REVISTA MEXICANA DE ANÁLISIS DE LA CONDUCTA 2003 NÚMERO 1 (JUN)
MEXICAN JOURNAL OF BEHAVIOR ANALYSIS **29**, 63-81 NUMBER 1 (JUN)

THE IMPORTANCE OF BRAINSTEM MECHANISMS IN THE NEUROBIOLOGY OF STRESS AND ANXIETY[1]

LA IMPORTANCIA DEL MECANISMO TALLO CEREBRAL EN LA NEUROBIOLOGÍA DEL ESTRÉS Y LA ANSIEDAD

MARCUS L. BRANDÃO, DANIEL V.M. VIANNA AND ANA CAROLINA GARGARO [2]
LABORATÓRIO DE PSICOBIOLOGIA, FFCLRP, CAMPUS USP, BRASIL

ABSTRACT

The medial hypothalamus, amygdala, and dorsal periaqueductal gray (dPAG) constitute the main neural substrates for the integration of aversive states in the brain. In fact, fear-like behaviors often result when these sites are electrically or chemically stimulated. We report here that different fear responses are generated by light, tones and contexts used as conditioned stimuli as well as by unconditioned stimulation of the dPAG. Recent data from this laboratory are also presented showing the influence of past experience with stressful situations on the performance of animals in the fear-potentiated startle and contextual fear procedure. Efforts have been made to characterize the neural circuits recruited in the organization of defensive reactions to these conditioned and unconditioned aversive stimulations. In this review we summarize the evidence linking the brain's defense response systems to the concept of fear-stress-anxiety. Successful preparatory processes of danger-orientation and preparedness to flee seem to be linked to anxiety. On the other hand, fear stimuli that originate active but incomplete forms of defensive response ensure emotional states of a different nature. As a working hypothesis, it is advanced

1. Artículo recibido el 20 de mayo de 2003 y aceptado el 10 de junio de 2003.
2. Correspondence: M.L. Brandão, Laboratório de Psicobiologia, FFCLRP, campus USP, av Bandeirantes 3900, 14049-901, Ribeirão Preto, SP, Brasil. Fax: 55 16 6330619. E-mail: mbrandao@usp.br

that past experience with stressors may lead to this emotional shift. When these animals are submitted to a contextual conditioning procedure there is a switch from the neural circuits responsible for the production of the usual defensive response to an aversive context towards thwarted defense responses related to panic attacks. Therefore, prior experience with stressor events may thwart instinctive, orientated and organized motor patterns of appropriate defensive behaviors to contextual aversive stimuli and result in incomplete and uncoordinated motor acts. This switch process may have the ventral PAG-medial hypothalamus-dorsal PAG circuit as its underlying neural substrate.

Key words: aversion, midbrain tectum, dPAG, superior colliculus, inferior colliculus.

RESUMEN

El hipotálamo medial, la amígdala y la sustancia gris periacueductal (dPAG) constituyen el principal substrato neural para la integración de los estados aversivos en el cerebro. De hecho, conductas como de miedo con frecuencia son resultado de la estimulación química o eléctrica de esos sitios. Reportamos aquí que diferentes respuestas de miedo son generadas por luz, tonos y contextos usados como estímulos condicionados así como incondicionadamente por la estimulación de la dPAG. También se presentan datos recientes de nuestro laboratorio mostrando la influencia de la experiencia pasada con situaciones estresantes sobre la ejecución de los animales en el procedimiento de sobresalto potenciado por miedo y de miedo contextual. Se han hecho esfuerzos por caracterizar los circuitos neurales reclutados en la organización de reacciones defensivas a éstas estimulaciones aversivas condicionadas e incondicionadas. En esta revisión resumimos la evidencia ligando los sistemas cerebrales de la respuesta de defensa con el concepto miedo-estrés-ansiedad. Procesos preparatorios exitosos de orientación-peligro y preparación al escape parecen estar ligados a la ansiedad. Por otro lado, dependiendo del grado de amenaza para la sobrevivencia del animal, estímulos de miedo originan estados emocionales de diferente naturaleza y consecuentemente activan distintos circuitos neurales. Como una hipótesis de trabajo, se ha avanzado en que la experiencia pasada lleva a este cambio emocional. Cuando los animales son sometidos al procedimiento de condicionamiento contextual hay un cambio de los circuitos neurales responsables de la producción de la respuesta defensiva usual a un estímulo aversivo hacia respuestas defensivas opuestas relacionadas con ataques de pánico. Por lo tanto la experiencia previa con eventos estresantes pueden contravenir patrones motores organizados, instintivos y orientados de conductas defensivas apropiadas a estímulos aversivos hacia actos motores incompletos y descoordinados

