Towards a translational model of panic attack

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Abstract

About 20 years ago, Deakin and Graeff proposed that whereas generalized anxiety disorder is produced by the overactivity of 5-HT excitatory projections from dorsal raphe nucleus to the areas of prefrontal cortex and amygdala which process distal threat, panic attacks are a dysfunction of 5-HT inhibitory projections from dorsal raphe nucleus to the dorsal periaqueductal gray matter, thereby releasing the responses to proximal threat, innate fear or anoxia. Besides, they suggested that the decrease in 5-HT1A neurotransmission in the hippocampus results in learned helplessness and depression. Accordingly, the Deakin Graeff hypothesis provided a unified frame to the widespread use of 5-HT selective reuptake inhibitors in generalized anxiety, panic disorder and depression. Competitor hypotheses implicate panic attacks with the abnormal functioning of locus coeruleus, basolateral amygdala, dorsomedial hypothalamus or an as-yet-unknown suffocation alarm system. Conversely, cognitive psychologists suggest that panic attacks result from the catastrophic (cortical) interpretation of bodily symptoms. In any event, translational models of panic attack are expected to reproduce the main features of clinical panic, namely, the patient's higher sensitivity to both lactate and CO_2, the drug specific sensitivity, the lack of stress hormone responses during panic attacks, the higher vulnerability of women and the high comorbidity with agoraphobia, major depression and childhood separation anxiety. Therefore, here we review the main steps in the experimental approach to anxiety disorders which are paving the route towards a translational model of panic attack. **Keywords:** Panic, Anxiety, Stress, Suffocation, Serotonin, Periaqueductal Gray Matter

A short story of panic disorder

The foundations of the modern nosology of anxiety disorders were laid down by Sigmund Freud's original classification of 'anxiety neuroses' (*Angstneuroses*) (Freud, 1896). In this early study, Freud attempted to distinguish anxiety disorders from 'melancholia' (major depression, MD) and a number of ill-defined psychiatric conditions which were at time subsumed under the label of 'neurasthenias'. Remarkably, Freud discerned two major syndromes of anxiety, i.e., the 'anxious expectation' or 'apprehension', which he considered the most essential form of anxiety disorder, and the less frequent 'attack of anxiety'. According to Freud, whereas the anxious expectation was '*a quantum of freely floating anxiety which controls the choice of ideas by expectation*', anxiety attacks consisted in the sudden irruption of anxiety '*into consciousness without being aroused by the issue of any idea*'. Freud emphasized that anxiety attacks could manifest either as '*the anxious feeling alone*' or the combination of

this feeling with '*the nearest interpretation of the termination of life, such as the idea of sudden death or threatening insanity*'. As well, anxiety attacks could be associated to '*some paresthesia ... [or] a disturbance of one or many somatic functions, such as respiration, cardiac activity, vasomotor innervation, and glandular activity*'. Remarkably, Freud pointed out that patients suffering from anxiety attacks '*put the feeling of anxiety to the background or [describe it] rather vaguely ... as feeling badly, uncomfortably, etc*', an observation corroborated by present-day psychiatrists. Freud was also aware about the high comorbidity of panic attacks and agoraphobia and linked the latter condition to a '*locomotion disorder*' caused by the presence of dizziness during panic attacks. (pp. 87-89) Freud's descriptions of 'anxious expectation' and 'anxiety attack' were very much the same of nowadays respective diagnoses of generalized anxiety disorder (GAD) and panic disorder (PD). However, it would take almost a century before the inclusion of PD as a definite psychiatric illness in the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-III, APA, 1980).

Presumably, Freud's clinical heritage was in some measure lost following the profound divorce of psychoanalysis from medicine. Consequently, anxiety disorders continued to be vaguely diagnosed as 'neurasthenias' up to the middle of the last century. Moreover, PD received a bewildering wealth of names

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including neurocirculatory asthenia, vasomotor neurosis, nervous tachycardia, effort syndrome, Da Costa's syndrome, irritable heart, soldier heart and others (Pitts & McClure, 1967). This scenario begun to undergo a profound change following the publication of Donald Klein's influential study showing that GAD and PD were treated by different classes of drugs (Klein, 1964). Klein observed that whereas the '*anxious expectation*' responded to the current time anxiolytics (barbiturates, meprobamate, chlordiazepoxide and low doses of phenothiazines), panic attacks were only treated by the chronic administration of the tricyclic antidepressant (TCA), imipramine.

Around the same time, Pitts and McClure (1967) showed that panic attacks had 'physiological markers' insofar that they could be precipitated by intravenous infusions of .5 M sodium lactate in patients prone to spontaneous panic but not in normal volunteers. Further studies provided ample evidence of the panicogenic properties of sodium lactate, but also of CO_2 and brain acting drugs such as yohimbine, cholecystokinin (CCK), flumazenil, β-carboline, caffeine, etc (for review, see Graeff, Garcia-Leal, Del-Ben, & Guimarães, 2005). The demonstration of the existence of physiological markers and drug-specific treatments of PD suggested that panic attacks were the outcome of a defective brain circuit discharging maladaptively.

The next breakthrough came a few years after the inclusion of PD in the DSM-III (APA, 1980). Indeed, the demonstration that clinical and lactate-induced panics do not activate the hypothalamus-pituitary-adrenal (HPA) axis was a remarkable finding inasmuch as it differentiated clinical panic from stress-like reactions and common fear (Hollander et al., 1989a, 1989b, 1989c; Levin et al., 1987; Liebowitz et al., 1985; Woods, Charney, Goodman, & Heninger, 1988; Woods, Charney, McPherson, Gradman, & Heninger, 1987;). These studies also brought into question the cognitive hypothesis which equated clinical panic to the fear produced by the catastrophic evaluation of bodily symptoms (Clark et al., 1997; Ehlers et al., 1986; Margraf, Ehlers, & Roth, 1986). The cognitive hypotheses were also challenged by studies showing that panic patients do not endorse somatic symptoms indiscriminately (Goetz et al., 1989).

These and other findings led Klein (1993) to suggest that clinical panic is bound to the fear of suffocation, of which dyspnoea (but not hyperpnoea) is the leading feature. According to Klein`s hypothesis, panic attacks are the outcome of the misfiring of an evolved suffocation alarm system. This results in a sudden respiratory distress followed by a brief hyperventilation, panic, and the urge to flee. Klein (1993) argued that the suffocation false alarm hypothesis (SFA) is an explanation both sufficient and consistent of CO_2 and lactate hypersensitivity of panic patients, of the occurrence of panic attacks during relaxation and sleep, of the increased frequency of panic attacks in the late luteal phase dysphoric disorder (LLPDD) and, conversely, its reduction in pregnancy, delivery and lactation. As a matter of fact, CO_2 and lactate-induced panic attacks remains the best model of clinical panic insofar that they are not precipitated in healthy subjects (Klein, 1993; Pitts & McClure, 1967) or patients suffering from obsessive-compulsive disorder (as cited by Griez & Schruers, 1998) and social phobia (Liebowitz et al., 1985b). Also, whereas the panic attacks produced by infusion of lactate or inhalation of CO_2 are blocked by chronic treatment with TCA (Liebowitz et al., 1985a; Rifkin, Klein, Dillon, & Levitt, 1981; Woods, Charney, Delgado, & Heninger, 1990; Yeragani et al., 1988), panics induced by β-carboline (Dorow, Horowski, Paschelke, & Amin, 1983) and yohimbine are not (Klein, 1993). The SFA hypothesis is also consistent with the high comorbidity of panic and respiratory diseases (Preter & Klein, 2008; Shavitt, Gentil, & Mandetta, 1992). However, patients with GAD and, more markedly, LLPDD are also highly sensitive to CO_2 (Kent et al., 2001; Lapierre, Knott, & Gray, 1984).

Following the advent of DSM-III (APA, 1980), experimental researchers became gradually aware about the similarity of panic attacks with the defensive behaviors and aversive emotions long known to be produced by electrical stimulation of the dorsal half of periaqueductal gray matter (DPAG) of animals (Fernandez de Molina & Hunsperger, 1962) and humans (Nashold, Wilson, & Slaughter, 1969), respectively. At the same time, the serotonin (5-HT) selective reuptake inhibitors (SSRI) begun to be widely prescribed for MD, GAD and PD. Therefore, Deakin and Graeff (1991) put forward a daring hypothesis according to which the 5-HT ascending systems of the brain evolved as a mechanism concerned with adaptive responses to aversive events. Briefly, the Deakin-Graeff hypothesis (DGH) suggests that whereas GAD is produced by the overactivity of 5-HT excitatory projections from dorsal raphe nucleus (DRN) to regions of prefrontal cortex (PFC), amygdala and hypothalamus which process distal threats, panic attacks would be the outcome of the malfunctioning of 5-HT inhibitory projections from that nucleus to the DPAG (Figure 1), thereby releasing the defensive responses to innate fear, proximal threat and anoxia. In addition, they proposed that 5-HT efferents from median raphe nucleus (MnR) to the hippocampus would be the substrate of a 'behavior resilience system' that uncouples stressful events from daily life routines (Figure 1). Reduction of $5-HT_{1A}$ neurotransmission of hippocampus would result in learned helplessness and depression. Besides providing a unified frame to the widespread use of SSRIs in GAD, PD and MD, the DGH suggested that DRN 5-HT efferents facilitate avoidance during anticipation of threat at amygdala while restrain the fight/flight components of proximal

defense system at DPAG. Finally, the DGH assumes that conflictive behaviors are processed via 5-HT and dopamine receptors of ventral striatum which mediate avoidance and approach behaviors, respectively (Figure 1). The latter proposal was a manifest rupture with Gray's well-known model which credited anxieties of conflict, frustration and novelty to the activation of classical limbic pathways connecting the medial septum to the ventral hippocampus (Gray, 1976; Gray, 1982; Gray & McNaughton, 2000).

Figure 1. A rat-brain model of the Deakin-Graeff Hypothesis: A) Deakin and Graeff (1991) suggested that whereas the generalized anxiety disorder is produced by the overactivity of 5-HT (black arrows) excitatory projections from dorsal raphe nucleus (DRN) to areas of prefrontal cortex (PFC) and amygdala (BLA) which process distal threat, panic attacks are a dysfunction of 5-HT inhibitory projections from that nucleus to the dorsal periaqueductal gray matter (DPAG), thereby releasing the responses to proximal threat, innate fear or anoxia. In turn, conflict anxiety is the outcome of the simultaneous activity of 5-HT projections from DRN and dopamine projections (thin gray arrows) from ventral tegmental area (VTA) targeting the striatum (STR) and mediating avoidance and approach behaviors, respectively. B) Deakin and Graeff (1991) also proposed that 5-HT efferents from median raphe nucleus (MnR) to the hippocampus are the substrate of a 'behavior resilience system' that uncouples stressful events from daily life routines. Stress increased levels of glucocorticoids (CORT) would downregulate the 5-HT$_{1A}$ receptors of hippocampus, thereby leading to learned helplessness and depression. Note the central place of lateral habenula (LHb) in the regulation of DRN and MnR activities. Other abbreviations: BLA – basolateral amygdala, BNST – bed nucleus of stria terminalis, CeA – central amygdala, CRH – corticotrophin releasing hormone, HPT – hypothalamus, ENT – nucleus entopeduncularis (internal pallidum), PVN – paraventricular nucleus of hypothalamus, S – septum.

Nevertheless, PD patients also complain of 'anticipatory anxiety', i.e., the expectant fear of lack of support when overwhelmed by a panic attack. As a matter of fact, because this condition very often aggravates in a disabling agoraphobia, the DSM-IV does not consider agoraphobia as an anxiety disorder on its own. Clinical evidence also suggests that sudden loss, bereavement and childhood separation anxiety are antecedents of 'spontaneous' panic attacks (Klein & Fink, 1962). The comorbidity of PD and childhood separation anxiety disorder is also supported by the proven effectiveness of imipramine in the latter condition (Bernstein et al., 2000; Gittelman-Klein & Klein, 1973). Moreover, although the agoraphobia was usually explained by the conditioned generalization of fear of having panic attacks in public places, crises rarely occur when patients go out escorted by relatives. The urge of an integrative explanation of these facts led Preter and Klein (2008) to extend the SFA. Thus, because the opioid systems have prominent roles in both breathing and separation distress, Preter and Klein (2008) suggested that panic attacks are produced by an episodic dysfunction of opioidergic systems which decreases the threshold of suffocation alarm system. This possibility was supported by a recent study showing that infusions of lactate in naloxone-treated healthy volunteers produce respiratory distress and feelings which mimic those of panic attacks either spontaneous or induced by lactate (Preter et al., 2010).

Therefore, although most models based on animal studies equate panic to the defensive behaviors of preys to a proximal threat (Blanchard, Griebel, & Blanchard, 2003; Deakin & Graeff, 1991; Mobbs et al., 2007), the development of a translational model of clinical panic is expected to reproduce the key features of PD, namely, drug treatment specificity, lactate and CO_2 hypersensitivity, lack of stress-hormone responses, the higher vulnerability of female sex and the high comorbidity with depression and separation anxiety, among other conditions. Indeed, the coming DSM-V puts a major emphasis on the comorbidity of psychiatric disorders (Holden, 2010). Therefore, the present study highlights the main breakthroughs in experimental research which are setting the stage for the development of a translational model of panic attack. The review considers first the main findings leading to the development of DGH and, secondly, the eventual participation of PAG in a suffocation alarm system. A reconciling hypothesis is then proposed.

Foundations of Deakin-Graeff Hypothesis

Because the main assumptions and implications of DGH have been intensively debated since its publication 20 years ago (Graeff, 2004; Graeff & Guimarães, 1999; Gray & McNaughton, 2000; McNaughton & Corr, 2004; Mobbs et al., 2007), the reader is asked to forego

any detail in here. Moreover, the DGH proposals on the mechanisms of anxiety and panic attacks are more emphasized than the mechanisms concerned with depression disorders.

