAG electrical/chemical stimulation model of panic. Thus, local infusion of the endogenous agonist 5-HT, the 5-HT_{1A} receptor agonist 8-OH-DPAT or the preferential 5-HT_{2A} agonist DOI consistently impaired escape in both tests. These agonists differentially affected the acquisition of inhibitory avoidance. Whereas 5-HT facilitated this response, 8-OH-DPAT impaired it and DOI was ineffective. Intra-dorsal PAG administration of the 5-HT_{1A} receptor antagonist WAY-100635 and of the 5-HT_{2A/2C} antagonists ketanserin or SDZ SER082 was ineffective in this test, corroborating previous evidence showing that in this brain region 5-HT exerts a phasic, rather than tonic, regulatory influence on defensive behaviors [43-45].

In support to the proposal that antidepressants cause a panicolytic effect by facilitating 5-HT-mediated neurotransmission at the level of the DPAG, it has been shown that repeated treatment with imipramine, sertraline or fluoxetine enhanced the inhibitory effect of intra-DPAG injection of 5-HT, 8-OH-DPAT or DOI on escape responses, either induced by local electrical/chemical stimulation or measured in the ETM [46-50]. This enhanced responsiveness of 5-HT_{1A} and 5-HT_{2A} receptors in the DPAG was not observed after short-term administration of these antidepressants, keeping correspondence to what is observed in clinics. The same facilitatory effect on 5-HT neurotransmission is also reported after long- but not short-term treatment with alprazolam, a highly potent benzodiazepine receptor agonist that is successfully used in the treatment of PD. Curiously, chronic treatment with alprazolam facilitates 5-HT_{1A}- or 5-HT_{2A}-mediated neurotransmission in the DPAG, without affecting the responsiveness of benzodiazepines receptors in the same brain area [51]. Differently from SSRIs, buspirone failed to enhance the inhibitory effects of 5-HT_{1A} or 5-HT₂ agonists on escape responses mediated by the DPAG [48, 49], what correlates with the lack of therapeutic efficacy of the same drug in PD.

More recently obtained evidence indicates that fluoxetine, besides enhancing the reactivity of 5-HT_{1A} and 5-HT_{2A} receptors in the DPAG, may also affect 5-HT efflux in the same brain area, with both processes having implications for its panicolytic effect. More appropriately, Zanoveli and colleagues [52] showed that chronic (21 days), but not acute, administration of this SSRI, at a dose that increases 5-HT receptor responsiveness in the DPAG, also increases 5-HT release in the DPAG, as measured by *in vivo* microdialysis. Long-term treatment with the anxiolytic buspirone was ineffective.

As mentioned earlier, the effect of repeated SSRI administration on 5-HT efflux in post-synaptic areas has been associated with the desensitization of presynaptic 5-HT_{1A} autoreceptors, which control by negative feedback the activity of serotonergic neurons [53-55]. As the DRN is the main source of 5-HT innervation to the DPAG [56], the rise in 5-HT levels caused by fluoxetine is assumed to reflect the blunted autoreceptor control of 5-HT neurons located at this particular raphe nucleus. Consistent with this notion is the observation that stimulation of 5-HT neurons within the DRN en-

hances 5-HT levels in the DPAG, also measured by *in vivo* microdialysis [57].

Not less interesting, Zanoveli *et al.* [52] have also shown that 21-day treatment with the same dose of fluoxetine that raises 5-HT concentration in the DPAG impairs escape performance in the ETM, in keeping with previous studies [50-58]. Intra-DPAG administration of the 5-HT_{1A} receptor antagonist WAY-10635 counteracted the anti-escape effect caused by chronic fluoxetine administration. Thus, by blocking 5-HT_{1A}-mediated neurotransmission at the level of the DPAG, the panicolytic-like effect of this SSRI, administered systemically, is cancelled. This finding is in line with the observation that intra-DPAG infusion of WAY-100635 also antagonizes the anti-escape effect generated by chemical (e.g. kanic acid) stimulation of the DRN [59], which by itself, as mentioned earlier, enhances 5-HT levels in the DPAG [57].

Therefore, by two different mechanisms, i.e., increase in local 5-HT levels and in responsiveness of post-synaptic receptors, repeated treatment with fluoxetine can cause a net enhancement of 5-HT_{1A}-mediated neurotransmission in the DPAG. A similar dual action of fluoxetine has been reported in the hippocampus and has been implicated in the antidepressant effect of this SSRI [53-60]. We are now investigating to which extend each of these two complementary mechanisms of action accounts for the panicolytic effect of other types and classes of antidepressants.

