PSYCHOLOGY & NEUROSCIENCE

Defense-related emotions in humans

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Abstract

The study of the role of serotonin in anxiety has led to the view that this neurotransmitter enhances anxiety, but inhibits panic. Validation of this hypothesis has been made using two experimental procedures that increase anxiety in human volunteers. One is classical conditioning of the skin electrical conductance response, which is assumed to represent anxiety. The other is simulated public speaking, which is believed to mobilize the same neural networks that are operative in panic and social anxiety disorders. In general, the results of these studies have fulfilled the predictions derived from the above hypothesis. The same procedures have been applied to panic disorder patients, and the obtained results have shown that these patients had a blunted anxiety response to public speaking. This speaking stress also did not activate the hypothalamic-pituitary-adrenal axis, which, in contrast, was activated by anticipatory anxiety. It may be concluded that anxiety and panic are qualitatively different emotional states, respectively related to the animal defense reactions to potential and proximal threat. In agreement, reported results of recent neuroimaging studies have shown that anxiety activates prefrontal cortical areas, whereas panic activates midbrain regions, particularly the periaqueductal gray matter. As a general conclusion, it may be said that anxiety, fear and panic do not belong to the same continuum of increasing intensity; instead, they are qualitatively different emotional states. **Keywords:** anxiety, panic, stress, serotonin, human experimental anxiety, brain neuroimaging.

Introduction

The involvement of our research group with the defense-related emotions – anxiety, fear and panic – started with the study of the role played by the neurotransmitter serotonin or 5-hydroxytryptamine (5-HT) in anxiety. Summarizing the reported evidence obtained with animal models of anxiety available in the late 1980s, it may be said that the reported results with conflict models, such as punished lever pressing maintained by water or food presentation in rats or key pecking maintained by food presentation in pigeons, indicated that 5-HT enhances anxiety. In contrast, results obtained with escape from aversive brain stimulation suggested that 5-HT inhibits anxiety (for a review see, e.g., Graeff, 2002). To overcome this seeming paradox, the hypothesis that 5-HT would play a dual role in defense has been formulated (Graeff, 1991). According to this view, 5-HT would facilitate inhibitory avoidance by acting on the amygdala, whereas it would inhibit escape by acting on the dorsal periaqueductal gray matter (dPAG) of the midbrain. This arrangement would have adaptive value because it allows the threatened animal to withhold active defensive responses in situations of either potential or distant threat, when such behavior would make the animal more conspicuous to the predator. As a consequence, more suitable responses such as cautious investigation (risk-assessment) or tense immobility (freezing), would be favored (see Blanchard & Blanchard 1988 for a discussion on defensive strategies).

Inconsistencies about the role of 5-HT in anxiety disorders have also been reported. For instance, a controlled clinical assay conducted in patients with a diagnosis of generalized anxiety disorder (GAD) showed that the 5-HT_2 (mainly 2A/2C) receptor antagonist ritanserin is as effective as the benzodiapine anxiolytic lorazepam in reducing clinical symptoms after 2 weeks of drug treatment (Ceulemans, Hoppenbrouwers, Gelders, & Reyntjens, 1985). In contrast, other clinical studies have shown that ritanserin either did not improve or even worsened the symptomatology of patients with panic disorder (PD) diagnosis (Deakin, Wang, & Guimarães, 1990; Den Boer & Westenberg, 1990). For this reason, Deakin and Graeff (1991) extended the above hypothesis to clinical conditions. Briefly, they proposed that the 5-HT pathway that originates in the median raphe nucleus (MRN) and projects to the hippocampus would be involved in depression; this piece of the theoretical model will not be analyzed in the present article. In addition, two 5-HT pathways arising in the dorsal raphe nucleus (DRN) are suggested to be involved in anxiety disorders: one, innervating prosencephalic structures such

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Table 1. Antipredator defense reactions, 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		
Proximal (US)	Flight or Immobility	DPAG	Panic	PD SAD PTSD	Antidepressants