The development of models of experimental conflict in which water- and food-rewarded behaviors are suppressed by the intermittent presentation of shocks had a major impact in drug research insofar that they enabled to discriminate the anxiolytic effects of barbiturates, meprobamate and chlordiazepoxide from their own sedative effects and the sedative effects of antipsychotics and opioids (Geller, Kulack, & Seifter, 1962). Consequently, much of the early research on the neural basis of anxiety sought to explain the disinhibitory effect of anxiolytics in experimental conflict. Most notably, the study of Graeff and Schoenfeld (1970) was a major breakthrough inasmuch as it showed that the 5-HT agonist alpha-methyltryptamine facilitated shock-induced suppression of a food-rewarded behavior, whereas the 5-HT antagonists, methysergide and bromolysergic acid, released punished behavior in pigeons (Figure 2). These observations were in line with contemporary studies carried out with the less specific method of 5-HT depletion by para-chlorophenylalanine (PCPA) (Geller & Blum, 1970; Hartmann & Geller, 1971; Robichaud & Sledge, 1969). The role of 5-HT in punishment, behavioral inhibition and anxiety seemed thus relatively established almost four decades ago. Consequently, even the anxiolytic actions of benzodiazepines were suggested to be mediated by a reduction in the turnover of 5-HT ascending fibers controlling aversive behaviors (Wise, Berger, & Stein, 1970, 1972, 1973) (Figure 3). Accordingly, the early models of behavioral suppression based on the cholinergic neurotransmission at the hypothalamus (Margules & Stein, 1969a, Margules & Stein, 1969b; Sepinwall & Grodsky, 1969) were very soon replaced by 5-HT-based models of punishment. Yet, other authors suggested that the benzodiazepine anxiolytic effects were due to a reduction in the turnover of norepinephrine (NE) (Taylor & Laverty, 1969).

On the other hand, the combination of operant conditioning and brain stimulation resulted in the discovery of rewarding and aversive systems of the brain (Delgado, Roberts, & Miller, 1954; Olds & Milner, 1954). Particularly, Olds and Olds (1963) showed that whereas the stimulation of the lateral hypothalamus (medial forebrain bundle) produced very strong approach responses, stimulation of periventricular hypothalamus and periaqueductal gray matter (PAG) yielded pure negative reinforcement regularly. Consequently, the brain aversive areas stood out as the presumptive substrates of fear and anxiety.

Mapping of brain aversive areas also provided the opportunity for a direct investigation of the role of neurotransmitters in these systems. Thus, in the mid 70's, researchers begun to examine the effects of drugs
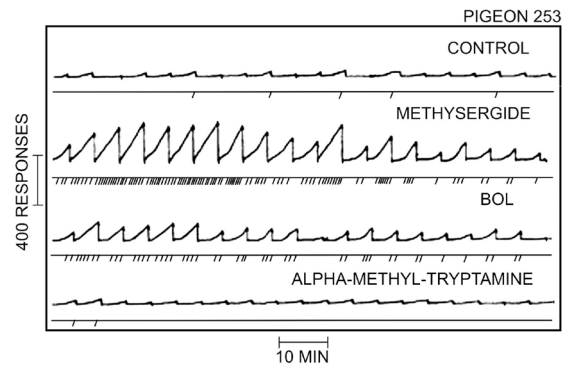
Figure 2. Effects of the 5-HT agonist alpha-methyl-tryptamine and the 5-HT antagonists methysergide and bromolysergic acid (BOL), on the punished behavior of a pigeon on a concurrent fixed-interval (5 min) food reinforced and fixed-ratio (30 responses) shock punishment schedule. Note the marked facilitation of punished behavior of methysergide and BOL sessions (middle recordings) as compared to the control session (top). Vertical pen deflections in event line indicate shocks applied to the pigeon (from Graeff & Schoenfeld, 1970).
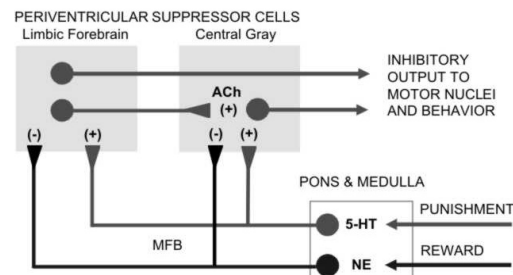
Figure 3. Diagram illustrating the presumptive roles of serotonin (5-HT) and norepinephrine (NE) in the punishment early model of Wise, Berger and Stein (1973). According to these authors, signals of positive reinforcement release behavior from cholinergic periventricular suppression cells through the activation of NE containing neurons in pons (locus coeruleus). In turn, signals of punishment increase behavioral suppression by activation of dorsal raphe and other 5-HT groups. The role of these transmitters in reward and punishment will be fully reinterpreted in the following years.

acting on 5-HT and gamma-aminobutyric acid (GABA) neurotransmission on bar-pressing escape behavior reinforced by the terminus of an electrical stimulus applied at the DPAG. Particularly, Schenberg and Graeff (1978) showed that DPAG-evoked bar-pressing escape was attenuated by peripheral administration of non-sedative doses of chlordiazepoxide (5.6-10 mg/kg) (Figure 4). These results supported the role of DPAG in both the anxiety and the anxiolytic action of benzodiazepines.

However, DPAG-evoked escape responses were unexpectedly facilitated by the 5-HT receptor antagonists cyproheptadine and methysegide, thereby suggesting an anxiolytic action of 5-HT (Figure 4). These experiments were replicated by Clarke and File (1982), using the same 5-HT antagonists and lorazepam, and agreed with
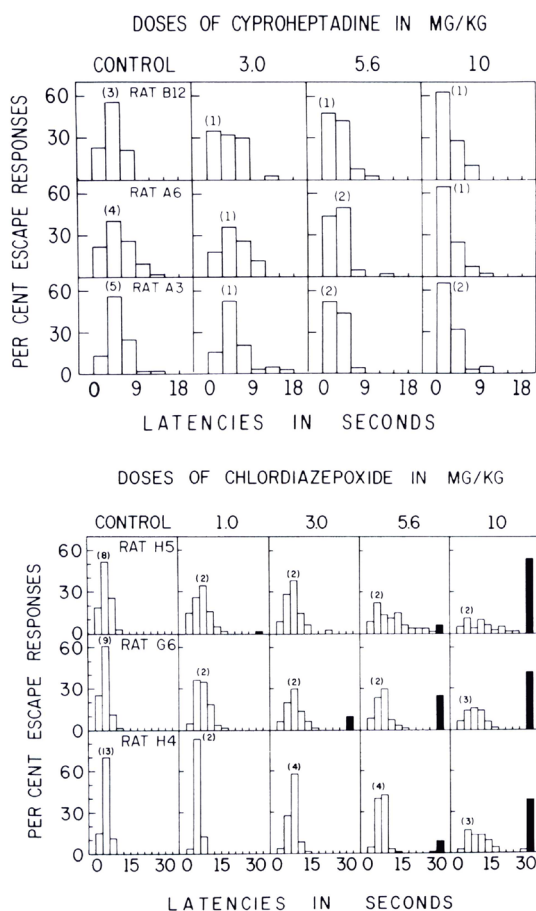
contemporary studies carried out with the model of 'decremental' escape in which each bar-press produced a fixed reduction in the intensity of an electrical stimulus applied to the DPAG (Kiser & Lebovitz, 1975; Kiser, German, & Lebovitz, 1978). Thus, whereas the DPAG-evoked decremental escape was facilitated by 5-HT depletion with PCPA, it was attenuated by the 5-HT precursor 5-hydroxytryptophan (5-HTP) and the SSRI clomipramine (CLM, 15 mg/kg) (Kiser & Lebovitz, 1975; Kiser et al., 1978). In contrast, the administration of 15 mg/kg protriptyline, a preferential inhibitor of NE reuptake, was ineffective (Kiser et al., 1978). In keeping with the study of Simon, Le Moal and Cardo (1976), Schenberg and Graeff (1978) also showed that the stimulation of DRN had rewarding effects.

While the latter studies were at variance with prevailing views suggesting that 5-HT mediated the behavioural effects of punishment (Graeff & Schoenfeld, 1970; Wise, Berger, & Stein, 1973), studies with 5-HT depletion with para-chlorophenylalanine (PCPA) or electrolytic lesion of

Figure 4. Opposite effects of the 5-HT antagonist cyproheptadine (upper panel) and the benzodiazepine chlordiazepoxide (lower panel) on bar-pressing escape responses from electrical stimulation of dorsal periaqueductal gray. Note the dose-dependent increase in short- or long-latency escape responses following the administration of cyproheptadine or chlordiazepoxide, respectively (from Schenberg Graeff, 1978).

5-HT neurons of MnR strongly supported the role of this transmitter in behavioral inhibition (Lorens, Sorensen, & Yunger, 1971; Srebro & Lorens, 1975). In addition, whereas the electrical stimulation of DRN with currents above 250 μA produced only arousal and orientation (Jacobs, 1973), the electrical stimulation of MnR with much lower intensities (40-200 μA) produced the suppression of food-reinforced bar-pressing behavior along with fear-like responses of crouching, dyspnoea, defecation, piloerection and teeth chattering (Graeff & Silveira Filho, 1978). Moreover, the latter authors showed that MnR-evoked behavioral suppression was abolished by the previous administration of PCPA and restored by the 5-HT precursor 5-HTP.

As a result, Schenberg and Graeff (1978) suggested that benzodiazepine anxiolytic effects involve the concomitant inhibition of periventricular/periaqueductal and raphe/septohippocampal systems thought to mediate the behavioral inhibition of punishment (Wise, Berger, & Stein, 1973) and conflict (Gray, 1976), respectively. As well, Schenberg and Graeff (1978) proposed that DPAG-evoked escape responses were inhibited by activation of GABA and 5-HT receptors. The existence of a two-system based behavioral inhibition was further supported by data showing that septal lesion increases responding punished by foot-shock while do not affect responding suppressed by electrical stimulation of DPAG (Graeff & Rawlins, 1980). In the same vein, Graeff and collaborators showed that 5-HT antagonists failed in releasing the water-reinforced bar-pressing behavior punished by electrical stimulation of DPAG (Morato de Carvalho, de Aguiar, & Graeff, 1981), thereby discarding the 5-HT pro-aversive effect at DPAG. Finally, Kiser, Brown, Sanghera and German (1980) showed that electrical stimulation of DRN inhibited DPAG-evoked decremental escape. The latter study was further corroborated by behavioral and electrophysiological evidence that the DRN inhibits the DPAG via $5-HT_{1A}$ receptor neurotransmission (Lovick, 1994; Pobbe & Zangrossi, 2005; Stezkha & Lovick, 1994).

Studies carried out with the microinjection of GABA- and 5-HT-acting drugs into the DPAG corroborated the data of peripherally injected drugs. Most often, these studies employed the shuttle-box escape paradigm in which the rat has to run to the opposite side of the box to turn off the intracranial stimulus. Data showed that the microinjection of GABA-A and 5-HT antagonists into the DPAG either produced the full-blown defense response or facilitated the escape induced by the stimulation of the same site, respectively. Conversely, the microinjection of drugs which facilitated GABA and 5-HT neurotransmission attenuated DPAG-evoked escape responses (Audi & Graeff, 1984; Brandão, de Aguiar, & Graeff, 1982; Graeff, Brandão, Audi, & Milani, 1986a, Graeff, Brandão, Audi, & Schutz, 1986b). Finally, Schenberg and collaborators showed that the DPAG-evoked cardiovascular and respiratory responses

were also attenuated by benzodiazepines injected either peripherally (chlordiazepoxide) or locally (midazolam). Conversely, the latter authors showed that these responses were readily induced by the microinjection of GABA-A antagonists (bicuculine and picrotoxin) into the DPAG (Schenberg, de Aguiar, Salgado, & Graeff, 1981; Schenberg, de Aguiar, & Graeff, 1983).

As a result, Graeff (1981) suggested that the antiaversive actions of benzodiazepines and 5-HT agonists were both mediated through the facilitation of GABA neurotransmission within the DPAG. Further studies found that 5-HT antiaversive actions are exerted either directly, through the activation of $5-HT_{1A}$ inhibitory receptors onto DPAG output neurons, or indirectly, through the activation of $5-HT_{2A}$ excitatory receptors onto GABA intrinsic neurons of DPAG (Brandão, Lopez-Garcia, Graeff, & Roberts, 1991; Griffiths & Lovick, 2002; Pobbe & Zangrossi, 2005).

These studies supported Graeff's contention that the brain aversive system was the substrate of anxiety (Graeff, 1981, 1987; Graeff et al., 1986a, 1986b). However, in the mid 80's, the growing importance of PD and the increased dialogue with clinical researchers (Gentil, 1988) had a profound impact on Graeff's ideas. Therefore, following a preliminary report (Graeff, 1988), the DPAG and the raphe-septo-hippocampal system were reinterpreted as the key structures mediating panic and depressive disorders, respectively (Deakin & Graeff, 1991). In the meanwhile, Jenck, Broekkamp and Van Delft (1990) showed that acute injections of the SSRI panicolytics sertraline and fluvoxamine (10 mg/kg), but not imipramine and CLM, attenuated the DPAG-evoked shuttle-box escape. Moreover, Jenck, Moreau and Martin (1995) showed that whereas the high-potency panicolytic benzodiazepines alprazolam (0.3-3.2 mg/kg) and clonazepam (0.1-1 mg/kg) produced a dose-dependent attenuation of DPAG-evoked shuttle-box escape, the putative panicogens yohimbine and CCK, had a facilitatory effect. Accordingly, even though the acute treatments with TCA and SSRIs are ineffective in PD, the inhibitory effects of SSRIs and high-potency benzodiazepines in DPAG-evoked escape were accepted as evidence of the DPAG mediation of panic attacks. These studies were recently extended for several SSRIs, including fluoxetine (FLX, ED_{50} 13.6 mg/kg), paroxetine (ED_{50} 2.9 mg/kg), citalopram (ED_{50} 6.6 mg/kg) and its active enantiometer, escitalopram (ED_{50} 3.1 mg/kg) (Hogg, Michan, & Jessa, 2006).

The role of amygdala in anticipatory anxiety

To this point, the reader is likely surprised by the omission of the amygdala. Indeed, although Deakin and Graeff (1991) suggested that this structure is crucial in anticipatory anxiety, evidence supporting this proposal stemmed from scientific traditions other than theirs.

The first evidence that the amygdala was involved in innate responses of fear and rage was reported by Klüver and Bucy (1939). These authors showed that monkeys whose temporal lobes were excised became unusually tame to approach. Further experiments showed that definite lesions of the amygdala and hippocampus tamed cats (Ursin, 1965) and monkeys (Smith, 1950; Thompson & Walker, 1951; Walker, Thompson, & McQueen, 1953). Conversely, while the electrical stimulation of the amygdala produced predominantly freezing, the responses of fight and flight were also eventually observed (Kaada, Anderson, & Jansen, 1954; Ursin & Kaada, 1960).