Studies in the ETM have also been employed to unveil the mechanisms through which antidepressants can affect anxiety; worsening it at the beginning of the treatment, but with beneficial therapeutic effects after continued administration. As mentioned before, the dual 5-HT defense hypothesis assumes that an enhancement of 5-HT-mediated neurotransmission in forebrain structures, such as the amygdala, septo-hippocampal system and prefrontal cortex, increases anxiety. In line with this assumption, Millan [61] has suggested that antidepressants would cause anxiolytic effects by down-regulating 5-HT_{2C} receptors and by enhancing effects mediated by post-synaptic 5-HT_{1A} receptors in these structures. Accordingly, studies in the ETM have shown that in three of these structures, namely amygdala, septum and hippocampus, 5-HT facilitates the acquisition of inhibitory avoidance, this effect being achieved through different subtypes of receptors, depending on the analyzed area. Thus, 8-OH-DPAT injection either into the lateral septum or the dorsal hippocampus has an anxiogenic effect upon inhibitory avoidance, without affecting escape performance. In the septum, the effect of 8-OH-DPAT on anxiety was opposed to that caused by the benzodiazepine midazolam. Microinjection of WAY-100635 in both areas had no effect on the two ETM defensive tasks [62, 63]. In contrast, in the basolateral nucleus of the amygdala (BLA) stimulation of 5-HT_{1A} and 5-HT_{2A} receptors by 8-OH-DPAT and DOI impaired, rather than facilitated, inhibitory avoidance acquisition, indicating an anxiolytic effect. Also different from the effect observed in the septo-hippocampal system, these compounds inhibited escape behavior (Christiana Villela de Andrade Strauss and Hélio Zangrossi Jr., unpublished results). However, in agreement with the dual 5-HT hypothesis, local injection of 5-HT or the 5-HT_{2C} receptor agonist MK212

caused an anxiogenic effect upon inhibitory avoidance, without changing escape performance. On the other hand, microinjection of the 5-HT_{2C} receptor antagonist SB242084 had the opposite effect on inhibitory avoidance, also without interfering with escape (Maria Adrielle Vicente and Hélio Zangrossi Jr, unpublished results).

Altogether, the results obtained in the BLA indicate that 5-HT plays a tonic and selective role in the mediation of inhibitory avoidance through the activation of 5-HT_{2C} receptors. Considering that this subnucleus has been proposed to assign emotional salience to both rewarding and aversive stimuli, the contradictory findings found after local injection of 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C}-related compounds may involve a complex interference on both approach and avoidance behavior [23]. Recently obtained evidence (Maria Adrielle Vicente and Hélio Zangrossi Jr, unpublished results) has shown that chronic, systemic administration of imipramine counteracts the anxiogenic effect on inhibitory avoidance caused by intra-BLA injection of MK212. This is indicative that repeated administration of antidepressants down regulates 5-HT_{2C} receptors in the BLA, opening the way to the emergence of an anxiolytic effect, as proposed by Millan [61]. It remains to be explored whether the same treatment, in addition, enhances 5-HT_{1A} receptor-mediated anxiolysis in this nucleus. This seems to be the case in the DPAG where, as mentioned before, activation of 5-HT_{1A} receptors by 8-OH-DAT causes anxiolysis, and chronic administration of sertraline, fluoxetine and, to a lesser extent, imipramine potentiates this effect [49, 50].