. Este proceso de cambio puede tener al circuito PAG ventral - hipotálamo medial - PAG dorsal como substrato neural subyacente.

Palabras clave: aversión, tectum, dPAG, colículo superior, colículo inferior.

THE ADAPTIVE VALUE OF ANXIETY

The word fear comes from the old English term for danger, while anxious derives from the Greek root angst, meaning to "press tight" or strangle. Anxiety has been defined as a subjective state induced by an aversive condition of unknown origin while fear has identifiable origin and causes. The dichotomy between anxiety and fear has become clearer when the first has been related to cognition and, hence, to higher brain structures such as amygdala, hippocampus and frontal cortex while fear has been considered as a more primitive reaction and elaborated in brainstem structures. When anxiety and panic are object of similar analysis several differences from the clinical viewpoint also come out. Anxiety is a subjective state of discomfort with behavioral and autonomic manifestations with a slow onset while panic is characterized by a sudden sensation of imminent death with more pronounced neurovegetative changes. Other evidence in favor of qualitatively different phenomenon is of the apparently poor therapeutic response of panic to benzodiazepines and good therapeutic response to monoamine oxidase inhibitors and selective serotonin reuptake inhibitors. Finally, an important related issue concerns the adaptive function of these clinical entities. Cognitive theorists believe that anxiety serves primarily to signal the brain to activate a physical response that will eliminate the source of anxiety. In this way, the role of anxiety is similar to that of pain. The experience of pain leads the individual to do something to stop it. The pain and anxiety are not the disease. They are symptoms of an underneath illness. On the other hand, it is very difficult to imagine what could be eliminated with a panic attack. It is highly doubtful the adaptive value of a primitive, involuntary, somatic and often immobilizing brainstem response as it is the panic attack. Indeed, it does not seem to be an efficient arrangement to have a disturbance in the processing of internal physiological stimuli along with neurovegetative alterations ('catastrophic interpretation') as a strategy to face imminent danger. Based on these arguments it seems that panic and anxiety are really dissociated entities with different mechanisms and neural substrates.

Many neurobiologists do not agree with this view on the basis that panic should be considered as a maladaptive response. Different from panic, there seems to be no considerable dispute on that anxiety has, indeed, an adaptive value insofar as it is a general disturbance in the processing of stimuli from the external world.

There has been good agreement that to understand anxiety and panic, the

appraisal of a situation is of fundamental importance. In this sense, a crucial issue for each condition is not the defensive reaction *per se* but may be related to the earlier stages of information-processing. As such, to interpret a given input as signifying danger/threat when it is not may determine an inappropriate or exaggerated response. Thus, models focusing on adaptive responses of normal animals to an actual/potential threat (virtually all models) may be not modeling panic or anxiety. The majority of the animal models of anxiety focus on its somatic-emotional component, because it is strong and clearly visible whereas the cognitive processing is not easily approached. On the other hand, to focus only on the cognitive aspects of anxiety, may not adequately approach the issue and overlook the fundamental role played by bodily responses and sensations in the experience of anxiety. In the beginning of the last century William James and Karl James drew our attention to the fact that the perception of the emotional stimulus automatically (without conscious participation) produces the responses that provide the feedback that defines the feeling. The meaning of the stimulus plays an important role in the cognitive assessment we make of dangerous situations. The physiological arousal triggered by the stimulus causes unique bodily sensations and the corresponding emotion with its unique quality. Together, both assessment and physiological activation, act in concert to produce our emotions. However, the statements we can have to the question "How do you feel?" simply do not fit William James theory. For instance, one of them could be that they are feeling strangulated in their throat; still another that their heart is leaping out of their chest; another that they have a knot in their gut. Other people might report that their neck, shoulders, arms, and legs are tight; others might feel ready for action, and still others that their legs feel weak or their chest collapsed. Another point of interest on this issue is related to the context in which we feel threatened and anxious. If we assess that we can escape or fight back; we will feel one set of physical sensations. If, on the other hand, we feel threatened and perceive that we cannot escape or fight back; then we feel something quite different. Both the assessment of danger and the perception of our capacity to respond are not primarily conscious.