Around the same time, Hunsperger and collaborators published an influential study showing that electrolytic lesions of PAG blocked the defensive behaviors induced by stimulation of the amygdala and hypothalamus, whereas the inverse did not hold (Fernandez de Molina & Hunsperger, 1962). Accordingly, these authors suggested a PAG-centered defense system in which the hypothalamus and amygdala played roles only modulatory. This proposal was at variance with old views which regarded the hypothalamus as the central structure of brain defense systems (Hess & Brugger, 1943). Deakin and Graeff (1991) also adopted a longitudinally organized defense system. However, although they admitted that the DPAG could get visual and auditory information directly from the superior colliculus (SC) (Redgrave & Dean, 1991), they suggested that the 'anticipatory anxiety' to distal threats, including olfactory cues, was due to the activation of a 'basolateral defense system' comprising the PFC and amygdala. Moreover, they proposed that while the freezing (tense immobility) and directed flight (trotting) to distal threats were mediated by the basolateral defense system and hypothalamus, the explosive flight (galloping, jumping) to a proximal threat was mediated by the DPAG. These assumptions relied mostly on phylogenetic and neuroanatomical considerations, but also on observations that stimulation of the hypothalamus evoked escape responses more directed than those elicited by stimulation of DPAG (Schmitt et al., 1986). In any event, Deakin and Graeff (1991) concluded that fear (and by then, phobias) and situational panic attacks were brought about by a dysfunction of the 'rostral defense system' comprising the medial PFC, amygdala and hypothalamus, whereas spontaneous panic attacks were due the malfunctioning of the 'caudal defense system' of DPAG.

The role of the amygdala in anticipatory anxiety was also supported by the evidence of its participation in aversive learning (Berg & Davis, 1983; Hitchcock & Davis, 1986, 1991; Iwata, LeDoux, & Reis, 1986; LeDoux, Iwata, Cicchetti, & Reis, 1988; LeDoux, Sakaguchi, & Reis, 1983; Tishler & Davis, 1983). These authors showed that nuclei of dorsal amygdala play a crucial role in conditioned fear to a light or tone which was previously paired to a shock. Moreover, they showed that the output of basolateral amygdala (BLA)

was mediated through distinct pathways from central amygdala (CeA) to the lateral hypothalamus and caudal ventrolateral PAG (VLPAG), in the case of conditioned fear to a tone, or to the ventral cochlear nucleus and nucleus reticularis pontis caudalis, in the case of fear-potentiated startle (Hitchcock & Davis, 1991; Iwata et al., 1986; LeDoux et al., 1988). Although these data were amply corroborated in the following years, recent studies showed that rhesus monkeys which retained and expressed fear-potentiated startle to a visual cue learned before the complete lesion of the amygdala, failed in acquiring the fear-potentiated startle to an auditory cue when training occurred after the lesion. Thus, the amygdala seems necessary for the acquisition of fear-potentiated startle but not its retention or expression (Antoniadis, Winslow, Davis, & Amaral, 2007, 2009).

Evidence also suggests that innate and conditioned fears are processed by distinct systems within the amygdala (Walker & Davis, 1997). Thus, infusions of a glutamate receptor antagonist into the CeA blocked fear-potentiated startle to a conditioned stimulus but not the light-enhanced startle to an unconditioned stimulus. Conversely, infusions into the bed nucleus of the stria terminalis blocked light-enhanced but not fear-potentiated startle. Infusions into the BLA disrupted both phenomena. These findings suggest that the neuroanatomical substrates of fear-potentiated and light-enhanced startle, and perhaps more generally of conditioned and unconditioned fear, may be dissociated. Likewise, Vianna, Graeff, Landeira-Fernandez and Brandão (2001) showed that lesions of the VLPAG which disrupted the conditioned freezing to a context associated with footshocks failed in changing the freezing and escape responses induced by electrical and chemical stimulations of DPAG.

Finally, recent data showed that whereas the lesions of CeA fail in attenuating the freezing of rats to the odor of the cat (Rosen, 2004), lesions of medial amygdala (MeA) did it effectively (Blanchard, Canteras, Markham, Pentkowski, & Blanchard, 2005). Based on this and previous studies, Blanchard et al. (2005) proposed that predator odor signals processed within the MeA are conveyed to the dorsomedial district of ventromedial hypothalamus (VMH) and then to the DPAG-projecting neurons of premammillary dorsal nucleus (PMD) of hypothalamus. Therefore, structures processing the odor of predators are the only system which retained a rostro-caudal organization (vomeronasal organ-amygdala-hypothalamus-PAG) akin to that proposed about 50 years ago by Fernandez de Molina and Hunsperger (1962).

Figure 5 illustrates the defense systems involved in innate and proximal threats (upper panel) or in the conditioned fear (bottom panel).

The behavioral inhibition system revisited

In the early 70's, the British experimental psychologist Jeffrey A. Gray put forward a neurocognitive model of

anxiety that became the most sophisticated interpretation ever made of Papez's and septo-hippocampal classical limbic pathways (Gray 1976, 1982, 1991). Based on Eysenck's (1967) early studies on human personality, Gray proposed that the 'dysthymic personality' of humans and the anxiety-like behaviors of animals were the expression of a 'behavioral inhibition system' (BIS) which core structures were the medial septum and the hippocampus. Gray suggested that BIS functioned as a 'comparator' (Gray, 1991). Thus, if actual stimuli
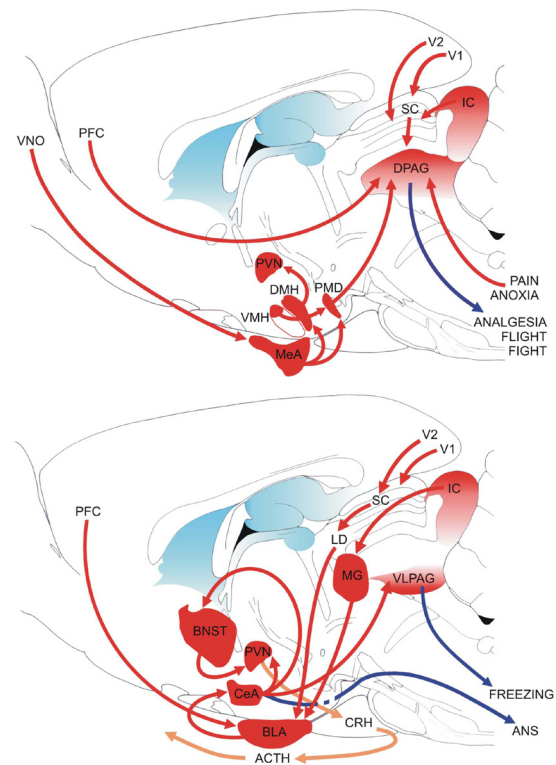
Figure 5. Defensive systems of unconditioned fear responses to proximal threats and interoceptive aversive stimuli (A) and of fear responses to distal threats and conditioned stimuli (B). (A) The dorsal periaqueductal gray matter (DPAG) is a supramedullary reflexive center that receives visual, auditory and somesthesic information from intermediate layers of superior colliculus (SC) which process information from retina, visual cortex (V1,V2), inferior colliculus (IC) and spinal cord. The DPAG also gets olfactory and interoceptive stimuli processed in medial amygdala (MeA), the dorsal 'cap' of ventromedial hypothalamus (VMH) and premmamilary dorsal nucleus of hypothalamus (PMD). DPAG is modulated by pre-frontal cortex (PFC) and produces analgesia and active defensive responses of flight and fight. B) The PFC and basolateral amygdala (BLA) are the key structures of the 'basolateral defense system' processing visual and auditory information from SC and medial geniculate (MG), and painful stimuli from spinal cord. BLA output is conveyed to CeA and then to bed nucleus of stria terminalis (BNST), hypothalamus and ventrolateral periaqueductal (VLPAG) areas which mediate the neuroendocrine, autonomic and freezing components of conditioned fear responses Other abbreviations: ACTH – corticotrophin, CORT – corticosterone, CRH - corticotrophin releasing hormone, DMH – dorsomedial hypothalamus, PVN – paraventricular nucleus of hypothalamus, (based on Deakin and Graeff, 1991).

match the expectancies of safety and normality, the BIS would operate in the 'checking mode' and behavior control would rest with brain mechanisms involved in foraging, ingestion, reproduction, grooming, sleeping, etc. However, if actual stimuli disagree from expected ones (novelty) or predict punishment, frustration or innate fear, the BIS would operate in 'control mode', inhibiting ongoing behavior via fornix efferents to hypothalamus and increasing arousal and attention. The BIS model was partly based on the disinhibitory effects of lesions of hippocampus and partly on the inhibitory effects of anxiolytic drugs on hippocampal theta rhythm, specially, of the 7.7 Hz rhythm associated with behavioral inhibition (Gray 1976, 1982, 1991; Gray & McNaughton, 2000).

Contrarily, however, Deakin and Graeff (1991) suggested that the disinhibition of hippocampus-lesioned rats (Jacobs, Trimbach, Eubanks, & Trulson, 1975) was due to the lack of information about spatial and/or temporal proximity. In this situation freezing was replaced by default primitive reactions of undirected escape leading to the failure of aversive stimuli to inhibit behavior. At that time, this proposal was substantiated by evidence showing that conditioned aversion to context was disrupted by lesions of hippocampus but not of the amygdala (Selden, Everitt, Jarrard, & Robbins, 1991).

Deakin and Graeff's (1991) relinquishment of Gray's model of BIS was a major rupture in Graeff's early scientific tradition. As a matter of fact, following Blanchard's etho-experimental analysis of antipredator defense levels (Blanchard, Flannelly, & Blanchard, 1986), Graeff (2004) rescued the BIS as the likely substrate of conflict-like risk-assessment behaviors to a potential threat. In the same vein, Kjelstrup et al. (2002) showed that lesions of the ventral hippocampus (subiculum) increase the exploration of open arms in the elevated plus-maze (EPM), a widespread accepted model of GAD, and reduce the neuroendocrine responses to a brightly lit chamber. Because the rat exploration of EPM implicates the risk assessment of open (dangerous) and closed (safe) arms, these data added new evidence of the role of hippocampus in conflictive behavior. Notably, the selective lesions of dorsal hippocampus and amygdala did not increase the exploration of open arms. Gray's BIS concept was also unexpectedly supported by findings showing that behaviors of male and female rats are predominantly controlled by anxiety and exploratory drives, respectively (Fernandes, Gonzalez, Wilson, & File, 1999). Indeed, Gray had long shown that the low threshold for the septum elicitation of the 7.7 Hz hippocampal theta rhythm (the 'theta dip') is a testosterone-dependent feature of male rats only (Gray, 1976). Accordingly, because this rhythm was associated with the behavioral inhibition of anxiety, it is tempting to speculate that the increased exploratory drive of female rats is due to the lack of theta dip.

LeDoux and collaborators presented additional evidence of the relative independence of fear-induced behavioral inhibition of Pavlovian conditioned freezing and Estes-Skinner conditioned suppression. Indeed, lesions of VLPAG which blocked the conditioned freezing failed in blocking the suppression of ongoing operant behavior (Amorapanth, Nader, & LeDoux, 1999). Actually, Gray and McNaughton (2000) argued that conditioned fear to stimuli previously paired to a shock is most probably a model of posttraumatic stress disorder (PTSD). This possibility was supported by recent studies showing that impaired inhibition of conditioned fear (fear-potentiated startle) may be a specific biomarker of PTSD (Jovanovic et al., 2009, 2010a, 2010b).

Finally, Schenberg and collaborators showed that the opposite responses of freezing (tense immobility plus exophthalmus) and flight (trotting, galloping and jumping) could be both produced by small variations in either the intensity or frequency of an electrical stimulus, or dose of an excitatory amino acid applied at a single site of the DPAG (Bittencourt, Carobrez, Zamprogno, Tufik, & Schenberg, 2004; Schenberg, Costa, Borges, & Castro, 1990). Further studies showed that DPAG-evoked freezing is also distinct from conditioned fear (De Oca, DeCola, Maren, & Fanselow, 1998; Vianna, Graeff, Landeira-Fernandez, & Brandão, 2001). Moreover, pharmacological evidence suggests that DPAG-evoked freezing is related to noradrenergic ascending systems involved in attention (Schenberg, Bittencourt, Sudré, & Vargas, 2001). Therefore, one can safely conclude that there are more systems involved in behavioral inhibition than those conjectured in Gray's BIS and DGH.

The interplay between anxiety and panic

The DGH states that whereas the 5-HT efferents of DRN facilitate the PFC and amygdala circuits which mediate the avoidance responses during anticipation of threat, they inhibit the circuits of DPAG mediating the fight/flight responses to a proximal threat (Deakin & Graeff, 1991). Clinical consequences implicate that anxiety inhibits panic. Indeed, these conditions are characterized by passive and active responses, respectively. This possibility is also supported by the precipitation of panic attacks by otherwise misguided techniques of behavioral relaxation (Adler, 1987; Barlow, Craske, Cerny, & Klosko, 1989). In the same vein, night panic attacks occur during sleep deepening from stage II to III (Hauri, Friedman, & Ravaris, 1989; Mellman & Uhde 1989a,b), i.e., just after REM sleep episodes in which the DRN cells are largely silent (Ginty & Harper, 1976; but see Lu, Jhou, & Saper, 2006).

Recent evidence from both rats and humans supports the GAD inhibition of PD. In rats, a study from our laboratory showed that whereas the short-term full-kindling of the amygdala enhances the natural

flight behaviors against capture, it attenuates the flight behaviors produced by electrical stimulation of DPAG (Tannure, Bittencourt, & Schenberg, 2009). Therefore, kindling-induced resistance-to-capture is most probably due to a sensitization of circuits other than the DPAG (probably, the 'basolateral defense system'). Yet, because kindling did not affect the performance in the EPM, Tannure et al. (2009) suggested that resistance-to-capture is most probably a model of phobia.

More important, however, Mobbs and colleagues (2001) investigated the spatial imminence of threat by developing an active avoidance paradigm in which volunteers are pursued by a virtual predator endowed with an ability to chase, capture, and inflict real shocks to the finger of the volunteer. Using functional magnetic resonance imaging, they found that as the virtual predator came closer, brain activity shifts from ventromedial PFC/BLA to CeA/PAG. This shift was maximal when a high degree of pain was anticipated. Moreover, PAG activity correlated with the subjective degree of fear and a decreased confidence of escape. Endorsing the DGH, Mobbs et al. (2007) suggested that GAD is mediated by PFC and BLA areas activated by distal threat, whereas panic is mediated by CeA and PAG circuits brought into action by proximal threat. Notably, Amano et al. (1978) have long reported that during a surgery for pain relief, a patient stimulated in the PAG uttered '*somebody is now chasing me, I am trying to escape from him*'. While this incidental observation was once regarded as a paranoid delusion of uncertain nature, nowadays it stands out as a sounding proof of the latter study.