The above results obtained in the ETM show that some interventions in the DPAG not only affect escape (panic), but also inhibitory avoidance (anxiety) and, conversely, manipulations in the amygdala can change escape, in addition to inhibitory avoidance. Therefore, they challenge the strict rostro-caudal hierarchy suggested by the dual 5-HT hypothesis, which ascribes the control of anxiety to the forebrain and that of panic to the midbrain. Nevertheless, these findings can be accounted for by the theoretical model proposed, more recently, by McNaughton and Corr [64], in which defensive responses are organized in two parallel, longitudinally organized systems, both extending from the forebrain to the midbrain. One of these, the “approach defense system” would deal with anxiety and be engaged mainly by approach-avoidance conflict. Its main neural representation involves the septum-hippocampus and other forebrain structures. The other, named the “avoidance defense system”, on the other hand, would be activated when there is no tendency to approach the source of danger. In this case, the experienced emotion is fear, not anxiety; fear or panic would be elicited depending on the defensive distance. Distal threat would activate forebrain structures and elicit fear while proximal threat would activate the periaqueductal gray and elicit panic. This proposal has recently received strong support from a study conducted by Mobbs and coworkers [65] in healthy volunteers using functional magnetic resonance imaging (fMRI), the results of which have shown that brain activity shifts from the PFC to the midbrain periaqueductal gray as a virtual predator capable of inflicting pain grows nearer to the virtual prey. In addition, the activity of the periaqueductal gray correlated with reported subjective degree of dread and decreased confidence of escape. A sche-

matic representation of the McNaughton and Corr’s model is shown in Fig. (1).

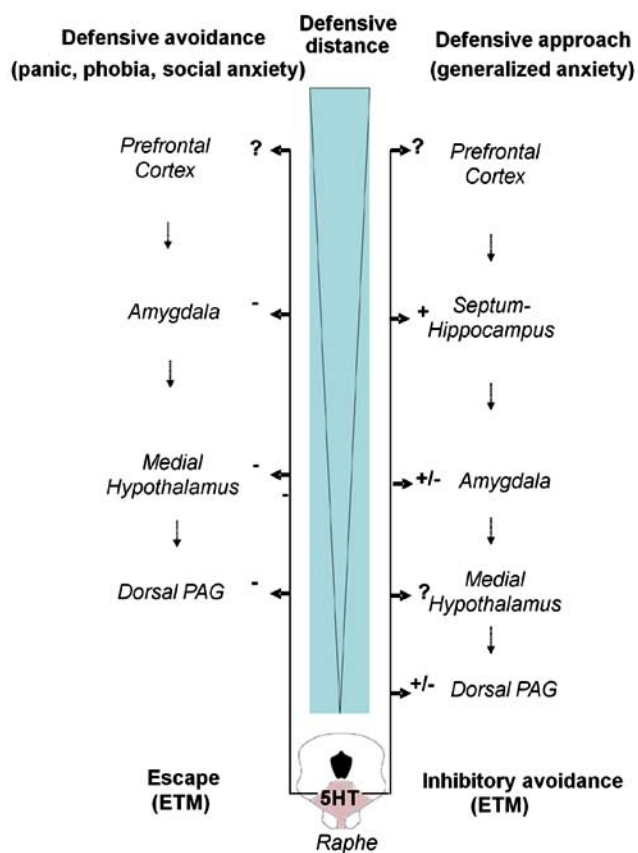


Fig. (1). McNaughton and Corr’s [64] theoretical model. The approach defense system would be engaged mainly by approach-avoidance conflict. Its main neural representation would involve forebrain structures. The avoidance defense system, on the other hand, would be activated when there is no tendency to approach the source of danger. In this case, the experienced emotion is fear or panic, depending on the defensive distance. Distal threat would activate forebrain structures and elicit fear, while proximal threat would activate the dorsal periaqueductal gray (PAG) and elicit panic. The figure also illustrates the results obtained after intracerebral injections of 5-HT agonists into the dorsal PAG, medial hypothalamus, septum-hippocampus, amygdala or medial prefrontal cortex on inhibitory avoidance and escape latencies of rats submitted to the elevated T-maze (ETM). The question mark (?) indicates areas where the effects of these drugs have not yet been tested. Modified from [7].

EXPERIMENTAL TESTS OF THE DUAL ROLE OF 5-HT IN DEFENSE HYPOTHESIS: HUMAN STUDIES

Two experimental procedures that generate anxiety in humans have been used to test this hypothesis: the simulated public speaking (SPS) and the conditioned skin conductance response (CSCR) tests. The SPS test consists in the preparation and performance of a speech in front of a videocamera. During the procedures the participant can see his/her own image on a TV screen. Anxiety and other subjective measures, as well as physiological measurements are taken before, during and after the speech. Since fear of speaking is highly