A significant caveat in the cognitive phenomenology of anxiety highlights its paradoxical nature. According to this reasoning, all forms of threat trigger the same body signal, which is relayed to higher brain structures. These higher structures are then somehow expected to decide on an appropriate course of action. The action dependent on cognitive analysis goes against the basic biological requirements for an immediate, precise, and unequivocal response to threat. It is a view that is quite confusing because it requires that distinctly different kinesthetic, proprioceptive and autonomic feedback be experienced as the same signal.

As it will be seen later a great deal of evidence points to the wider role of

instinctive, bodily responses in elaborating, organizing and integrating the defensive responses to threatening situations. A specific combination of autonomic and motor patterns may be used for conditioned responses, a different combination for unconditioned freezing, and a still different pattern for escape. In the following paragraphs of this article we will report that when the individual has experienced prior stress events in the same context where he faces dangerous situations high levels of fear ensues with a fall in the motor capacity to deal with it. What is the real biological meaning of these defensive responses? Is it stress, fear, anxiety or panic? We present in this review recent evidence collected in this laboratory that bring some contribution to the answers to these questions.

Visual, Auditory and Contextual Signals for Conditioned Freezing

Nowadays, a procedure called contextual fear conditioning has been widely used as a model of anxiety. In this test the time animals spent freezing when they are placed in a context in which they had previously received footshocks is considered a good index of anxiety. Biologically, immobility is a potent adaptive strategy used by animals when they are re-exposed to dangerous situations or are presented with neutral signals that had been previously paired with an unconditioned stimulus, such as footshock (Fanselow, 1994). Animals are immobilized in a sustained pattern of neuromuscular activity and high autonomic and brain wave activity. Sympathetic and parasympathetic responses are also concurrently activated, like brake and accelerator, working against each other. Ethologists have found wide adaptive value in these immobility responses. Indeed, freezing makes prey less visible avoiding attacks from predators.

In our laboratory, using simple conditioning procedures we observed that contextual conditioned freezing (background stimuli) is stronger than that obtained in classical conditioning paradigms with the use of tone or light (foreground stimuli). With the use of explicit signals as CS light-CS cause weaker conditioned freezing than that elicited by tone-CS (Fig. 1). This is confirmed by the studies consulted in the literature. It is thought that light is a good CS that elicits some form of conditioned responses other than freezing or some form of unconditioned response that interferes with freezing. Alternately, the light might, in fact, condition more poorly than the noise, while the freezing index is insensitive to differences in the strength of conditioning of the two CS. Also, light might elicit more unconditioned activity than the noise to interfere with a conditioned response of freezing. Specifically, when paired with shock unconditioned stimuli (USs), auditory CSs often evoke more freezing (defensive immobility) than do visual CSs (Sigmundi et al., 1980; Sigmundi and Bolles,