Challenging the model

The contention that DPAG mediates GAD became untenable by the end of the last century. Particularly, in the mid 80's we showed that doses of diazepam as high as 10 mg/kg (i.p.) failed in attenuating the DPAG-evoked shuttle-box escape (Schenberg, unpublished results). Although these results were accounted to the specific features of shuttle-box in which the rat has to move to the box side in which it was previously punished (Gray, 1976), they also raised the suspicion that the model was not appropriate. On the other hand, the effects of locally injected drugs were difficult to interpret owing to the lack of correlation between intracranial and peripheral doses. For instance, considering that the mean weight of the rat midbrain is about 130 mg and that 98% of midazolam is bound to plasma proteins, the microinjection of 1 nmol midazolam into the midbrain tectum would correspond to an intravenous dose of 170 mg/kg. Finally, at that time the evidence supporting the DPAG mediation of panic attacks was only based on the acute effects of drugs injected either locally or peripherally (Graeff, 2004). Nevertheless, panicolytic effects of TCAs and SSRIs are known to develop gradually along several weeks.

These problems prompted us to the development of threshold logistic analysis as a convenient way to model the probabilities of intracranially-induced behaviors (Schenberg et al., 1990). Since then, this method has been progressively improved and extensively employed in the mapping of brain defense areas (Bittencourt, Carobrez, Zamprogno, Tufik, & Schenberg, 2004, Bittencourt, Nakamura-Palacios, Mauad, Tufik, & Schenberg, 2005) and in the assessment of acute and chronic effects of anxiolytics, panicogens and antidepressants on DPAG-evoked defensive behaviors (Schenberg, Bittencourt, Sudré, & Vargas, 2001; Schenberg, Capucho, Vatanabe, & Vargas, 2002; Vargas & Schenberg, 2001).

Briefly, studies with acute injections of antidepressants showed that CLM had variable effects on the thresholds of galloping which were either increased or decreased by the respective doses of 5 and 10 mg/kg. In contrast, the acute injection of FLX produced a moderate but definite increase in the thresholds of immobility (22±2%) and galloping (25±3%) with 1 and 5 mg/kg, respectively. Galloping attenuation by 5 mg/kg FLX was but paralleled by a facilitation of trotting (Schenberg et al., 2002). Therefore, FLX seems to downgrade the DPAG defensive repertoire towards low-level antipredatory defensive behaviors of freezing and trotting (directed escape). Contrarily, galloping was markedly facilitated by a subconvulsant dose of pentylenetetrazole (60 mg/kg) (Schenberg et al., 2001), a chloride channel blocker which is a putative panicogen in humans (Gentil, 1988). Moreover, drugs lacking panicolytic actions failed in attenuating DPAG-evoked behaviors. Therefore, neither the acute administration of 1.8 mg/kg diazepam (Schenberg et al., 2001), nor the acute or 10-day administrations of 10 mg/kg buspirone (Vargas, 2002) produced any increase in the thresholds of DPAG-evoked defensive responses. Accordingly, in spite of the different approaches of intracranial stimulation in the shuttle-box and the open-field, SSRI acute effects were very often converging, supporting the 5-HT inhibition of DPAG-evoked panic-like behaviors.

More important, these studies evaluated the effects of peripheral administrations of CLM and FLX in doses and regimens similar to those employed in the therapy of PD (Schenberg et al., 2001; Vargas & Schenberg, 2001). Thus, whereas the 21-day administration of saline produced only a moderate but significant increase (35%) in the thresholds of galloping, the chronic treatment with CLM produced significant increases in the thresholds of immobility (24%) and, markedly, of trotting (138%), galloping (75%), jumping (45%) and micturition (87%). Although these effects were variable in both timing and response selectivity, they were produced with doses of CLM (5 and 10 mg/kg) near to those of clinical range (1 to 3 mg/kg) (Lotufo-Neto, Bernik, Ramos, Andrade, Gorenstein et al., 2001). Moreover,

because CLM attenuated both passive (freezing) and active (flight) behaviors, effects were most likely due to an antiaversive action. Although the affinity of CLM for the 5-HT transporter is about 130 times that for the NE transporter, its main metabolite (norclomipramine) is 16 times more selective for the NE transporter. Thus, CLM chronic effects were compared to those of the 21-day treatment with FLX doses (1 and 5 mg/kg) already within the clinical range (0.2 to 2 mg/kg) (Schneier et al., 1990). Remarkably, the 21-day treatment with 1 mg/kg FLX produced the selective suppression of galloping without changing the remaining responses (Figure 6).

On the other hand, pre-clinical evidence suggests that anxiety and fear are both related to an increase in the activity of brain noradrenergic neurons. In addition, clinical studies suggest the abnormal regulation of noradrenergic systems in PD (Charney et al., 1990). Nevertheless, electrical stimulation of human locus coeruleus (LC) which caused a 4 to 9 fold increase in NE plasma metabolites produced only alertness (Libet & Gleason, 1994). Although the latter data makes unlikely the LC contribution to aversiveness, NE may play roles either modulatory or permissive in panic attacks as suggested by several reports of successful treatments of PD with selective or preferential inhibitors of NE reuptake (Bertani et al., 2004; Dannon, Iancu, & Grunhaus, 2002; Kalus et al., 1991; Lydiard, 1987, Lydiard et al., 1993; Nardi et al., 2003; Sasson et al., 1999; Seedat et al., 2003; Versiani et al., 2002;). More important, whereas the α_2-adrenergic antagonist yohimbine is an established panicogen (Bremner,

Krystal, Southwick, & Charney, 1996; Charney, Woods, Goodman, & Heninger, 1987; Charney, Woods, Krystal, Nagy, & Heninger, 1992), appropriate doses of the α_2-adrenergic agonist clonidine promote a rapid but transient improvement of PD patients (Liebowitz, Fyer, McGrath, & Klein, 1981). Finally, the DPAG sends dense excitatory projections to the LC (Sandkuhler, 1991; Sandkuhler & Herdegen, 1995).

The DPAG-evoked defensive responses were thus evaluated following the administration of maprotiline (MPT), a NE selective reuptake inhibitor (NSRI) antidepressant that lacks conspicuous panicolytic actions (Westenberg & Den Boer, 1993). Moreover, these responses were evaluated 6 and 24 hours following the administration of DSP-4, a NE synthesis inhibitor that produces the selective depletion of dorsal noradrenergic bundle (Fritschy & Grzanna, 1989). To this end, effects were assessed following acute and chronic peripheral treatments with MPT (10 mg/kg, i.p.), and the acute intracerebroventricular (i.c.v.) injections of MPT (19.5 µg/15 µL, i.c.v.) and DSP-4 (100 µg/15 µL, i.c.v.). Therefore, doses of MPT were about 10 times those employed in the treatment of MD (around 1 mg/kg, p.o.).

Acute injections of MPT inhibited the rat spontaneous behaviors and DPAG-evoked defecation (90% threshold increase) (Schenberg et al., 2001). In anesthetized rats, Ceci and Borsini (1996) showed that MPT (1-10 mg/kg, i.v.) excites prefronto-cortical neurons while inhibits the LC. Inhibition of LC is likely due to the activation of autosomic alpha-2 adrenergic receptors (Abercrombie & Jacobs 1987). The excitation of prefronto cortical neurons is but surprising insofar that the cortical application of NE predominantly inhibits the spontaneous activity while enhances the evoked responses, whereby it increases the signal-to-noise ratio of cerebral cortex (Aston-Jones, Rajkowski, & Cohen, 2000; Bunney & Aghajanian 1976; Servan-Schreiber, Printz, & Cohen, 1990). Accordingly, MPT inhibition of rat spontaneous behaviors was most probably due to the enhancement of cortical activity secondary to the prevalent inhibition of LC.

The MPT attenuation of DPAG-evoked defecation could have been due to effects either central or peripheral at gastrointestinal tract. MPT central effects were but confirmed by the marked increases (50%) in the thresholds of defecation following the i.c.v. injections. In turn, acute treatment with MPT produced only light to moderate increases in the thresholds of immobility (22.5%), trotting (23.6%), exophthalmus (23.3%) and galloping (32.5%) (Lugon, 2006). Although the central dose of MPT (10 µg/g, i.c.v.) was proportionate to that employed in peripheral injections (10 mg/kg, i.p.), brain drug levels following i.c.v. injections were most likely higher than those of the latter injections in which the drug is extensively bound to plasma proteins. Likewise, NE depletion with DSP-4 produced significant increases in the thresholds of all defensive responses 6 hours and

Figure 6. Long-term effects of fluoxetine (1 and 5 mg/kg/day, i.p.) and saline (.9% NaCl, i.p.) administered daily for 21 days, on the thresholds of defensive responses induced by electrical stimulation of dorsal periaqueductal gray. Values are the percent variation of median threshold intensities in relation to pre-drug session (day 1). ---- and * p < .05, significantly different from pre-drug controls (regression likelihood ratio coincidence tests and maximum output Pearson's χ^2, respectively) (r = responders, n = number of stimulated rats) (modified from Schenberg, Bittencourt, Sudré, & Vargas, 2001).

24 hours after the drug injection. Thresholds were but partially recovered 1 week after drug injection (Lugon, 2006). The converging effects of MPT and DSP4 support the inhibition of LC through distinct mechanisms, i.e., stimulation of autosomic α_2-adrenergic and NE depletion of dorsal noradrenergic bundle.

Notably, the 21-day treatment with MPT caused marked selective increases in the thresholds of immobility (116±6%) (Figure 7). Therefore, because the LC has a prominent role in visually-guided responses (Aston-Jones et al., 2000; Delagrange, Canu, Rougeul, Buser, & Bouyer, 1993), the DPAG-evoked immobility is most likely a NE-mediated attentional shift from nearby targets (e.g., food) to distal ones requiring visual fixation (e.g., predators). The sedative-like effects of the chronic administration of MPT is also supported by previous observations showing that DPAG-evoked immobility is only attenuated by doses already sedative of midazolam (5.0 mg/kg) (Schenberg et al., 2001). Conversely, the lack of effects of the chronic administration of FLX in DPAG-evoked immobility makes it unlikely the relevant role of 5-HT in this response (Vargas & Schenberg, 2001).

Effects of central injections of MPT and DSP-4 are compatible with the reports of successful treatment of PD with NE reuptake inhibitors both selective and preferential, with the panicolytic action of clonidine and, conversely, the panicogenic properties of yohimbine. Moreover, because DSP-4 promotes the selective depletion of dorsal noradrenergic bundle, the present data suggest that LC ascending projections are involved with the increased attentiveness of anticipatory anxiety which precedes panic attacks.

The attenuation of DPAG-evoked defensive behaviors following the chronic administration of antidepressants bears a close resemblance with the FLX attenuation of predator-induced flight behavior in both mice and rats (Blanchard, Griebel, & Blanchard, 1993; Blanchard, Griebel, Henrie, & Blanchard, 1997; Griebel et al., 1995; Griebel, Blanchard, & Blanchard, 1996). It is noteworthy, however, that imipramine (10 mg/kg, i.p.) was ineffective in these experiments (Vargas, 2002). In any event, the above studies suggest that whereas the DPAG-evoked immobility is a NE-mediated attentional response, galloping is the panic attack best candidate response (Schenberg et al., 2001).

Teasing out panic from stress-related disorders

The concept of stress was coined by Hans Selye (1936, 1976) to denote a complex syndrome produced by a diversity of 'nocuous' agents thereafter known as 'stressors'. Selye's hypothesis had a widespread influence on the medical world, but mostly on immunology and psychiatry. Particularly, clinical and experimental studies implicate stress in MD, PD and PTSD (Jovanovic et al., 2009, 2010a, 2010b; Keen-Rhinehart et al., 2009; Strohle & Holsboer, 2003).
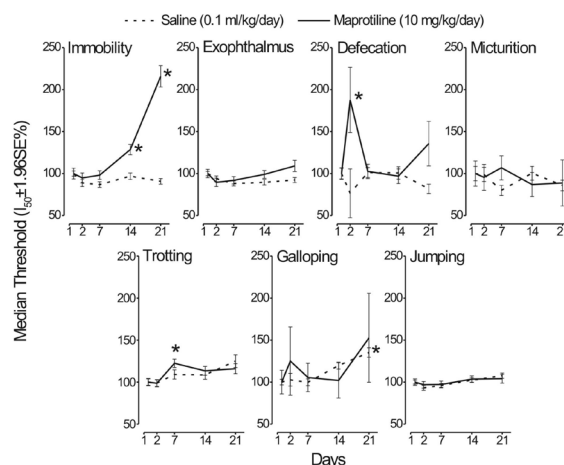
Figure 7. Acute and long-term effects of the 21-day administration of maprotiline (10 mg/kg/day, i.p.) or saline (0.9% NaCl, i.p.) on the thresholds of defensive responses induced by electrical stimulation of dorsal periaqueductal gray. Values are the percent variation of median threshold intensities in relation to pre-drug session (Day-1). Acute effects were assessed after the daily drug injections (Day-2) and chronic effects before injections (Days 7, 14 and 21) so as to separate acute and chronic effects. Other details as in Figure 6 (modified from Schenberg, Bittencourt, Sudré, & Vargas, 2001).

Selye's hallmark was but the doctrine of the non-specificity of the '*alarm reaction*', i.e., the early activation of the HPA axis that follows the exposure of mammals to a perplexing number of stressors. As a result, there happens a marked increase in plasma levels of corticotrophin (ACTH) and the glucocorticoids cortisol (humans) and corticosterone (rats and other species) (glucocorticoids are both abbreviated as CORT). Further studies showed that prolactin (PRL) is likewise released in response to a great number of stressors both physical and psychological (Dijkstra, Tilders, Hiehle, & Smelik, 1992; Neill, 1970; Siegel, Conforti, & Chowers, 1980).