prevalent and generates anxiety in healthy persons irrespective of trait anxiety level, it is supposed to be a species-specific fear. Pharmacological studies have shown that benzodiazepine anxiolytics decrease anxiety, measured by the anxiety factor of the Visual Analog Mood Scale (VAMS), before and after the speech, but do not attenuate the increase of VAMS anxiety during speech preparation or performance (speaking fear). In contrast, and in agreement with the predictions of the dual 5-HT hypothesis, drugs that facilitate 5-HT-mediated neurotransmission decrease, whereas drugs that impair 5-HT function increase speaking fear. The CSCR test consists in the presentation through ear phones of 10 neutral tones (habituation phase), followed by a neutral tone paired with a loud white noise (acquisition phase) and by the presentation of the same 10 tones, which after the pairing became conditioned aversive stimuli – CS (extinction phase). During the procedures, measures of skin conductance (level, SCL; response, SCR and spontaneous fluctuations, SF) are taken. Anxiolytic drugs, such as diazepam and the 5-HT_{1A} partial agonist buspirone, impaired conditioning, while anxiogenic drugs, such as the 5-HT agonist methyl-chlorphenylpiperidine (mCPP) and the 5-HT releaser d-fenfluramine tend to facilitate conditioning (For a review, see [66]).

The pharmacological profile of the above experimental human tests keeps some similarities with the pharmacological response observed in the clinical practice of patients with the diagnoses of anxiety disorders. Thus, both diazepam and buspirone, which are effective in the treatment of GAD [67], but not of PD [68], had an anxiolytic effect in the CSCR, without decreasing speaking fear in the SPS test. Some antidepressants have also been tested in these models of human experimental anxiety. The acute administration of clomipramine [69] and nefazodone [70] increased speaking fear in the SPS test, and this effect has been related to the worsening of anxiety symptoms frequently observed at the beginning of the treatment with antidepressants. It has been proposed that the fear-enhancing effect of a single dose of antidepressants in SPS is due to a lack of 5-HT inhibition of brain systems that generate panic attacks, likely to be localized in the DPAG. Microdialysis studies in animals have shown a greater increase of extracellular 5-HT in the raphe nuclei than in the neocortex [71]. Therefore, it is possible that acute doses of antidepressants preferentially increase 5-HT concentration near the cell bodies of serotonergic neurons. This decreases their firing rate due to the activation of somatodendritic 5-HT_{1A} autoreceptors, reducing 5-HT release and lowering the postsynaptic concentration of 5-HT. Taken together, the pharmacological data obtained so far with these human experimental tests are compatible with the hypothesis that 5-HT enhances anxiety, evaluated by the CSCR test, whereas it inhibits fear, accessed by the SPS test.

From the available pharmacological evidence, it is expected that panic patients would perform in SPS differently from healthy volunteers, but not in CSCR, since the former test would engage the neural substrates that would be implicated in the panic attack, but the latter would not. It is important to highlight that the SPS is not viewed as a model of panic attack or a challenge aimed at provoking panic attacks in susceptible individuals. The rationale of the relationship between the experimental model and the anxiety disorder is that the public speaking is believed to engage the brain

mechanisms involved in proximal defense, which would be impaired in PD. As predicted, the response of panic patients to the CSCR test has been shown to be similar to that of controls, except for the occurrence of more spontaneous fluctuations of skin conductance along the experimental session in panic patients, a finding that is consistent with their high baseline level of subjective anxiety. In the SPS test, panic patients show higher levels of anxiety than normal controls along the whole session, but are less responsive to the speaking challenge [72-74]. The profile of panic patients during the SPS test resembles the effect of the nonselective 5-HT-receptor blocker metergoline in healthy volunteers, which increases anxiety before and after the speech, but not during the speech preparation or performance [75]. These results are in agreement with the suggestion that 5-HT deficiency, and therefore less inhibition of the DPAG, may be present in PD. Interestingly, the above SPS task does not increase cortisol secretion [73]. Accordingly, neither panic attacks [76] nor the electrical stimulation of the DPAG of the rat [77] seems to activate the hypothalamic-pituitary-adrenal axis.