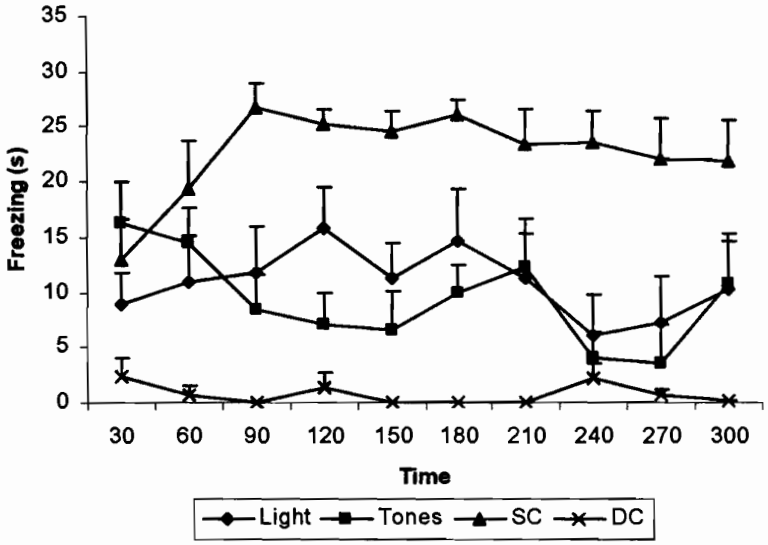

Fig.1. Time course of the freezing response in rats submitted to testing sessions in the chamber where they had been previously paired with foot-shocks (same context group) and in a different chamber where they were exposed to light-CS, tone-CS or no stimuli. The sessions were divided in ten blocks of 30-sec each. SC: same context. DC: Different context. All curves were significantly different from each other (ANOVA followed by Newman-Keuls test).

1983; Kim et al., 1996). An interesting explanation for this difference is that tones and lights have similar conditioned values but they support different form of defensive behavior (Sigmund and Bolles, 1983; Kim et al., 1996). Although there is no direct evidence to support this idea, probably, light and noise might control conditioned responses that differ in form or control different forms of behavior.

The mechanisms underlying this dissociation could be related to those that influence response topography. Taking into account the consideration advanced above that the unconditioned responses to the two CSs could differ in their ability to interfere with conditioned freezing, the modality effect would be a matter of peripheral response competition between the conditioned and unconditioned responses to each CS. This makes sense if we consider that audition, has evolved as a primary receptor for detecting predators at a distance so that many auditory CSs tend to control antidetection behavior such as freezing in rats. On the other hand, the rat's visual system may have been

phylogenetically ineffective as a distal sensor for predators. By the time a rat can see a predator, it might be too late to perform successful antidetection behaviors. If this were the case, then in the presence of visual danger signals, the rat should implement another defensive strategy such as moving to a safe place or preparing a counterattack. Such strategies would be difficult to recognize when testing the rat in a conditioning chamber that has no escape routes or places of safety.

Recent evidence suggests that the conclusions drawn in the precedent paragraph that feared CSs in different modalities may evoke defensive CRs of different forms may also apply to unconditioned stimuli and responses. The characteristic organization of the unconditioned defensive behavior may be illustrated by an imaginary situation, in which an animal unexpectedly gets in an unknown territory and suddenly hear the characteristic sound of its predator. Instinctively all movements are arrested; reflexively it crouches; the eyes narrow somewhat as to increase the perception of what is going on through the activation of the parasympathetic autonomic nervous system. Afterwards, its head turns automatically in the direction of the sound in an attempt to localize and identify it. The neck, back, legs, and feet muscles coordinate so that the whole body turns. This initial two-phase action pattern is an instinctive orientation preparing the animal to respond adequately to many possible contingencies. The initial arrest-crouch response minimizes detection by possible predators. Primarily though, it provides an arousal reaction that interrupts any motor patterns that were already in execution and then prepares the animal, through scanning, for the defensive behaviors.

If the initial signal in the open field had been from the sight of the predator itself rather than from the sound, a very different preparedness reaction would have been evoked-the preparation to flee. This is because the contours and features of the approaching animal cast a particular light pattern upon the retina of the eye. This stimulates a pattern of neural firing that is registered in phylogenetically primitive brain regions. This "pattern recognition" triggers preparation for defensive responding before it is registered in consciousness. Previous experiences and the access to memory information related to similar situations influence these responses activating preset patterns of defensive posturing. These outcomes will depend on the particular set of neural regulatory mechanisms that trigger the corresponding set of muscles, viscera, and autonomic nervous system that cooperate in the preparation of the defense. This pattern of defense reaction is also based on genetic predispositions so that arousal, immobility, freezing or escape may be the appropriate choice depending on the particular danger stimulus the animal faces.