As already mentioned, the demonstration that stress hormones (ACTH, CORT and PRL) remain unaltered during panic attacks is both intriguing and an important clue for the understanding of the neurobiology of PD (Hollander et al., 1989a, 1989b; Kellner, Knaudt, Jahn, Holsboer, & Wiedemann, 1998; Levin et al., 1987; Liebowitz et al., 1985; Woods et al., 1987, 1988). However, because drugs which induce anxiety (β-carboline, yohimbine, metrazol and fenfluramine) and panic (CCK-related peptides) release stress hormones, the unresponsiveness of HPA axis in panic attacks has been hotly debated up to the present days (Graeff et al., 2005; Hollander et al., 1989c). For instance, it has been argued whether the lack of HPA responses is a feature of CO_2- and lactate-induced panics or may be extended to spontaneous panic as well. Yet, in a rare study with spontaneous panic attacks, Cameron, Lee, Curtis and McCann (1987) failed in recording consistent changes in either the CORT or PRL plasma level at the peak or

10 and 60 minutes after 9 spontaneous panic attacks presented by 4 patients. Likewise, stress hormones did not change following the provocation of situational panic attacks of 15 drug-free agoraphobic patients (Woods et al., 1987). Contrarily, PRL responses tended to be even smaller in patients as compared to controls. Although the latter authors suggested that recurrent panic attacks might have caused an adaptation of the neuroendocrine mechanisms, Anegg et al. (2002) reported that PRL plasma levels did not change, whereas CORT saliva levels in fact decreased in healthy scuba divers who experienced a panic attack during an emergency training in a natural setting. In turn, while the decrease in CORT saliva levels could have been due to mouth rinsing during dive, PRL plasma levels of panic-resistant divers were unexpectedly higher than those of the panicking subjects. This study is particularly important given the straightforward relationship of diving-induced panic attacks and SFA (Klein, 1993; Preter & Klein, 2008). In addition, a recent study showed that panic patients are remarkably unresponsive to stress despite normal HPA responses to awakening (Petrowski, Herold, Joraschky, Wittchen, & Kirschbaum, 2010). Finally, stress hormones are not increased in panic-like responses of rats (escape from the open arm of the elevated T-maze, Graeff & Zangrossi, this issue) and humans (simulated public speaking, Garcia-Leal et al., 2005).

There is some controversy, as well, about the HPA axis responsiveness to CO_2. Indeed, whereas the CORT plasma levels of PD patients did not change during or after the panic attacks induced by inhalation of 5 or 7% CO_2 (Sinha et al., 1999; Woods et al., 1988), a single breath of 35% CO_2 produced a significant increase in ACTH, CORT and PRL plasma levels of healthy volunteers (Kaye et al., 2004; van Duinen, Schruers, Maes, & Griez, 2005). The latter effects were similar in PD patients and controls, and were not correlated with the severity of panic attacks (van Duinen, Schruers, Maes, & Griez, 2007).

Studies in anesthetized dogs showed that neither the ACTH, nor the CORT are increased following the 20 minute exposure to 6% CO_2. In contrast, the 20 minute exposure to hypoxia produced an increase of 175% in ACTH plasma level that was attenuated (albeit not abolished) by chronic chemodenervation of carotid body (Raff, Shinsako, & Dallman, 1984). However, the HPA responses did occur to hypoxia and hypercapnia of higher magnitude (Raff, Shinsako, Keil, & Dallman, 1983a, 1983b). In conscious rats, the normocapnic hypoxia (7% O_2) caused increases of 100% in ACTH plasma levels, whereas the acute exposure to 8% CO_2 and the hypercapnic hypoxia produced increases of approximately 200% and 300%, respectively (Raff & Roarty, 1988). Responses were but quite different in sustained hypoxia. Thus, the 42 hour exposure to hypoxia (10 and 12.5% O_2) failed in changing the levels of both

the ACTH and CORT in conscious rats (Raff, Sandri, & Segerson, 1986). Overall, these studies suggest that the HPA responds differently to low and moderate levels of acute and sustained hypoxia and hypercapnia.

In any event, in a recent review, Graeff et al. (2005) found that neither the real-life panic attacks nor those triggered by physiological panicogens (sodium lactate and CO_2) are likely to activate the HPA axis. Contrarily, ACTH and CORT are consistently released by panicogens known to produce anticipatory anxiety. Accordingly, anticipatory anxiety and comorbid affective disorders are likely the major confounding factors in clinical studies of HPA responses in human patients (Garcia-Leal et al., 2005; Hollander et al., 1989c).

Although the CCK-related peptides (CCK-4, CCK-8S, pentagastrin) promote panic attacks and activate the HPA axis robustly (Abelson & Liberzon, 1999; Bradwejn et al., 1992; De Montigny, 1989), these effects might be due to the direct stimulation of the hypothalamus through mechanisms unrelated to panic itself. Thus, *in vivo* and *in vitro* studies suggest that CCK neuroendocrine responses are brought about by the stimulation either direct or indirect (peripherally-mediated) of paraventricular (PVN) and dorsomedial hypothalamus (DMH) (Kamilaris et al., 1992; Kobelt et al., 2006). Also, whereas the chronic treatment with the clinically-effective panicolytic citalopram decreased the intensity of CCK-induced panic attacks, citalopram did not attenuate the HPA axis response to this peptide (Shlik et al., 1997). Finally, CCK-induced panic attacks were shown to be blocked by the 5-HT$_3$ antagonist odasentron (Depot et al., 1999), vagotomy (Kamilaris et al., 1992) and CCK-A antagonists (Chen, Deutsch, Gonzalez, & Gu, 1993; Kamilaris et al., 1992), thereby suggesting the contribution of a peripherally-mediated component.

The demonstration of the lack of HPA axis and PRL responses in an animal model of panic attack is thus an invaluable evidence of a similar mechanism in PD. Therefore, a recent study of our group examined whether the 1-minute stimulation of DPAG of freely-moving rats is accompanied by increases in ACTH and PRL plasma levels. Data showed that neither the freezing reaction, nor the vigorous flight behavior is likely to activate the hypothalamus-pituitary axes (Schenberg, Reis, Póvoa, Tufik, & Silva, 2008) (Figure 8). Moreover, because panic attacks last 10 minutes or so, we further examined whether the 5-minute stimulation of DPAG (ten 30s on-30s off periods) with the flight threshold intensity activates the HPA axis. Yet, not even the extenuating exertion of repetitive flight bouts produced a significant increase in the ACTH plasma level.

These data are worthy of note for a number of reasons. First, they bring Selye's (1936, 1956, 1976) hypothesis of non-specificity into question. Although criticized since its very beginning, the concept of non-specificity was tested only recently by Pacak et al. (1998). These authors showed that different stressors
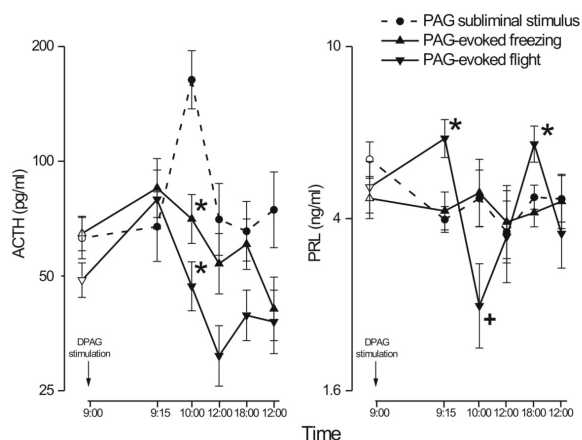
Figure 8. Effects of the 1-min electrical stimulation of dorsal periaqueductal gray matter (DPAG) on corticotropin (ACTH) and prolactin (PRL) plasma levels of Wistar male rats. Hormones were assayed in separate groups. Rats were stimulated with either a subliminal stimulus (ACTH, *n* = 8; PRL, *n* = 11) or the freezing (ACTH, *n* = 7; PRL, *n* = 8) and flight (ACTH, *n* = 7; PRL, *n* = 5) threshold intensities. Data represent log-transformed hormone data (mean±SEM). Open symbols represent the pre-stimulus baseline levels. Ordinate and abscissa are in logarithmic scales. * P < .05, different from subliminal stimulation (repeated measures ANOVA followed by Bonferroni's t-test of single degree of freedom contrasts for time/group interactions) (Schenberg et al., 2008).

give rise to specific patterns of neuroendocrine responses. Moreover, hormones (ACTH and adrenaline) and transmitters (NE) did not respond proportionally to the same change in the intensity of stress. Therefore, Pacak et al. (1998) suggested that each stress has its 'own signature'. Indeed, Mason (1971) has long shown that whereas the HPA axis was reliably activated by cold and hemorrhage, responses to other stressors were either abolished (fasting, exercise) or reversed (heat) by minimization of psychological stress. Accordingly, Mason (1971) proposed that the apparent non-specificity of HPA axis activation was due to the superimposition of a psychological component (anxiety, suffering) common to most kinds of stressors. Surprisingly, however, we did not observe any significant increase in the stress hormones following the undisputable emotional arousal of the aversive stimulation of DPAG. Second, our findings help in clarifying some clinical studies in which results might have been biased by anticipatory anxiety and comorbid disorders. Lastly, we showed that not even the extreme effort of the prolonged flight behavior activated the HPA axis significantly. Therefore, as far as we know, the lack of HPA axis activation during the intense emotional arousal seems to be a feature unique of DPAG-evoked defense reaction and panic attacks.

Panic attacks are very often misdiagnosed as coronary heart disease (Potokar & Nutt, 2000), even when symptoms do not involve "fear of dying" (Fleet, Martel, Lavoie, Dupuis, & Beitman, 2000). Panic patients are also very often referred for pulmonary

function testing of chronic obstructive pulmonary disease (Pollack, Kradin, Otto, Worthington, Gould et al., 1996). On the other hand, DPAG stimulation is unquestionably unpleasant in both humans and animals. Thus, there can be little doubt about the subjective experience of panic attack and DPAG stimulation as true emotional stress. Accordingly, HPA and PRL responses are most likely inhibited during panic attacks. Indeed, although the inhibition of the HPA axis during emotional behaviors is rarely observed, the ACTH response to shocks was attenuated when the rat was allowed to fight another rat during the application of the same shock schedule (Conner, Vernikos-Danellis, & Levine, 1971; Erskine & Levine, 1973). In the same vein, Bhatnagar et al. (2000) showed that the HPA axis of chronically stressed rats may be inhibited by PAG CCK projections to the posterior paraventricular nucleus of the thalamus. During DPAG-evoked behaviors, the HPA axis could be likewise inhibited by the VLPAG projections to the PVN (Floyd, Keay, Arias, Sawchenko, & Bandler, 1996). Finally, in the specific case of lactate-induced panic attacks, Kellner et al. (1998) presented evidence that HPA axis could be inhibited by the augmented release of atrium natriuretic hormone, an endogenous antagonist of ACTH.

Whatever the mechanism may be involved, the lack of ACTH and PRL responses following the DPAG-evoked defensive behaviors endorse the DPAG-evoked defensive behaviors as a model of panic attack.

Translational modelling of panic attacks

Besides the pharmacological (Schenberg et al., 2002; Vargas & Schenberg, 2001) and endocrine (Schenberg et al., 2008) studies above discussed, few research groups carried out animal studies specifically designed to reproduce the unique features of clinical panic.

Among these, Shekhar and collaborators reported a series of experiments that sought to model lactate-induced panic attacks in rats (Johnson & Shekhar, 2006; Johnson, Truitt, Fitz, Lowry, & Shekhar, 2008, Johnson et al., 2010; Molosh et al., 2010; Shekhar, 1994; Shekhar, & Keim, 1997; Shekhar, Keim, Simon, & McBride, 1996; Shekhar et al., 2006). These studies were based on the previous observation that the inhibition of GABA transmission in the posterior hypothalamus produced increases in locomotor activity 'suggesting an escape reaction' and facilitated the behavioral suppression in experimental conflict and Sidman's non-signaled avoidance schedule (Shekhar & DiMicco, 1987; Shekhar, Hingtgen, & DiMicco, 1987; 1990). Conversely, injections of the GABA-A receptor agonist muscimol in the posterior hypothalamus released punished behavior (Shekhar et al., 1990). Although the involvement of DMH was discarded in the earlier studies, further experiments identified the effective sites in this nucleus properly (Shekhar, Sims, & Bowsher, 1993). The latter studies also showed

that blockade or enhancement of GABA-A transmission in the DMH produced increases or decreases in anxiety as measured in the EPM, respectively.

Shekhar's next study was a turning point in these investigations insofar that it showed that DMH-evoked behaviors were blocked by subchronic treatments with the clinically effective panicolytics imipramine (5 and 15 mg/kg, 7 days) and clonazepam (5 mg/kg, 3 days). Besides supporting the DMH mediation of panic attacks (Shekhar, 1994), these data were corroborated by studies showing that long-term osmotically-driven infusions of a GABA synthesis inhibitor (allylglycine) in the DMH turned rats sensitive to an intravenous infusion of .5 M sodium lactate (10 ml/kg/15 min), as shown by the significant increases in heart rate, blood pressure and anxiety measured through the social interaction (SI) and the EPM tests (Shekhar et al., 2006). Lactate-induced responses were also blocked by glutamate antagonists of ionotropic or metabotropic receptor microinjected into the DMH, and by microinjections of tetrodotoxin in the organum vasculosum lamina terminalis (OVLT) (but not in the subfornical organ) (Shekhar & Keim, 1997). Furthermore, lactate-induced responses in panic-prone rats were facilitated by peripheral injections of yohimbine (Shekhar, Sajdyk, Keim, Yoder, & Sanders, 1999, Shekhar et al., 2006), but blocked by alprazolam (Shekhar & Keim, 2000). The involvement of amygdala in lactate-induced autonomic and behavioral effects (SI) were reproduced in rats subjected to chemical kindling of the amygdala through the repeated microinjections into the BLA of antagonists of GABA-A receptor or urocortin, an analogue of corticotrophin releasing hormone (CRH) (Sajdyk, Schober, Gehlert, & Shekhar, 1999; Sajdyk & Shekhar, 2000).