NEUROIMAGING DATA

In the last two decades there has been a growing use of non-invasive imaging studies to investigate the neurobiology of psychiatric disorders and the mode of action of psychotherapeutic drugs. As already exemplified by the above mentioned experiment by Mobbs and coworkers [65], brain neuroimaging is giving an important contribution to clarify the neural substrate of defense-related emotions by showing that anxiety involves the activation of forebrain structures, whereas panic engages the midbrain PAG. In the same vein, Rachel Berkowitz and coworkers [78] have undertaken a comprehensive review of neuroimaging studies and concluded that anxiety disorders characterized by intense fear and panic, such as PD, SAD and PTSD, show PFC underactivity, with consequent disinhibiting of the amygdala and insula. In contrast, conditions such as GAD and OCD, which involve worry and rumination, are characterized by PFC overactivity. In addition, the results of a study carried out by Sakai and coworkers [79], aimed at assessing the effect of cognitive behavior therapy (CBT) in PD, using positron emission tomography (PET) have shown positive correlation between decreased activity in the left medial PFC and clinical improvement of anxiety and agoraphobia, as well as between decreased activity in the midbrain (possibly the DPAG) and reduction in the number of panic attacks during the four weeks separating the first scan, made before treatment and the second, made after CBT. This study also exemplifies an important contribution of neuroimaging, which is the demonstration that psychotherapy can change brain functioning in the same way as medication.

In regard to 5-HT, functional studies using PET, fMRI and single-photon emission computed tomography (SPECT), have detected several changes in 5-HT-mediated neurotransmission. For instance, individuals with one or two copies of the short (SLC6A4) allele of the serotonin transporter (5-HTT) promoter polymorphism show greater neuronal activity in response to fearful stimuli compared with individuals homozygous for the long allele. This makes sense, since the same polymorphism has been associated with reduced 5-HTT expression and function, and with increased

anxiety-related behavior [80]. Since the finding has been confirmed using a large sample of healthy volunteers, it has been suggested that this polymorphism is related to vulnerability for affective and anxiety disorders by biasing the reactivity of the amygdala to stressful life experiences [81].

This bias towards the detection of aversive stimuli is believed to be a crucial vulnerability factor for anxiety disorders. In this regard, it has been reported that SAD patients with one or two copies of the short allele in the promoter region of the human serotonin transporter (5-HTT) gene have increased levels of anxiety-related traits, state anxiety, and enhanced right amygdala responding to anxiety provocation [82]. Successful treatment with citalopram has reduced the activity of the anterior and lateral part of the left temporal cortex; the anterior, lateral and posterior part of the left mid frontal cortex; and the left cingulum [83]. The same drug treatment had also been reported to decrease the regional cerebral blood flow response to public speaking, bilaterally in the amygdala, hippocampus, and in the periamygdaloid, rhinal, and parahippocampal cortices [82]. Increased cortical activity and decreased basal ganglia activity in patients with GAD have also been shown by functional imaging data [84], and chronic treatment with citalopram that decreased self-reported anxiety has been reported to reduce responses elicited by worry sentences in prefrontal regions, striatum, insula and paralimbic regions [85]. In synthesis, these and other reported studies have indicated brain areas with a characteristic pattern of reduced or increased activity in distinct anxiety disorders. Since these changes are usually corrected by successful treatment with SSRI, they may be relevant for the therapeutic action of these compounds. This hypothesis is supported by reported results obtained in healthy volunteers, as for instance in those showing that escitalopram significantly decreased activation in bilateral posterior and middle insula during the anticipation condition irrespective of stimulus valence, and in medial prefrontal and ACC during anticipation of aversive vs. pleasant images [86]. Thus, reduced insular and ACC activation in healthy controls during anticipation of aversion may be integral to the therapeutic efficacy of SSRIs.

Another line of evidence showing the action of antidepressants on threat detection comes from studies on the perception of emotional expression in human faces, since the ability to identify facial expressions of emotion is important for social functioning and adaptation. Neuroimaging studies have provided substantial data about the neural substrate of emotional face recognition. One of the most consistent findings is the amygdala being activated by the perception of fearful faces, although it can also be activated by other kinds of facial expressions, such as disgust, sadness, happiness and anger. However, activation of the amygdala in response to fearful faces is greater than in response to angry faces [87].