Different types of fear

Another way in which the unconditioned responses to a CS could influence the form of behavior of a prey would be if they functioned as preparatory responses to attenuate the aversiveness of the unpleasant encounter or situation. This consideration is inside the understanding of the response selection of defensive behaviors from a phylogenetic perspective, which varies with the proximity of the predator. In other words this notion is based on differences in behavior arising as phylogenetic adaptations to different selection pressures. When the predator is very far away one can expect little defensive behavior from the prey. At a closer distance, the rat might engage in antidetection behavior such as freezing or hiding. As predator draws nearer, flight might predominate, but when the predator is in contact with the rat, the rat should exhibit violent counterattack and escape behavior, possibly followed by tonic immobility (Klemm, 1990). Figure 2 presents a general model with sequential defensive responses as the predator approaches the prey.

In terms of what was presented in the precedent section, the light might exert more powerful control than the noise or context over either a conditioned or an unconditioned preparatory response to danger. This is clearly seen in the model called fear-potentiated startle. The light condition very well in the sense of becoming a reliable signal for shock but condition poorly as to its ability to elicit overt conditioned defensive behavior. By inducing arousal light would turn on the motor systems for the preparatory response which could reduce the magnitude of conditioned freezing, which varies directly with shock intensity, to a level below that supported by the noise or context. This state of affairs is not easily understood for those that only see a positive correlation between the strength of the conditioning stimulus and the conditioned response.

We enlist with those that think that a specific combination of autonomic and motor patterns will be used for escape, a different combination for freezing, and a still different pattern for fainting or any other pattern of defensive behavior. This assumption seems to contradict both evolutionary imperative and subjective experience and also do not fit into the James-Lange theory. However, in support to this there is empirical evidence from the animal laboratory. We report here some of the evidence favoring dissociated fear responses to different threatening conditions. Electrolytic and excitotoxic lesions of the ventrolateral periaqueductal gray (vPAG) reduced freezing evoked by re-exposure of rats to the same box in which it has previously received electric foot-shock (LeDoux, 1995; Vianna et al., 2001). Immunohistochemical studies have shown that contextual fear is associated with marked increase in Fos expression in the vPAG (Carrive et al., 1997). vPAG activation (after GABA receptor blockade or EAA receptor activation) markedly reduces fear-potentiated startle (Fendt et al., 1994). Indeed, the immobility induced by contextual fear, which is integrated in the vPAG

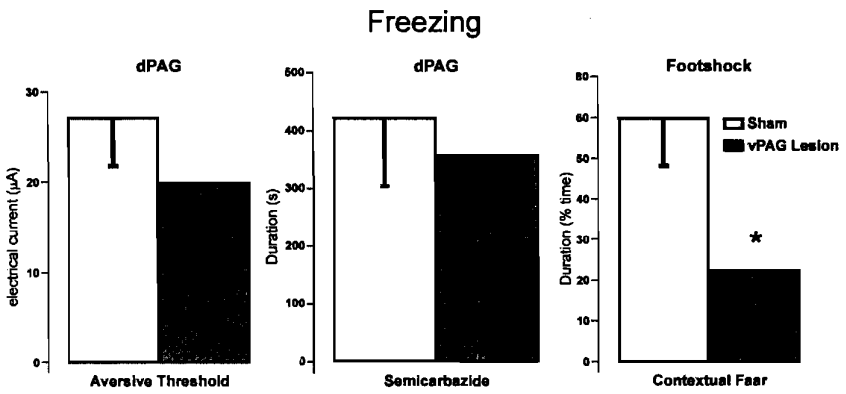

Fig 2. Freezing threshold and percentage of time spent freezing by rats with sham (open columns) or vPAG (hatched columns) lesions and submitted to electrical stimulation of the dPAG at freezing threshold (left), chemical stimulation of the dPAG with local injections of semicarbazide- a glutamic acid decarboxilase blocker - (middle) or placed in a contextual conditioning procedure using footshocks as unconditioned stimuli (right).

courses with little muscle tone, and the animal does not appear ready to jump or run as it occur when the freezing is induced by electrical or chemical stimulation of the dPAG. As can be seen in Fig. 2 lesions of the vPAG significantly reduce the conditioned contextual freezing but does not change the freezing and escape responses induced by electrical or chemical stimulation of the dPAG (Vianna et al., 2001). Besides, different from the dPAG, activation of the vPAG causes immobility along with cessation of ongoing movement. This vPAG-evoked immobility is a hyporeactive type of immobility, which has been described as "quiescent immobility" and is considered to be phasic (Walker and Carrive, 2003). Thus, it is unlikely that the increase in muscle tone responsible for the tense freezing posture is mediated by vPAG. On the other hand, several studies using c-fos have shown that the dorsal parts of the PAG are activated by immediate threatening stimuli (Sandner et al., 1993; Canteras and Goto, 1999; Lamprea et al., 2002).