CS: conditioned stimulus, US: unconditioned stimulus, PFC: prefrontal cortex, VAPG: ventral periaqueductal gray, DPAG: dorsal periaqueductal gray, GAD: generalized anxiety disorder, PD: panic disorder, SAD: social anxiety disorder, PTSD: post-traumatic stress disorder. Modified from Deakin and Graeff (1991).

as the amygdala and the prefrontal cortex would enhance anxiety, whereas the other, innervating the dPAG, would inhibit panic. A further suggestion was that fear would be integrated in the hypothalamus and the amygdala, fear pathology being represented by the specific phobias. The overall picture is shown in Table 1.

Experimental tests in healthy volunteers

Most of the experimental tests of the 'dual role of serotonin in defense hypothesis' (DRSDH) have been carried out in an animal model of anxiety specially designed for such purpose and referred to as the elevated T-maze (ETM). This apparatus is composed of one arm enclosed by walls except at is end that is perpendicular to two open arms of equal dimensions, all being elevated from the floor. In the ETM, the same rat is trained to perform inhibitory avoidance and escape from the elevated and unprotected arms of the maze, which are naturally aversive to the rat. The former response is anxiety, whereas the second response is assumed to represent panic. Because the present article is focused on human data, the ETM results are not going to be discussed here. Suffice it to say that nearly all the tests reported so far have fulfilled the predictions derived from the DRSDH (for reviews, see Graeff, 2002, 2004).

Although far more complex than animal experiments, some critical tests of the DRSDH have been conducted in human volunteers and anxiety disorder patients, as described in the following. In these studies, two experimental procedures have been used: conditioning of the skin conductance response (CSCR) and simulated public speaking (SPS), which have been assumed to represent anxiety and panic, respectively.

In the CSCR test, participants sitting inside a quiet room have earphones for sound presentation and electrical wires attached to the phalanxes of two adjacent fingers for skin electrical conductance recording. During an experimental session, a sequence of ten tones of 80 dB is presented along the first, habituation phase. As expected, the intensity of the SCR starts at a high level and rapidly declines. The 11th tone is immediately followed by a 100 dB noise that startles the participant, in a classical associative conditioning paradigm. The noise (unconditioned stimulus – US) elicits a marked SCR. Following the stimulus pairing, the tone – now an aversive conditioned stimulus (CS) – is presented ten times unaccompanied by any further presentation of the noise, in an extinction procedure. As a result, the SCR is reinstated at a high intensity after the 12th tone (first CS) and progressively declines as in the habituation phase, although at a slower pace. Typically, anxiolytic drugs such as the benzodiazepine diazepam facilitate extinction, making the CSCR test representative of GAD or anticipatory anxiety (for a review, see Graeff, Parente, Del-Ben, & Guimarães, 2003).

In the SPS test, the participant sits in front of a videocamera and sees his/her own image on a television screen. After habituation, recorded instructions about preparing and then performing a speech about a given topic are presented through the video. In an experimental session, psychological and physiological measures are taken at the following phases: B - Beginning: 10 min after arriving to the laboratory and before drug intake. P - Pre-stress: just before instructions. A - Preparatory: preparing the speech. S - Speaking: during speech. F - Final: post-stress measure. Only the psychological measure related to anxiety, consisting of the anxiety factor of the Visual Analog Mood Scale – VAMS (Norris, 1971), will be discussed here. In healthy volunteers under placebo, this index starts at a high level at B because the participant is in a novel and potentially harmful situation, declines to a basal level after habituation (P), then rises during speech preparation (A) and performance (S), and finally lowers down to nearly the basal level at the final phase (F). Pharmacological results have shown that anxiolytic drugs enhance habituation to the laboratory environment, but fail to affect the rise of the anxiety index determined by speech preparation and performance. Conversely, drugs that alter 5-HT function affect the response to the speaking stress in both directions. Because fear of speaking in public occurs in every individual, independent of the trait anxiety level, it may be considered a species-specific innate kind of fear. This type of fear is supposed to mobilize the same brain mechanism that participates in social anxiety disorder (SAD), of which fear of speaking in public is the main symptom, PD and, possibly, post-traumatic stress disorder (PTSD). For a review of the evidence on the SPS test see Graeff et al. (2003).