However, Shekhar's recent studies raised serious doubts about the homology of lactate-induced panic attacks in rats and humans. First, Johnson and Shekhar (2006) reported that whereas the lactate-evoked responses were facilitated by the microinjection of angiotensin-II in the DMH, they were attenuated by the angiotensin-II antagonists saralasin (non-selective) and losartan (type-1 selective). As a result, these authors concluded that lactate activates angiotensinergic projections from the OVLT to DMH neurons which project in turn to the BNST and CeA to mediate anxiety-like behaviors, and to the brainstem sympathetic and parasympathetic nuclei to mediate the cardiovascular and respiratory components of panic responses. Next, Johnson et al. (2010) showed that lactate-induced responses were blocked by antagonists and gene silencing of orexin-1 receptor. Altogether, the latter studies suggest that lactate-induced responses in panic-prone rats were mediated by angiotensin and orexin peptides believed to play a crucial role in drinking (Antunes-Rodrigues, Elias, Valença, & McCann, 2004; Fitzsimons, 2008), feeding (Rodgers, Ishii, Halford, &

Blundell, 2002) and, in the case of orexin, wakefulness (Siegel, 1999; Taheri, Zeitzer, & Mignot, 2002). The relationship of lactate-induced responses in rats and drinking mechanisms was further suggested by the puzzling finding of Molosh et al. (2010) showing that panic-like behaviors of panic-prone rats were also produced by the intravenous infusion of .5 M sodium chloride. Because iso-osmolar infusions of D-mannitol were ineffective, they concluded that panic attacks were the result of the activation of osmosensitive pathways by increased concentrations of sodium, but not lactate. Nevertheless, Kellner et al. (1998) had long shown that neither the PD patients, nor controls presented panic attacks during the intravenous infusion of sodium chloride solutions (2.7%) iso-osmolar to .5 M sodium lactate. Therefore, although we cannot discard the involvement of angiotensin-II and orexin in anxious states accompanying thirst, hunger and eating disorders, the lactate-induced responses in 'panic-prone' rats are unlikely to reproduce the clinical panic. Actually, the behavioral effects of lactate infusions were evaluated in procedures believed to model GAD but not PD.

There are but two more reasons which make unlikely the participation of DMH in panic attacks. First, although the chemical stimulation of DMH in pentobarbital anesthetized rats produced only small increases in ACTH (70%) and corticosterone (80%) plasma levels, its stimulation in conscious rats increased ACTH plasma levels in about 600% (Zaretskaia, Zaretsky, Shekhar, & DiMicco, 2002). Second, recent studies from our laboratory showed that whereas the electrical stimulation of DMH with low-resolution intensity-varying sine-wave pulses elicited all defensive responses of the rat (including the panic-like behaviors of galloping and jumping), stimulation with high-resolution frequency-varying square-wave pulses produced only exophthalmus in the DMH *pars diffusa* (DMHd) or exophthalmus, immobility, defecation and micturition in the DMH *pars compacta* (DMHc). The latter responses were also observed following a low-resolution chemical stimulation of DMHd with NMDA. However, in the presence of food, electrical and chemical stimulations of DMHd elicited intensity- and dose-dependent robust ingestive behaviors in sated rats. Conversely, the entire defensive repertoire of the rat, including the panic-like behaviors of galloping and jumping, was produced in three rats which electrodes were inadvertently implanted in the dorsomedial division of VMH (Alves, 2007). These data suggest that the defensive behaviors induced by stimulation of DMHd were most probably due to the current spreading to the latter nucleus. While these data support the involvement of VMH 'cap' in the expression of defensive behaviors (Blanchard et al., 2005), they suggest a limited participation of DMHc if any. As a matter of fact, a recent study reported that the

electrical stimulation of human VMH produces panic attacks (Wilent et al., 2010).

In turn, Lovick and collaborators carried out several studies showing that the neural excitability of PAG of female rats fluctuates across the estrous cycle (for review see Lovick, 2006, 2008; Lovick & Devall, 2009). Particularly, neurons of dorsolateral PAG (DLPAG) show a significant increase in the expression of alpha4/beta1/delta subunits of GABA receptors in the late diestrus as compared to the other phases of the estrous cycle or to their expression in male rats (Lovick, Griffiths, Dunn, & Martin, 2005). Moreover, these authors showed that most alpha4/beta1/delta GABA receptors of DLPAG are localized in GABA neurons (autoreceptors) as revealed by the double labeling for the corresponding subunits and glutamic acid descarboxilase (Griffits & Lovick, 2005a, 2005b). Therefore, because the alpha4/beta1/delta GABA receptors show an outstanding sensitivity to iontophoretic injections of GABA (Lovick et al., 2005), the upregulation of alpha4/beta1/delta autoreceptors is likely to reduce the GABA inhibitory tonus within the DLPAG. Lovick and collaborators suggested that the increased expression of alpha4/beta1/delta autoreceptors may contribute to both the development of LLPDD and to the high comorbidity of LLPDD and PD (Yonkers, Pearlstein, & Rosenheck, 2003). These mechanisms may also explain the similar susceptibility of PD and LLPDD patients to CO_2 (Gorman et al., 2001; Kent et al., 2001) and the higher susceptibility of LLPDD patients to panicogenic challenges in late luteal phase (Le Mellédo, Van, Coupland, Lott, & Jhangri, 2000). In the same vein, Lovick and collaborators showed that the pressor response, the tachycardia and the tachypnoea produced by systemic injections of a CCK-related panicogen (pentagastrin) were all enhanced in late diestrus. For rats in oestrus, the pressor response and tachycardia but not tachypnoea were also significantly larger than the response evoked in early diestrus (Brack, Jeffery, & Lovick, 2006). In addition, extracellular recordings from output neurons in the DPAG showed that the increase in firing rate produced by the intravenous administration of pentagastrin or the iontophoretic injection of the GABA-A receptor antagonist bicuculine, is significantly increased during estrus and late diestrus as compared with proestrus and early diestrus (Brack & Lovick, 2007).

These mechanisms may be complementary to those suggested to underlie LLPDD. Women's bimodal incidence of catamenial epilepsy suggests that brain excitability increases in the middle of the cycle, between the peak of estradiol and the early surge of progesterone, and during the sharp fall of progesterone just before menses (Smith & Wooley, 2004). However, while the neuroexcitant properties of estradiol are responsible for the increased excitability around the midcycle peak, the higher excitability in early and late luteal phase are most probably associated with changes in the level of the progesterone

neuroactive metabolite allopregnanolone ($3\alpha,5\alpha$-THP). However, whereas the acute injections of progesterone and allopregnanolone have conspicuous anxiolytic effects in rats, the 72 hour exposure to progesterone produces increases in anxiety akin those observed in women's early surge of progesterone. Thereafter, anxiety decays to baseline levels despite the sustained exposure of rats to progesterone. Anxiety is but reinstated following the sudden withdrawal of progesterone which mimics the rapid decay of this hormone preceding menses (Gallo & Smith, 1993; Smith & Wooley, 2004). Anxiety increases in early and late luteal phases seem to be due to the allopregnanolone upregulation of alpha4 subunit of GABA-insensitive receptors (Gulinello, Gong, Li, & Smith, 2001, Gulinello, Gong, & Smith, 2002; Smith, 2002). Indeed, progesterone withdrawal anxiety increases in rats can be prevented by indomethacyn blocking of the breakdown of progesterone to allopregnanolone. Thus, allopregnanolone rather than progesterone is the active compound (Smith et al., 1998). In fact, allopregnanolone levels of LLPDD treated patients were significantly lower in the improved compared with the unimproved subjects. Improvement was also significantly associated with lower allopregnanolone levels for premenstrual depression and appetite changes (Freeman, Frye, Rickels, Martin, & Smith, 2002). Therefore, the increased incidence of anxiety and panic in late luteal phase seem to be mediated by distinct effects of allopregnanolone in GABA transmission, i.e., the upregulation of alpha4 GABA-insensitive receptors in areas controlling anxiety, or the upregulation of alpha4/beta1/delta GABA-sensitive autoreceptors in DPAG, respectively.

A third group of experiments examined the effects of hypothyroidism in DPAG-evoked defensive responses (Siqueira, Rossoni, & Schenberg, 2010a; Siqueira, Rossoni, Tiengo, Tufik, & Schenberg, 2010). The relationship between panic attacks and hormone imbalance was originally suggested by Klein (1964) in his pioneering study of the pharmacotherapy of panic and anxiety disorders. Indeed, 12 of 14 patients of the latter study had the first panic attack in a context of endocrine disturbance, including oophorectomy, hysterectomy, post- or pre-partum conditions and unbalanced therapy of hypothyroidism. In order to clarify the conflicting clinical data about the comorbidity of PD and thyroid diseases, recent experiments from our laboratory showed that the systemic injections of low doses of thyrotropin-releasing-hormone (TRH, 1 µg/kg, i.p.) produced significant increases in the thresholds of DPAG-evoked responses of immobility (40%), trotting (33%), galloping (34%), jumping (39%) and exophthalmus (43%). In contrast, TRH had no effects on EPM arm exploration (Siqueira, Rossoni, & Schenberg, 2010). In another study, the effects of the anti-thyroid agent methimazole (MTZ) were evaluated either on the panic-like behaviors induced by electrical stimulation of DPAG or the anxiety-like behaviors of rats exposed to

the EPM. Data showed that the 10-day treatment with MTZ produced marked increases in the thresholds of exophthalmus (65%), immobility (75%), trotting (63%), galloping (56%), jumping (47%), defecation (114%) and micturition (85%). Effects outlasted the drug discontinuation. In contrast, MTZ had variable effects in the EPM which did not correlate with biochemical indices of hypothyroidism (Siqueira, Rossoni, Tiengo, Tufik, & Schenberg, 2010b). These studies support the attenuation of panic attacks in hypothyroidism. It remains to be elucidated whether these effects are mediated by DPAG-projecting TRHergic neurons of DRN or by the unique population of TRH neurons in the VLPAG.In any event, the above studies suggest that experiments devised to reproduce the clinical features of PD or the comorbidity of this disorder with other conditions are an obligatory step in the investigation of the neurobiology of PD.

The smothered brain

Panics induced by lactate, hypercapnia and anoxia were barely discussed in DGH (Deakin & Graeff, 1991). Conversely, they are the very core of Klein's SFA hypothesis (Klein, 1993; Preter & Klein, 2008). Although these hypotheses might refer to the otherwise distinct situational and spontaneous panic attacks, the present section examines whether the PAG might harbor a suffocation alarm system.

Breathing is under the control of a widespread network involving structures from caudal medulla up to the hypothalamus, limbic system and cortex. Whereas the latter structures are most probably involved in the thermoregulatory, emotional and volitional respective aspects of respiration, the NTS is the recipient of glossopharyngeal afferents which convey blood gas information from carotid body chemoreceptors (Chitravanashi & Sapru, 1995; Finley & Katz, 1992). Among several suprapontine targets (Ricardo & Koh, 1978), the NTS second order respiratory neurons project to the ventral respiratory nuclei of medulla that control the phrenic nerve activity (Ter Horst & Streefland, 1994). However, the respiratory drive is likewise dependent on the chemosensitive cholinergic neurons of ventral surface of medulla which senses pH and CO_2 levels of cerebrospinal fluid (Loeschcke, 1958, 1982; Pappenheimer, Fencl, Heisey, & Held, 1965; Schlaefke, 1981).

In any event, while the peripheral and central chemoreceptors monitor blood/brain gases continuously, panic attacks occur only sporadically, being extremely rare in healthy people. Accordingly, the SFA hypothesis presumes that gas sensors and suffocation alarm systems may operate separately eventually. Uncoupling of these systems would result in panic attacks either spontaneous (upon normal air conditions) or induced by chemicals (lactate, CO_2, CCK, etc). Klein's SFA also predicts that panic attack effectors (i.e., the suffocation alarm system output) are activated by degrees of hypoxia and hypercapnia that produce manifest feelings of breathlessness and 'hunger for air' in either PD patients or healthy volunteers. In mechanically ventilated humans, Banzett, Lansing, Evans and Shea (1996) showed that air hunger threshold is 43 Torr, i.e., only 4 Torr above resting end tidal PCO_2 ($P_{ET}CO_2$). In turn, the 50 Torr $P_{ET}CO_2$ produces an unbearable hunger for air. Thresholds and tolerance to CO_2 varied among individuals, but did not correlate significantly with the ventilatory response. Hypoxia ($P_{ET}O_2$ 60-75 Torr) shifted the $P_{ET}CO_2$ threshold and tolerance by 2 Torr only and breathing greatly reduced the hunger for air experienced at any $P_{ET}CO_2$, increasing the thresholds by 5 Torr and decreasing the sensitivity in about 50%. More important, while Moosavi et al. (2003) found that hypercapnia and hypoxia have equal potency in producing hunger for air in healthy subjects matched by ventilatory drive, Beck, Shipherd, & Ohtake (1999) reported that panic patients present increased panic symptoms to both hypercapnia and normocapnic hypoxia. Overall, these data support the presence of a single suffocation alarm system endowed with the capability to integrate the main respiratory inputs (H^+/CO_2, O_2) and, possibly, lactate brain levels and toxic gases as well. Contrarily, studies carried out in subjects either curarized or quadriplegics suggested that mechanoreceptor afferents play a minor role in producing hunger for air (Banzett, Lansing, Reid, Adams, & Brown, 1989; Gandevia et al., 1993). Nevertheless, it has been extremely difficult to tease out the suffocation alarm system from respiratory centers controlling eupnoea.

Feelings of breathlessness and hunger for air are most likely processed by supramedullary structures activated during hypoxia and hypercapnia. Cognitive hypotheses suggest that the urge for air arises from 'corollary discharges' of medullary respiratory nuclei conveyed to forebrain structures (Moosavi, Banzett, & Butler, 2004). This model predicts that the development of hunger for air should parallel the central respiratory responses to both hypoxia and hypercapnia. Because the ventilatory response to sustained hypoxia is a sharp ventilatory increase followed by a slow decay beyond 5 minutes, Moosavi et al. (2004) tested the corollary discharge hypothesis comparing the time course of hunger for air of sustained isocapnic hypoxia in mechanically ventilated healthy subjects to the time course of the ventilatory response of the same subject in separate free-breathing trials. Data showed that during sustained hypoxia of mechanically ventilated healthy subjects, the initial increase in hunger for air is followed by a progressive decline mirroring the biphasic pattern of reflex respiratory response. Admittedly, a mismatch of respiratory drive and forebrain corollary processing could trigger a suffocation false alarm, producing a panic attack.