Exposure to the context where the rats 24-h earlier received footshocks is also an aversive manipulation that causes freezing behavior and fear-potentiated startle (McNish et al., 1987; Walker and Carrive, 2003; Silva et al., 2003). This can be easily observed by the increase in the amplitude of the startle reflex observed in rats submitted to contextual fear conditioning alone (Phillips and Le Doux, 1992; Frankland and Yeomans, 1995; McNish et al., 1997). That is, animals non pre-exposed to stressful stimuli increase the amplitude of acoustic

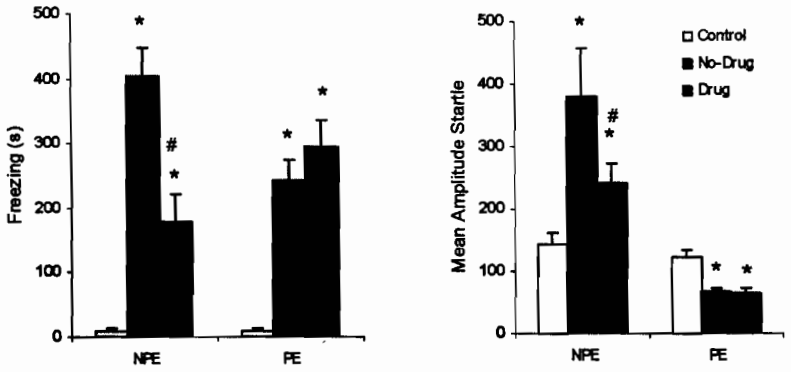

*Fig.3. Left: Effects of midazolam (0.5 mg/kg) given to rats non-preexposed (NPE) or pre-exposed (PE) to stressors and submitted to the context conditioning procedure on time (sec) of freezing. Control animals received no-shock in the conditioning sessions. Mean ± S.E.M. * p<0.01, one-way ANOVA. Right: Effects of midazolam (0.5 mg/kg) given to rats non-pre-exposed (NPE) or pre-exposed (PE) on the mean amplitude startle response as compared to control animals (No-shock). * Different from noise-alone condition, and # different from the respective control group (P<0.05, Newman-Keuls test).*

startle. Rats given the same shock treatment but in a context different to that used for test, did not respond more than non-shocked groups. This latter result clearly suggests that shock sensitization of startle is due to contextual conditioning. Additionally, we have also shown that a singular experience of traumatic stress occurs only where the normally active defensive responses have been unsuccessful, that is, when a situation is both dangerous and inescapable. Indeed, animals possess a variety of orientation and defensive responses that allows them to respond automatically to different, potentially dangerous situations rapidly and fluidly. However, a complete different pattern of response results when the animal is pre-exposed to stressful stimuli (Fig. 3).

The observed decline of startle reflex in rats with previous experience of stress more likely reflects a performance deficit. If this response reflected a lack of fear, one would expect this to be equally evident across other fear measures. However this is not the case here since when freezing was recorded as an additional measure of conditioned fear, animals trained after exposure to stress events also presented significant freezing behavior, despite lower levels of startle in the same animals. These results suggest that fear itself is maintained, indeed elevated, in the preexposed group, and that the loss of startle reflex in this group reflects a performance rather than learning deficit.