To understand the functional meaning of the brain activation patterns depicted by neuroimaging evidence, it would be necessary to know the adaptive meaning of each facial expression. Considering the processing of anxiety and related emotions, the facial expressions of fear and anger are of particular interest. A plausible assumption is that faces expressing fear signal potential threat in the environment; on the other hand, an angry face may represent proximal threat. If

these assumptions are true, the hypothesis of a dual role of 5-HT in defense allows the following predictions: 1) increased 5-HT function should facilitate the identification of fearful faces, whereas reduced 5-HT function should impair the same process; 2) increased 5-HT should impair, whereas lack of 5-HT should enhance, the identification of angry faces. To test these predictions, a recent review of the literature about the effects of 5-HT-acting drugs on the perception of fearful and angry faces was carried out by Del Ben and coworkers [87]. Although the hypothesis that angry faces would be processed as a proximal threat was not supported by the reviewed results, most of the studies have shown that increased 5-HT neurotransmission facilitates the recognition of fearful faces, whereas its decrease impairs the same performance. These results agree with the hypothesis that fearful faces are processed as potential threats and that 5-HT enhances this brain processing. Consistent with this hypothesis, it has been reported that daily administration of citalopram for seven days reduces threat-relevant processing – that is opposite to the effects of acute administration. In particular, seven days of citalopram reduced the perception of fearful, angry and disgusted facial expressions [88]. These results support the aforementioned argument that early increases in fear processing with acute administration of antidepressants may reflect initial anxiogenic actions of these agents, which reverse with repeated treatment. Coherent with the behavioral results, imaging studies with fMRI have shown that antidepressant treatment for seven days attenuates the activation of the amygdala to facial expressions of fear [89]. In these studies, the effect of antidepressants on facial emotion recognition is unaccompanied by conscious emotional changes, measured by rating scales of subjective state. This lack of drug effect on subjective states suggests that the serotonergic modulation of facial expression processing can occur independently of changes in the conscious recognition of the feelings and mood states aroused by emotional faces, suggesting a specific effect on preconscious cognitive-affective processing, which may relate to later clinical improvement.

HOW DO ANTIDEPRESSANTS IMPROVE GENERALIZED AND PANIC DISORDERS?

Although the precise role of 5-HT in anxiety is not yet completely understood, the reviewed evidence clearly shows that this neurotransmitter: 1) modulates normal defensive responses; 2) is altered in anxiety disorders; and 3) is involved in the therapeutic effects of anxiolytic drugs, particularly the SSRIs. It also indicates that the modulatory action of 5-HT on anxiety-related behavior depends on several factors, including the nature of the threatening stimulus, the defensive strategies available and the brain structures engaged. There is good evidence that 5-HT inhibits a defense system engaged by proximal threat that is akin to fear/panic symptoms in humans. In this sense, facilitation of 5-HT-mediated neurotransmission in brain structures related to this system, such as the DPAG, could be the mechanism of the therapeutic effects of SSRIs in PD. In this way, a recently reported study has investigated if 5-HT neurons in the DRN would be activated in rats submitted to a model of PD [90]. The obtained results have shown that rats submitted to chronic inhibition of GABA synthesis in the dorsal hypothalamus present defense responses when injected with so-

dium lactate, a well-known panicogenic agent in PD patients. These animals failed to activate a subset of serotonergic neurons in the DRN that project to the DPAG, an effect induced by lactate in control animals that do not display defensive responses. Therefore, it may be suggested that chronic administration of antidepressant agents, by increasing the sensitivity of 5-HT_{1A} and 5-HT_{2A} receptors and by increasing release of 5-HT in the DPAG (see above section on animal studies), corrects the deficient inhibitory processes that allow panic attacks to occur in PD patients.

The role of 5-HT in anticipatory anxiety and GAD, however, is not as clear. Although several studies have been compatible with the proposal that 5-HT facilitates anxiety while inhibiting panic, these effects may involve interference in longitudinally organized systems [64] instead of the rostral-caudal hierarchy originally proposed by Deakin and Graeff [36]. Even so, some contradictory data still persist. Despite several pieces of evidence suggesting that facilitation of 5-HT_{2C}-mediated neurotransmission in forebrain areas facilitates anxiety, the results regarding the involvement of forebrain 5-HT_{1A} receptors are much less clear. In any case, neuroimaging data suggest that antidepressants act on forebrain structures, such as medial prefrontal cortex, insula and amygdala, decreasing their activation by stimuli or contexts that signal danger. This may correct the bias toward enhanced recognition of warning signals that is characteristic of anxious patients.

Hopefully, future studies combining the traditional approaches (behavioral, pharmacological, anatomical and electrophysiological) with the new molecular genetic and functional neuroimaging techniques will help us to further unveil the complex role of serotonin in anxiety and allow a better understanding of how antidepressant medication acts to improve anxiety disorders.

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