In order to test the DRSDH in healthy volunteers, predictions have been made assuming that the CSCR test represents anxiety, whereas the SPS test addresses panic. More specifically, drugs that decrease 5-HT action on the amygdala are expected to impair aversive conditioning in the CSCR test; the same action on the dPAG would enhance speaking fear in the SPS test. Contrariwise, drugs that increase 5-HT action in the amygdala would enhance conditioned anxiety, whereas the same action in the dPAG would reduce speaking fear.

For decreasing 5-HT activity, the above-mentioned 5-HT2 antagonist ritanserin has been used. As expected, ritanserin accelerated extinction in the CSCR test without affecting habituation, thus having a selective anxiolytic effect (Hensman, Guimarães, Wang, & Deakin, 1991). Also as expected, the same drug prolonged speaking fear in the SPS test, indicating a panicogenic action (Guimarães, Mbaya, & Deakin, 1997). This evidence agrees with the results of the above-mentioned clinical assays showing that ritanserin improves GAD (Ceulemans et al., 1985), but not PD (Deakin, Wang, & Guimarães, 1990; Den Boer & Westenberg, 1990).

Another way of lessening 5-HT activity is to administer a single dose of antidepressant drugs. Although these drugs are believed to improve anxiety disorders and depression by enhancing 5-HT neurotransmission, the therapeutic effect occurs only after several weeks of repeated administration. The accepted explanation for this delay is that when given acutely these drugs overstimulate autosomic $5-HT_{1A}$ receptors situated in the cell bodies and dendrites of 5-HT neurons; their stimulation reduces cell firing and, consequently, decrease 5-HT release in innervated areas. Only after these receptors become desensitized, what occurs within approximately 3 weeks of repeated drug administration is that 5-HT concentration in the synaptic cleft is increased (Blier & de Montigny, 1998).

Three antidepressants have been used to validate the DRSDH in volunteers undergoing the SPS test. Two of these, chlorimipramine (Guimarães, Zuardi, & Graeff, 1987) and nefazodone (Silva, Hetem, Guimarães, & Graeff, 2001), increased the magnitude of the anxiety index rise determined by speaking; the other, escitalopram, prolonged the same response (Garcia-Leal, Del-Ben, Leal, Graeff, & Guimarães, 2010), as described above after ritanserin (Guimarães et al., 1997). Nefazodone has also been studied in the CSCR test and, as expected, tended to facilitate extinction and decrease reported anxiety (Silva et al., 2001).

To enhance 5-HT activity, D-fenfluramine has been used. This drug has been shown to selectively release 5-HT from the thin 5-HT fibers that come from the DRN, being thus particularly suited for testing the DRSDH. Although D-fenfluramine was later withdrawn from clinical use because of untoward collateral effects, at the time of the reported study it was clinically used as an appetite suppressant. As expected, D-fenfluramine has markedly reduced the amplitude of the anxiety rise determined by speech preparation and performance in the SPS test, whereas it tended to impair extinction in the CSCR test (Hetem, de Souza, Guimarães, Zuardi, & Graeff, 1996).

In conclusion, the results so far obtained in healthy volunteers fulfill the predictions derived from the DRSDH, suggesting that 5-HT enhances anxiety while inhibiting panic.