This performance hypothesis had already been advanced by Davis and Astrachan (1987) with the use of light CS instead of background stimuli as in the present work. Based on their data it was proposed that fear may be linearly related to the intensity of shock used during training, but that the magnitude of potentiated startle may be non-monotonically related to fear. For example, moderate fear levels may produce maximal fear-potentiated startle, whereas higher levels might switch animals to a different mode of defensive responding that do not include fear-potentiated startle as a component behavior. In conformity with this possibility the present results clearly show that a previous history of stress sensitizes defensive mechanisms with weakening of physical strength in reaction to subsequent submission to other aversive stimuli. Animals previously exposed to stress-inducing procedures have been reported to display subsequent behavioral passivity, even when these animals are submitted to only a mild stressor (Van Dijken et al., 1992). This is not to say that the stressor exposure procedure causes a general debilitating effect on motor activity since animals submitted to the forced swim test after stressful experience displayed higher immobility but the struggle phase of the test was even enhanced (Molina et al., 1994). As further confirmation of these findings, sensitization to subsequent aversive stimuli in animals with a past history of stress has been largely documented by hormonal, neurochemical and immunological studies (Weiss et al., 1981; Irwin et al., 1986; Caggiula et al., 1989; Antelman et al., 1990).

Developing still further the above mentioned hypothesis that low levels of aversion trigger behavioral mechanisms different from those triggered by higher levels the present findings can be considered in the theory stated elsewhere that defensive behaviors are hierarchically organized and different behaviors within this class are provoked by aversive stimuli of different intensities (Blanchard et al., 1969, 1972). For example, arousal/immobility is the result of mild or distant threatening stimuli whereas freezing behavior is the outcome of stronger or nearer stimuli. Likewise, in the present experiment, conditioned contextual fear alone triggered mechanisms similar to those that mediate moderate immobility/arousal while conditioning with high level of stress may trigger an intense freezing that precede more vigorous behaviors. Consequently, the first case would make the animals more able to receive all kinds of sensorial information and more prone to a motor activity whereas in the second case the animal could have already made great physical struggle what would make the animals less able to exert further physical activity in response to the loud noise. Consistent with this hypothesis, a recent study from this laboratory demonstrated that rats exposed to a standard elevated plus-maze with conventional wooden walls showed higher levels of freezing than when exposed to a modified plus-maze with transparent walls in which they display more exploratory activity and lower levels of fear (Anseloni et al., 1995). Also, we

have recently reported that prior electrical stimulation of the midbrain tectum, at aversive thresholds, caused an increase in fear-like behaviors with a concomitant reduction in the exploratory activity of rats in the elevated plus-maze (Pandossio et al., 2001).

Another hypothesis is a parallel between the non-monotonic function and the generation of two distinct states of anxiety generated by stress associated to contextual fear conditioning. Pre-exposure to stressful stimuli seems to paradoxically bring the alterations lower than the baseline level. Importantly, baseline responses (i.e. startle to noise alone) were unaffected. Thus, the relative ineffectiveness of contextual stimuli paired with footshock in pre-exposed rats cannot be attributed either to ceiling effects produced by a baseline elevation or to a general suppression of startle responses. These behavioral effects may be related to fear states different from the anxiety state induced by contextual conditioned stimuli alone acquired by the previous pairing to footshocks. Indeed, midazolam caused an anxiolytic-like effect on the contextual fear-potentiated startle at a dose that did not produce any effect al all on the depression of the startle in pre-exposed rats. This may represent an emotional shift from low to high fear levels, which leads to change in the responsivity of the animals to benzodiazepines. From a neurological point of view, it is recognized that there could be two different types of anxiety: anxiolytic-sensitive and anxiolytic-insensitive. Although, much work still need to be done we can not help comparing the present results with the phenomenon of one-trial tolerance to midazolam in the elevated plus-maze test, which also has been attributed to the development of anxiolytic-insensitive fear states (Cruz-Morales et al., 2002). Where the flight-or-fight response is appropriate, freezing will be relatively maladaptive; where freezing is appropriate, attempts to flee or fight are likely to be maladaptive. Biologically, immobility is a potent adaptive strategy where active escape is prevented. When, however, it becomes a preferred response pattern in situations of activation in general, it is profoundly debilitating.