Otherwise, the malfunctioning of supramedullary nuclei which take part/modulate the suffocation alarm system could produce panic attacks irrespective of the forebrain processing of respiratory corollary discharges. Indeed, *in vivo* and *in vitro* experiments showed that the LC, PAG, raphe nuclei and posterior hypothalamus (PH) have neurons intrinsically sensitive to either hypoxia or hypercapnia (Coates, Li, & Nattie, 1993; Dillon & Walldrop, 1992; Kramer, Nolan, & Waldrop, 1999; Pineda & Aghajanian, 1997; Ryan & Waldrop, 1995). Thus, although we cannot discard the abnormal functioning of any respiratory structure, including the peripheral and central chemoreceptors, the unusual sensitivity of PD patients to CO_2 and lactate is most likely due to the dysfunction of supramedullary gas sensors. Be this as it may, it remains to be elucidated why these nuclei become so sensitive in PD patients. Lastly, because gas sensors and suffocation alarm systems are continuously interacting, Klein's hypothesis also predicts that panic attacks could be also susceptible to respiratory maneuvers (exposure to hypercapnia and hypoxia, paced respiration, active hyperventilation, lactate challenge) or respiratory-acting drugs (opioids, progesterone, thyroid hormones, cholinergic agents, etc) (Battaglia, 2002; Klein, 1993; Preter & Klein, 2008). At any rate, the brain defense structures placed at the crossroad of respiratory pathways from medulla to forebrain are in a strategic position to trigger a panic attack.

Brain areas activated in hypoxia and hypercapnia

Neuroimaging studies of hypercapnia can provide valuable information about the main structures of the suffocation alarm system. Most often, these studies were performed with the co-registration of magnetic resonance imaging (MRI) and positron emission tomography (PET) scans of suprapontine structures of healthy volunteers which presented definite feelings of smothering, breathlessness or hunger for air during inhalation of CO_2 (Brannan et al., 2001; Corfield et al., 1995; Liotti et al., 2001). Particularly, Brannan and colleagues (2001) carried out a detailed study which contrasted the PET scans of hypercapnia (8% CO_2/92% O_2) with those of oxygen inhalation (9% N_2/91% O_2). Data revealed the activation of a vast array of structures either cortical or subcortical, in one or both sides of the brain. Significant increases in regional cerebral blood flow (rCBF) were found in pons, PAG, midbrain tegmentum, hypothalamus, amygdala and sublenticular periamygdalar region, hippocampus (BA28) and parahippocampal areas (BA 20/36), subgenual anterior cingulated gyrus (BA24), fusiform gyrus (BA19/37), middle temporal gyrus (BA9), anterior insula, pulvinar, putamen/caudatum and several sites in midline and lateral cerebellum. Brain activated areas were also likely to involve the LC, PBA and DRN. In contrast, there were significant deactivations in dorsal anterior cingulated gyrus (ACg, BA24), posterior cingulated cortex (BA23/31) and lateral pre-frontal cortex (BA9/46).

In contrast, structures lighted up during lactate- and CCK-induced panic attacks were only a small subset of the extensive network associated to breathlessness and hunger for air. Most notably, the only structures activated in both the hypercapnia and panic attacks were the subgenual ACg, insula, hypothalamus, PAG, midline cerebellum and, possibly, LC (note that PBA was not activated in either the lactate- or CCK-induced panic attack).

Corroborating the PET pioneering study of lactate-induced panic attacks (Reiman et al., 1989b), exposure to CO_2 also activated the temporopolar cortex (Liotti et al., 2001). Because the latter authors employed high-resolution MRI-coupled PET, the activation of temporopolar cortex could not be ascribed to changes in extracerebral blood flow recorded in CCK-induced panics (Benkelfat et al., 1995; Drevets, Videen, MacLeod, Haller & Raichle, 1992; Javanmard et al., 1999). Thus, the study of Liotti et al. (2001) both rekindles the controversial issue of the activation of temporopolar cortex in lactate-induced panic attacks and suggests that this activation may be due to the lactate conversion to CO_2. However, whereas the region of insula/claustrum was only activated in lactate- and CCK-induced panic attacks, the temporopolar cortex and parahippocampal gyrus were also activated during anticipatory anxiety of both volunteers and panickers (Reiman, Fusselman, Fox & Raichle, 1989a).

Because the PET signal is a function of rCBF, the vasodilator properties of CO_2 and sodium lactate are a major drawback of above studies (Corfield et al., 1995; Gorman, Kent, Sullivan, & Coplan, 2000). Actually, the only PET study of a spontaneous panic attack (a single-patient record) raises a major concern insofar that it reported only deactivations in the right orbitofrontal (BA11), prelimbic (BA25), anterior temporal (BA15) and anterior cingulate (BA32) cortices (Fischer, Andersson, Furmark, & Fredrikson, 1998).

Otherwise, because c-fos protein expression does not depend on rCBF, c-fos immunohistochemical studies of hypoxia and hypercapnia in animals may be a reliable control of corresponding PET studies in humans. Unfortunately, however, PET human studies were most often focused on suprapontine structures, whereas c-fos animal studies examined pontine and medullary regions (Erickson & Milhorn, 1994; Haxhiu, Yung, Erokwu, & Cherniack, 1996; Hirooka, Polson, Potts, & Dampney, 1997; Sato, Severinghaus, & Basbaum, 1992; Teppema et al., 1997). Yet, some studies examined c-fos expression up to the rostral midbrain and diencephalon of rats and cats (Berquin, Bodineau, Gros, & Larnicol, 2000; Larnicol, Wallois, Berquin, Gros, & Rose, 1994; Teppema et al., 1997). A second difficulty concerns to the rather different protocols of PET and c-fos studies. Thus, whereas the PET human studies employed short exposures (minutes)

to moderate degrees of hypercapnia (8 %), c-fos animal studies employed long exposures (hours) to varying degrees of hypoxia (5% to 11 %) or to a moderate hypercapnia (15%) (Berquin et al., 2000; Larnicol et al., 1994; Teppema et al., 1997). Brain activations of rats exposed to hyperoxic hypercapnia (60% O_2/15% CO_2/25% N_2) were also examined (Teppema et al., 1997).

Exposure to moderate degrees of hypoxia (8% O_2) produced a significant increase in fos-like immunoreactivity (FLI) in medullary nuclei that play a crucial role in eupnoea (Berquin et al., 2000), including the commissural and caudal NTS and the parapyramidal neurons lying just above the ventral surface of the medulla. Teppema et al. (1997) reported additional labeling in ventrolateral groups of medulla, including the C1 adrenergic and A1 noradrenergic groups following a similar degree of hypoxia (9% O_2) (labeled cells were found mostly in rostral and caudal ventrolateral nuclei, and none in paragigatocellularis lateralis). The NTS was also activated by 5% (Berquin et al., 2000) and 15% CO_2 (Teppema et al., 1997). However, the number of labeled cells in rostroventrolateral medulla (RVLM) following the exposure to 15% CO_2 was almost 2.5 times greater than those observed in the 9% O_2 group, corroborating the long known sensitivity of these neurons to hypercapnia (Mitchell & Hebert, 1974). Interestingly, addition of 60% O_2 to 15% CO_2 greatly reduced the number of CO_2 activated cells in both the NTS and RVLM (Teppema et al., 1997). Hypercapnic rats also presented a superficial column of labeled cells in the ventral surface of the medulla (0-100 μm depth) which was absent in hypoxic rats (Teppema et al., 1997). These neurons are part of retrotrapezoid nucleus and may correspond to the paraolivary cell group reported by Berquin et al. (2000).

Neurons of ventral surface of the medulla seem to correspond to the caudal and rostral central sensors of H^+/CO_2 formerly suggested by Loeschcke and Koepchen (1958). Indeed, Loeschcke (1982) pointed out that pH changes of ventral surface of cats produce '*maximal drive of ventilation … in two areas, one medial to vagal root and the other medial to the hypoglossal root but lateral to the pyramids*'. (p.7) Surprisingly, however, pre-Bötzinger/ ventrolateral areas of the medulla which are supposed to play a critical role in eupnoea (Feldman, Mitchell, & Nattie, 2003) were only activated by the higher level of hypercapnia (15%) (Teppema et al., 1997).

In dorsolateral pons, the external division of lateral parabrachial area (LPBA) and the Kölliker-Fuse nucleus (KFN) were activated by both the hypercapnia and the hypoxia (Berquin et al., 2000; Teppema et al., 1997). Remarkably, however, midbrain structures not belonging to the established brainstem respiratory nuclei showed significant increases in FLI. Particularly, the DLPAG and VLPAG, as well as the LC and nucleus subcoeruleus, were intensely labeled by moderate

degrees of both hypoxia and hypercapnia (yet, despite the high counts of labeled cells in DLPAG of hypoxic rats, values were not statistically significant) (Berquin et al., 2000). Importantly, c-fos labeled neurons of LC of hypercapnic rats were double-stainned for tyrosin hydroxylase (Teppema et al., 1997). These finding agree with *in vitro* experiments showing that LC neurons are sensitive to CO_2 (Pineda & Aghajanian, 1997). Conversely, the LC was not stained in hypoxic rats (Berquin et al., 2000; Teppema et al., 1997; but see Erickson & Milhorn, 1994).

More rostrally, Berquin et al. (2000) found significant increases of FLI in DMH, PH and in a ventrolateral area extending from the mammillary nuclei to the retrochiasmatic area (ventral hypothalamic zone). Although not significant, there were also FLI increases of 184% in the PVN of hypoxic rats and of 460% in the SON (supraoptic nucleus) of hypercapnic rats. Activations of PVN, SON and DMH are likely involved in HPA responses to CO_2. Activation of PH is particularly important insofar that this nucleus harbors hypoxia- and hypercapnia-sensitive neurons that project to the PAG (Ryan & Waldrop, 1995). In fact, the latter authors suggested that PAG and PBA may be key relays connecting the PH with medullary nuclei involved in the ventilatory response to both hypoxia and hypercapnia. Lastly, because burrowing species (mole rats) are able to tolerate high levels of hypoxia (7.2% O_2) and hypercapnia (6.1% CO_2) (Shams, Avivi, & Nevo, 2005), it is worth stressing that these conditions lead to similar results in rats and cats (Berquin et al., 2000; Larnicol et al., 1994). Actually, the only difference was the lack of label in the retrotrapezoid nucleus of hypoxic rats.

Evidence of a suffocation alarm system within the periaqueductal gray matter

During hypercapnia, the only structures activated in both the PET and c-fos studies were the PH, PAG, PBA and, possibly, LC. Except for the PBA, these areas had chemosensitive neurons to H^+/CO_2 and/or O_2. In contrast, although the cerebellum was examined in both kinds of studies, the regular activation of midline and cortical areas of cerebellum observed during the hypercapnia and experimental panic attacks in humans was not corroborated in c-fos studies of animals exposed to hypercapnia. Indeed, Coates et al. (1993) showed that phrenic nerve activity was markedly increased by microinjections of acetazolamide (an inhibitor of carbonic anhydrase) in several brainstem regions in servo-ventilated paralyzed anesthetized rats and cats which were both vagotomized and glomectomized. Responsive sites at tissue pH compatible with an end-tidal PCO_2 of approximately 36 Torr were found within 800 μm of the ventrolateral medullary surface, within the vicinity of the NTS and LC. Authors concluded that

central chemoreceptors are distributed at many locations within the brainstem, all within 1.5 mm of inner and outer brainstem surface.

The sensitivity PH to both hypoxia and hypercapnia was also supported by whole-cell patch-clamp and extracellular recordings from brain slice preparations (Dillon & Waldrop, 1992). Extracellular recordings showed that hypoxia stimulated over 80% of tested neurons in a dose-dependent manner. In addition, more than 80% of cells excited by hypoxia retained this response during synaptic blockade. In contrast, hypercapnia increased the discharge frequency in only 22% of neurons. Similarly, whole-cell patch recordings showed that whereas hypoxia produced a depolarization in 76% of these neurons, depolarization and/or increase in discharge rate were observed in only 35% of neurons during hypercapnia. These results suggest that separate subpopulations of caudal hypothalamic neurons are sensitive to hypoxia and hypercapnia. Ryan and Waldrop (1995) further demonstrated that PAG-projecting PH neurons were sensitive to both hypoxia (53%) and hypercapnia (27%). Many of these sites were usually referred as belonging to the brain defense system.

More important to the present study, Kramer et al. (1999) showed that the majority (39 of 53) of PAG neurons of rat brain slices responded to hypoxia, 92% of which with an increase in firing rate. Notably, as well, hypoxia-sensitive neurons in DLPAG/LPAG regions increased firing rates to a greater extent than those located in VLPAG. The former neurons retained the response to hypoxia after tissue perfusion with a low Ca^{2+}/high Mg^{2+} solution to block synaptic transmission, thereby supporting their intrinsic sensitivity to a fall of O_2 tissue levels. A much smaller proportion (7/33) of neurons responded to hypercapnia.

Although the PAG is not required for respiratory rhythmogenesis, its stimulation is long known for its prominent effects on respiration. Indeed, in the first study ever published with electrical stimulation of PAG, Sachs (1911) reported that among all stimulated regions in the thalamus and midbrain, the PAG yielded the most pronounced respiratory responses, being '*undoubtedly ... a respiratory center*'. (p.418) These pioneering observations were corroborated by extensive mapping of electrically-induced respiratory responses (Kabat, 1936). Recent studies showed that chemical stimulation of PAG predominantly affects the respiratory frequency, decreasing the duration of both phases of respiration (Hayward, Swartz, & Davenport, 2003). Because the chemical stimulation of DPAG produces a marked increase in FLI of LPBA, PAG-evoked respiratory responses are most probably mediated by the latter nucleus (Hayward, Castellanos, & Davenport, 2004). Moreover, bilateral microinjections of GABA-A agonists or glutamate antagonists in the LPBA produced marked attenuations of DPAG-evoked changes in

respiration (-65% to -90%), heart rate (-53% to -72%) and, to a lesser degree, blood pressure (-34% to -57%). These results support a significant role of LPBA in the DPAG-mediated respiratory and cardiac components of defensive behaviors (Hayward et al., 2004; Zhang, Hayward, & Davenport, 2007).