Studies in patients with anxiety disorders

If the SPS procedure really probes brain mechanisms that are operant in PD and in SAD, the performance of patients with such diagnoses is expected to be different from that of healthy controls. In contrast, their performance in the CSCR test, supposedly related to GAD and anticipatory anxiety, is expected to be similar to that of healthy controls. One study by Del-Ben et al. (2001) was aimed at testing these predictions in panic patients. The obtained results showed that panic patients did not

differ from controls regarding CSCR, except that they tended to be more responsive to the unconditioned aversive stimulus. In the SPS test, however, panic patients had a higher level of anxiety than controls at the beginning of the experimental session and before the speech (phases B and A) and, chiefly, did not show any increase of the VAMS anxiety index during speech preparation and performance (phases A and S). Because they already had started at a high level of anxiety, this lack of response could be due to either decreased reactivity or, simply, a ceiling effect. Further results obtained in SAD patients tend to exclude the latter alternative. Although these patients also show a high level of initial anxiety, their response to the speaking challenge is far higher than that of healthy controls (Bergamaschi et al., 2011).

In the same study (Del-Ben et al., 2001), it has been pointed out that the performance of panic patients in the simulation of public speaking test resembles the early reported effect of metergoline in healthy volunteers (Graeff et al., 1985). since in both cases the anxiety index was significantly higher than control at pre-stress (P) and post-stress (F) and similar during speech preparation (A) and performance (S). Therefore, panic patients may have impaired 5-HT inhibition of the brain mechanisms that trigger panic attacks. This suggestion is supported by recent preclinical evidence obtained by Shekhar and co-workers (Johnson, Lowry, Truitt, & Shekhar, 2008). Their results have shown that rats made susceptible to lactate – a chemical known to induce panic attacks in panic patients but not in healthy controls – through chronic inhibition of GABA synthesis in the dorsomedial hypothalamus fail to activate the mesolimbic 5-HT system earlier described by Lowry and coworkers (2005). This system originates from a group of 5-HT-containing neurons localized in the mid-rostrocaudal and caudal parts of the DRN, the axons of which project to both the dPAG and the rostroventrolateral medulla, an autonomic regulation center. In this way, it can inhibit both the behavioral (at the dPAG) and neurovegetative (at the medulla) manifestations of the panic attack. In normal rats, lactate administration activates the mesolimbic 5-HT system, and the animals do not show the effects observed in susceptible rats, which are viewed as representative of the human panic attack.

A further study with the SPS test in panic patients included a group of patients who became symptomless as as result of pharmacological treatment with antidepressants, in addition to a group of symptomatic panic patients and another of healthy controls (Garcia-Leal et al., 2005). Interestingly, the profile of the asymptomatic patients was intermediate between symptomatic panic patients and healthy controls in regard to the VAMS anxiety index. Furthermore, the same study explored the response of the hypothalamic-pituitary-adrenal (HPA) axis by measuring salivary cortisol at different phases of the experimental session. The results showed that the level of cortisol at the beginning of the experimental session was significantly higher in panic patients, either symptomatic or asymptomatic, than in controls. Also, in every group, cortisol was elevated at the beginning of the experimental session (phase B) and decreased after 70 min (phase P). This parallels the decline of the VAMS anxiety factor and appears to reflect habituation of the initial, anticipatory anxiety. Accordingly, there has been a positive correlation between the initial level of cortisol and VAMS anxiety for the three groups, taken together. As expected, preparation and performance of speech raised the anxiety index to nearly the initial level, but failed to increase salivary cortisol, measured for 60 min starting at the end of the speech. Therefore, SPS does not seem to activate the HPA axis, in contrast to anticipatory anxiety. This evidence strengthens the view that SPS mobilizes the same neural networks that operate during the panic attack because, stressful as they are, panic attacks also fail to activate the HPA axis (for a review, see Graeff, Garcia-Leal, Del-Ben, & Guimarães, 2005).

Because the dPAG has been implicated in panic attacks, activation of the HPA axis has been explored in preclinical studies using electrical stimulation of the dPAG as an animal model of panic. In this experimental situation, Schenberg and coworkers (2008) showed that intensities of electrical stimulation that elicit vigorous flight did not increase plasma levels of ACTH, indicating no activation of the HPA axis.

Summary

From the evidence discussed so far it may be concluded that anxiety and panic are qualitatively different emotional states, which are related to the defense reactions to potential and proximal threat, respectively. Equally distinct are the related pathologies, GAD and PD, which differ both in symptomatology and in drug response. Hence, the neurobiological systems involved in each emotion are likely to be different, anxiety being mainly integrated at the forebrain and panic at the midbrain. In addition, these conditions promote differential mobilization of stress hormones. Anxiety activates both the HPA and the sympathoadrenal axes, and the panic attack causes major sympathetic activation but has little or no effect on the HPA axis.

Neuroimaging data

The development of non-invasive neuroimaging techniques allows the exploration of brain morphology and function in healthy volunteers as well as in anxiety disorder patients. It is understandable that these methods have been used to investigate which brain structures are involved in anxiety and panic.

In this regard, a paradigmatic experiment has been carried out by Mobbs and coworkers (2007) in healthy volunteers using functional magnetic resonance imaging (fMRI). Based on the concept of defensive distance taken from animal studies on predatory aggression (Blanchard & Blanchard, 1988; Fanselow & Lester, 1988), these researchers have designed an experimental session where a virtual predator, with the ability to chase, capture and inflict pain, pursues a virtual prey within the alleys of a labyrinth, both being visualized by the participant on a video screen. In this situation, the participant can avoid the virtual predator, represented by a red dot, by manipulating a keyboard to move a triangle, representing the prey. There were two levels of threat consisting of either one or three electric shocks being administered to one finger when the virtual prey was caught. The main finding was that brain activity shifted from prefrontal cortex (PFC) areas (anterior ventromedial PFC and cingulated and orbitofrontal cortices) to the midbrain PAG as the predator grew closer to the prey. When the predator was far, the PFC and the lateral amygdala were activated more clearly when the expected threat level was low (one shock). In contrast, the activation shifted to the central amygdala and the PAG when the predator was close. This shift was maximal when the highest level of pain was anticipated. In addition, the PAG activity correlated with reported subjective degree of dread and of decreased confidence of escape.

A subsequent study carried out by the same research team (Mobbs et al., 2009) used a similar experimental condition to test the MacNaughton and Corr's two dimensional theory of fear/anxiety (McNaughton & Corr, 2004). One dimension is defensive distance, and the other, defensive direction, which distinguishes fear from anxiety. As a model of fear, the same escape task described above was used. Anxiety has been measured after the virtual prey had been caught by the predator, and the persecution was stopped – a post-strike condition (Fanselow & Lester, 1988) of potential threat. In addition to regional activity, the fMRI also explored connectivity among brain structures. The results have shown that anxiety activated the medial PFC, which actively inhibited the PAG. The opposite occurred when the predator was close to the prey – the PAG was activated and restrained forebrain activation.

Together these results give strong support to the above discussed view that anxiety is related to potential or distant threat, being integrated in the forebrain; in turn, panic is associated with immediate danger and is mainly organized in the midbrain.

A functional glucose utilization study carried out in panic patients (Sakai et al., 2006) further supports this view. Brain scans have been performed before and after successful treatment with cognitive-behavior therapy (CBT), and changes in regional activity have been correlated with clinical improvement. In this way,

significant correlations were found between the percent change relative to the pretreatment value of glucose utilization in the left medial PFC and those of anxiety and agoraphobia-related subscale of the Panic Disorder Severity Scale, and between that of the change in the midbrain (probably the PAG) and that of the number of panic attacks during the 4 weeks before each scan in all 12 patients analyzed.

Morphometric MRI studies in panic patients have also found abnormalities in brain regions related to defense. Two of these studies (Protopopescu et al., 2006; Uchida et al., 2008) reported an increase in the gray matter volume in the midbrain (likely to be the PAG) of panic patients compared to healthy controls. In one of these studies (Uchida et al., 2008) carried out by our research group, a major increase in gray matter volume has also been found in the insular cortex and a decrease in the anterior cingulated gyrus. Both areas receive input from interoceptive pathways. Functional neuroimaging studies have also shown abnormal activation of the insula and anterior cingulate in PD (see, e.g., Boshuisen, Ter Horst, Paans, Reinders, & den Boer, 2002). Because PD patients overreact to bodily signals, and one of the main goals of cognitive behavior therapy is to build up tolerance to interoceptive stimulation, the suggestion has been made that the observed abnormalities of the insula and anterior cingulate underpin the interoceptive false alarm that renders patients vulnerable to panic attacks (Uchida et al., 2008). The key role played by the insula in anxiety disorder has been thoroughly discussed by Paulus and Stein (2006).