In spite of the above evidence implicating the PH, PAG, LPBA and LC in the suffocation alarm system, panic patients could present a respiratory dysfunction at any level of respiratory circuits, including central or peripheral chemoreceptors. Indeed, studies in decerebrated cats have long suggested that whereas the baroreceptor activation inhibits the sham-rage behaviors (Bartorelli, Bizzi, Libretti, & Zanchetti, 1960), chemoreceptor inputs have a facilitatory effect (Bizzi, Libretti, Malliani, & Zanchetti, 1961). As a matter of fact, incidental observations of Franchini and Krieger (1993) showed that the selective stimulation of carotid chemoreceptors by low doses of potassium cyanide (KCN, 30 µg/kg) produces defensive behaviors which are reminiscent of those evoked by electrical and chemical stimulations of DLPAG. Recent observations from our laboratory showed that these responses are predominantly alerting, scanning, sniffing, raising and trotting (Schimitel, 2009). However, gallops were also induced at higher doses. The involvement of arterial chemoreceptors in KCN-induced behavioral and respiratory responses was demonstrated by the lack of these responses in rats either sinus-denervated or subjected to the ligation of carotid body artery (Franchini & Krieger, 1993; Franchini, Oliveira, & Krieger, 1997). Reciprocally, electrical stimulation of PAG and hypothalamus defense areas was shown to facilitate the chemoreceptor ventilatory response to both KCN and dinitrophenol (Hilton & Joels, 1965). Finally, Hayward and von Reitzentstein (2002) showed that whereas the chemoreceptor stimulation with KCN induces c-fos expression throughout the DLPAG and LPAG, baroreceptor stimulation labels only the caudal districts of dorsomedial and lateral columns. This pattern is similar to that induced by systemic hypoxia (Berquin et al., 2000). Hayward and von Reitzentstein (2002) also showed that less than 10% of neurons of caudal PAG which expressed c-fos after KCN injections were retrogradely labeled from the A5 region of pons which is believed to mediate the pressor component of chemoreceptor reflex (Guyenet & Koshiya, 1995). Overall, these results support the chemoreceptor facilitation of DPAG-evoked defensive behaviors and, reciprocally, the DPAG facilitation of chemoreceptor ventilatory reflex. More important, however, because Hayward and von Reitzentstein (2002) carried out their experiments in urethane anesthetized rats, KCN activation of DPAG was not secondary to emotional responses. Rather, they suggest the DPAG intrinsic sensitivity to hypoxic states.

The DPAG-evoked cardiovascular and respiratory responses have been long associated with the visceral adjustments supportive of fight and flight behaviors (Carobrez, Schenberg, & Graeff, 1983; Hilton, 1982; Lovick, 1996; Schenberg et al., 1981, 1983; Subramanian, Balnave, & Holstege, 2008). The detailed study of DPAG-evoked respiratory responses was but carried out only recently by Paydaphar and Eldridge (1987). These authors examined the DPAG-induced facilitation of phrenic nerve activity of sino-aortic denervated vagotomized anesthetized cats kept at constant $P_{ET}CO_2$ (30-35 Torr). Data showed that whereas the electrical stimulation of DPAG with supraliminal intensities produces inspiratory apneusis, brief stimulations (1-s trains) with threshold stimuli invariably reset the cycle to inspiratory or expiratory phase. Following cycle resetting, the respiratory rhythm resumed with predictable periodicity. There was but a unique stimulus applied at a definite timing of inspiratory-expiratory transition which resulted in unpredictable time recovery of respiratory cycle (random cophase duration). This response is exactly what would be expected in dyspnoea, the leading symptom of panic attack (Klein, 1993; Preter & Klein, 2008). Paydaphar and Eldridge (1987) also showed that critically timed stimuli with appropriate intensity resulted in prolonged inspiratory apneusis that lasted up to 3 times the duration of a respiratory cycle. These data show that subtle stimulations of DPAG are able to produce prolonged episodes of dyspnoea which are typical of panic attacks. Altogether, the above data strongly support an anoxia-sensitive suffocation alarm system within the DPAG.

A reconciling hypothesis

Recently we examined the behavioral effects of CO_2-enriched air (0-13%) or of the cytotoxic anoxia of chemoreceptors by low doses of KCN (10-80 µg, i.v.), administered either alone or combined with electrical stimulation or the electrolytic lesion of DPAG (Schimitel, 2009).

Exposure to 13% CO_2 alone produced a manifest increase in respiration. However, rats either explored the open-field slowly or remained quiet with eyes opened and muscles relaxed as indicated by the lowering of the trunk, head and tail. Similarly, Teppema et al. (1997) had already shown that CO_2 concentrations as high as 14% failed in inducing any defensive behavior in rats. Although hypoactive, rats exposed to low concentrations of CO_2 seem not sleepy as indicated by the full opened eyes and the reported activation of LC (Berquin et al., 2000; Teppema et al., 1997).

Exposure to 8 and 13% CO_2 also produced a significant facilitation of defecation and micturition both spontaneous and induced by electrical stimulation of DPAG. Unexpectedly, however, thresholds of DPAG-evoked immobility, trotting and galloping were markedly increased in the presence of CO_2. Because the DRN and the VLPAG were shown to be markedly activated by hypercapnia (Berquin et al., 2000; Teppema et al., 1997), the CO_2 inhibitory effects of spontaneous behaviors may correspond to the 'hyporeactive immobility' produced by chemical stimulation of VLPAG (Morgan & Carrive, 2001). For similar reasons, the CO_2 attenuation of DPAG-evoked defensive responses is most likely due to the activation of VLPAG and DRN inhibitory projections to the DPAG (Brandão, Lopez-Garcia, Graeff, & Roberts, 1991; Kiser et al., 1980; Lovick, 1992, 1994; Pobbe & Zangrossi, 2005; Stezkha & Lovick, 1994).

The respiratory activity was also markedly increased by KCN. However, the KCN alone produced all defensive responses of the rat in a dose-dependent fashion similar to that produced by stepwise stimulation of DPAG (Bittencourt et al., 2004; Schenberg et al., 2005). Exceptions were jumping, which was elicited in one rat only, and defecation and micturition which effective doses were unusually low as compared to the electrical and chemical effective stimuli. Because the NTS sends profuse efferents to the DPAG (Bandler & Tork, 1987) and the KCN-induced defensive behaviors are blocked by both the sinus denervation and the ligation of carotid body artery (Franchini & Krieger, 1993; Franchini et al., 1997), the KCN-evoked defensive behaviors are most probably due to the NTS-mediated chemoreceptor inputs of DLPAG and LPAG (but not VLPAG, see Hayward and von Reizenstein, 2002).

KCN effects on DPAG-evoked defensive responses were much the opposite of those of hypercapnia. Indeed, KCN injections facilitated all defensive responses but micturition. Moreover, in spite of the cytotoxic effects of KCN, energy demanding responses of trotting and galloping were more facilitated than the immobility.

Finally, KCN-evoked defensive responses were either blocked (immobility, galloping and jumping) or markedly attenuated (immobility, exophthalmus, defecation and micturition) following the electrolytic lesion of the same sites whose stimulation in screening sessions evoked the defensive responses with intensities below 50 µA. Although the damage of fibers of passage cannot be discarded, these effects support the existence of an anoxia-sensitive suffocation alarm system within the DPAG.

Evidence suggests that hypoxia and hypercapnia are both sensed through 'tandem acid sensitive potassium channels' (TASK-1 and TASK-3 channels) at carotid body chemoreceptor cells. Indeed, while the TASK inhibitory currents are inhibited by acid pH and hypoxia, they are activated in alkalosis (Buckler, Williams, & Honore, 2000; Buckler, 2007; Duprat et al., 1997; Kim, Bang, & Kim, 2000; Rajan et al., 2000). TASK genes are also expressed in brain chemosensitive areas, including the RVLM, the raphe nuclei and LC (Bayliss, Talley, Sirois, & Lei, 2001; Talley, Solorzano, Lei, Kim, & Bayliss, 2001; Washburn,
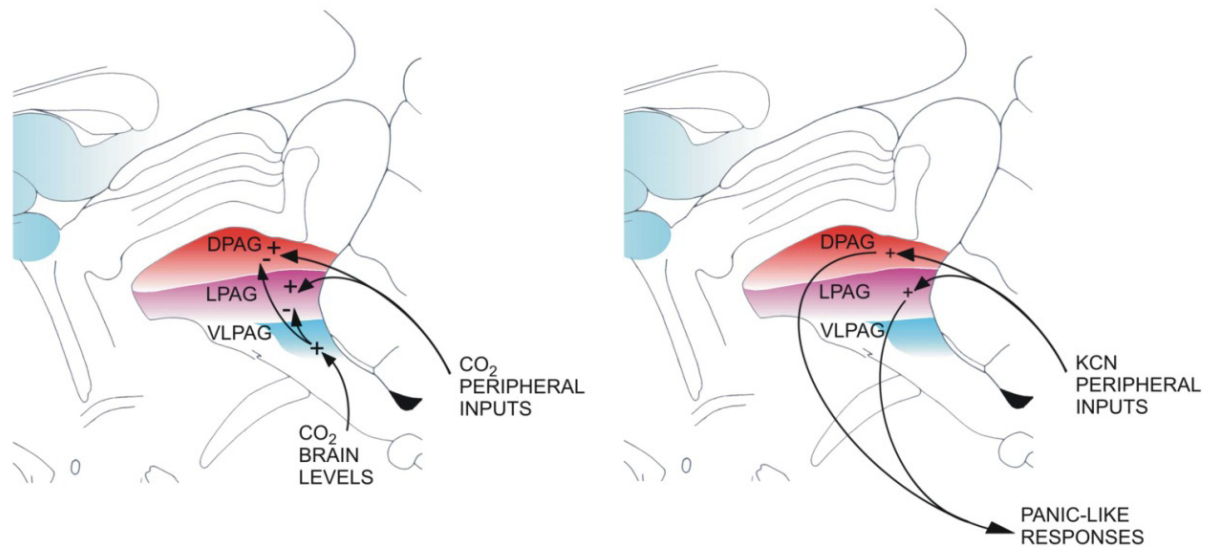
Figure 9. Presumptive mechanisms of sedative- and panic-like responses induced by carbon dioxide (CO_2) and potassium cyanide (KCN), respectively. Peripheral CO_2 inputs to dorsolateral (DLPAG) and lateral (LPAG) periaqueductal gray (PAG) are counteracted by the CO_2 direct stimulation of the ventrolateral PAG (VLPAG). In contrast, KCN activates only the DLPAG and LPAG, thereby producing panic-like responses.

Bayliss, & Guyenet, 2003; Washburn, Sirois, Talley, Guyenet, & Bayliss, 2002). Therefore, TASK channels may also sense pH/brain gas levels in areas involved with the detection of both hypoxia and hypercapnia. Yet, evidence above discussed suggests that the suffocation alarm system of the rat triggers distinct behaviors to deal with hypoxia (flight) and hypercapnia (hyporeactive immobility). Thus, it remains to be elucidated why panic patients react to CO_2 as it were KCN.

NTS projections to PAG are likely to convey the chemoreceptor information of both hypoxia and hypercapnia. However, while the KCN activated the DLPAG and LPAG (Hayward & von Reitzenstein, 2002), the hypercapnia activated the LPAG but also the VLPAG and DRN (Berquin et al., 2000). Behavioral and electrophysiological studies suggest that the stimulation of the latter nuclei inhibits the DLPAG and LPAG (Brandão, Lopez-Garcia, Graeff, & Roberts, 1991; Kiser et al., 1980; Lovick, 1992, 1994; Pobbe & Zangrossi, 2005; Stezkha & Lovick, 1994). Therefore, it is tempting to speculate that the CO_2 activation of VLPAG and NDR aborted the defensive responses to peripheral signals of hypercapnia. Conversely, hypoxia activates mostly the DLPAG and LPAG, thereby producing the freezing and flight behaviors. Be this as it may, the CO_2 hypersensitivity of PD patients could be due to a dysfunction of CO_2-sensitive VLPAG/DRN inhibitory projections to the LPAG/DLPAG (Figure 9).

It should be noted, however, that whereas the DLPAG gets extereoceptive stimuli via SC and PMD and projects to nuclei involved in attention (LC) and motor pattern generation (cuneiform nucleus), LPAG and VLPAG project predominantly to the nuclei of the medulla which may mediate the visceral responses that

are the hallmark of clinical panic. As a matter of fact, LPAG and VLPAG (but not DLPAG) showed similar activations to the exposure of rats to 5% CO_2 only (Berquin et al., 2000). It is thus suggested that DLPAG and VLPAG outputs are funneled through the LPAG, the very gatekeeper of clinical panic. Conversely, situational panic attacks would activate predominantly the DLPAG, LC and cuneiform nucleus.

The latter proposal is supported by the robust activation of HPA axis following the exposure of preys to predators for periods either short (Figueiredo, Bodie, Tauchi, Dolgas, & Herman, 2003; Roseboom et al., 2007) or prolonged (Bateson & Bradshaw, 1997). Therefore, although the predator-induced flight presents a pharmacological profile compatible with panic (Blanchard et al., 1993, 1997; Griebel et al., 1995, 1996), they cannot be equated to clinical panic proper. Most probably, spontaneous panic attacks are, on one hand, due to the activation of LPAG projections to nuclei of the medulla which mediate the visceral responses and, on the other hand, the activation of an 'extra-hypothalamic' loop comprising the DPAG projections to the LC and thence to the orbitomedial prefrontal cortical areas, of which the ACg projects back to the DPAG (Floyd, Price, Ferry, Keay, & Bandler, 2000). These circuits are only a subset of a larger circuitry activated during the exposure of preys to predators.

Conclusion

Even though the animal models are not expected to be the exact reproduction of human psychiatric disorders, the translational modelling of panic attacks is expected to reproduce the main clinical features of PD, including the CO_2 and lactate hypersensitivity, the drug treatment

specificity, the lack of stress hormone responses, the women's higher vulnerability and the high comorbidity of panic with depression and separation anxiety disorders. Thus far, the DPAG model of panic attack fulfilled many requirements, including behavioral, pharmacological, neuroendocrine and sex-dependent features alike those seen in women. The role of DPAG in panic attacks is also supported by complementary data from stimulation and neuroimaging studies in humans. Moreover, KCN experiments support the existence of an anoxia-sensitive suffocation alarm system within the DPAG, thereby corroborating the SFA. Nevertheless, CO_2 did not facilitate the DPAG-evoked defensive responses. Also, the DPAG-evoked responses were not challenged with lactate. Whether the rat lack of sensitivity to CO_2 is a feature of burrowing species or not (Shams, Avivi, & Nevo, 2005), it remains to be elucidated why panic patients react to CO_2 as it were KCN. In any event, the development of animal models of PD allow invasive methods that may reveal brain abnormalities underlying the higher sensitivity of PD patients to both CO_2 and lactate. On the other hand, modelling of the comorbidity of PD with separation anxiety and depression implicate the combination of extant experimental models of panic with those of latter disorders. At any rate, there is still a long track ahead before the development of a clinically validated model of panic attacks.

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