Starting with Charles Darwin himself (Darwin, 1872) and becoming systematic after the standardization carried out by Ekman (1993), the perception of emotional expressions in human faces has become an important tool for the study of emotional processing. For instance, a recent study performed by our research team (Del-Ben et al., 2010) used fMRI to explore brain regional activation in male healthy volunteers exposed to faces expressing either fear or rage. The emotional intensity of the expression varied from 0 (neutral) to 100% at 10% steps by morphing. When inside the resonance apparatus, the participants were requested to identify the gender of the face (male or female), not the type of emotion, in a masked task of emotion recognition. In addition, a pharmacological tool – single administration of the benzodiazepine anxiolytic diazepam (10 mg, *per os*) – has been used to identify brain structures related to anxiety. As a working hypothesis it was assumed that faces expressing fear would signal potential danger somewhere in the environment and, thus, would arouse anxiety. In contrast, faces expressing rage would represent actual danger, eliciting fear. Diazepam was expected to reduce activation of brain structures related to anxiety in response to fearful, but not angry, faces. In full agreement with this prediction the obtained results

showed that diazepam decreased activation of the right amygdala and bilateral anterior insula by fearful faces. In contrast, the activation by angry faces was increased in the left posterior insula and decreased in the left anterior cingulate gyrus by diazepam.

A similar fMRI study conducted by Harmer and coworkers (2006) evaluated the effect of 7 days administration of the selective serotonin reuptake inhibitor (SSRI), citalopram, on amygdala responses to masked presentations of fear. The results showed that citalopram decreased amygdala responses to masked presentations of fearful faces compared with subjects receiving placebo. Citalopram also reduced responses within the hippocampus and medial prefrontal cortex (mPFC) specifically during the perception of fearful faces. The authors concluded that these results suggest an effect of serotonin on amygdala response to threat-relevant stimuli in humans, and that such effects may be important for the therapeutic actions of antidepressants in depression and anxiety. Within the theoretical context of the present review, it may be further suggested that the reduction of sensitivity to threat stimuli determined by chronic administration of antidepressants is likely to underlie the reduction of the pervading anxiety shown by GAD patients and of the anticipatory anxiety present in panic patients (Graeff & Zangrossi Jr., 2010).

A further contribution of neuroimaging studies has been to explore the role of the PFC in human anxiety. In an enlightening review, Berkowitz and coworkers (2007) have highlighted the structural and functional peculiarities of the human PFC, which is responsible for our remarkable cognitive abilities. Specifically, the ability to foresee future events and the consequences of one's actions would cause worrying, which is the main symptom of GAD. In addition, this development of the PFC allows for enhanced cognitive control of emotions, as testified by several functional neuroimaging studies showing that cortical forebrain regions are able to attenuate emotional responses at subcortical levels, suggesting a neural basis for modulating emotional experience through interpretation and labeling. These findings may help to understand how psychotherapy works. Indeed, in a recent review of functional neuroimaging data in several anxiety disorders, Etkin and Wager (2007) concluded that a finding that was common to all these disorders is reduced activity in medial PFC areas that inhibit limbic structures, together with increased activity in the insula and amygdala, indicating impaired cognitive control of emotions.

As general conclusion, it may be said that the biological evolutionary approach to the study of emotions illustrated in the preceding sections of this article indicates that anxiety, fear and panic do not belong to the same continuum of increasing intensity and, instead, constitute qualitatively different emotional states (for further discussion, see Graeff, 2010).

Acknowledgments

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