Neural Substrates

Evidence from many sources has been obtained in support of the widely recognized notion that amygdala, medial hypothalamus and the dorsal periaqueductal gray (dPAG) constitutes the main neural substrates for the integration of aversive states in the brain (Graeff 1990, 1994, Brandão et al. 1994, 1999). Gradual increases in the intensity of electrical stimulation of these structures causes in progressive manner alertness, turnings, freezing and escape in the same way as the predator approaches the prey from a distant to a nearer or proximal position (Fig 4). As this set of structures shares this functional role in the organization of defensive behaviors and have two-way projections it has been called "brain aversion system" or "arousal system" or

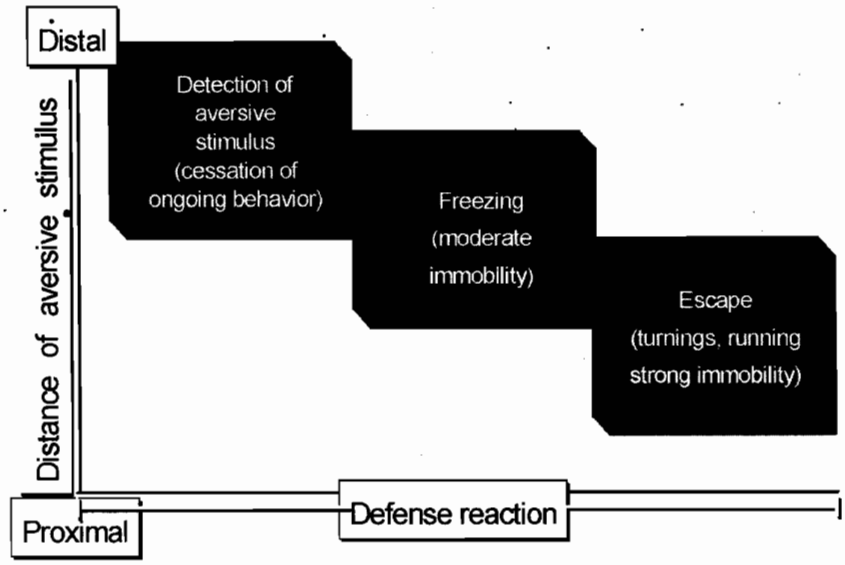

Fig. 4. Hierarchical organization of defensive behaviors displayed by preys to increasing proximity of the predators. Similar pattern of defense reaction is observed to increasing intensity of electrical current applied to the dorsal periaqueductal gray (dPAG).

still fight-flight system (Graeff 1990, 1994; Gray and MacNaughton, 2000). This circuit acts to generate the basic changes necessary for the expression of any emergency response, from arousal to full-blown fight or flight (Brandão et al. 1994, 1999; Graeff, 1990, 1994). Orientation and turning toward the source of dangerous signal and other defensive preparations are activated just prior to the onset of freezing. This freezing behavior, however, unlike that described previously, is tonic and accompanied of hypertension, tachycardia, piloerection, micturition and defecation. This tonic freezing is prevalent and also thought to be linked to the activation of neural circuits of panic. The fight or escape is sequentially the usual defense response of the activation of this system. It has been shown that four interesting features make the arousal system unique (Schmitt et al., 1985; Brandão et al., 1986): 1) turning and freezing are not common responses induced by activation of the medial hypothalamus, which, by its turn, cause an oriented, flexible and well-coordinated escape response; 2) stimulation of the dPAG causes uncoordinated and explosive escape response; 3) sensory changes, such as sensorial neglect to tactile stimulation and serotonergic analgesia, are fragrant responses specific for the neural

substrates of aversion in dPAG; 4) as already described a tonic immobility that outlasts the dPAG stimulation has characteristics distinct from the conditioned freezing. In this latter case, it could well be that where fight and escape have been unlikely, the nervous system reorganizes to tonic immobility.

We believe that when orienting and defensive behaviors are carried out smoothly and effectively anxiety-related neural substrates of the medial hypothalamus are activated. This generates a normal pattern of reaction to aversive situations that incorporates the curiosity, approach or avoidance. Otherwise, high levels of fear or past experience with traumatic events destructure this defensive strategy and lead to the defensive pattern characteristic of the dPAG activation, generating a panic-related, thwarted and disorganized anxiety response. Therefore, the ability in becoming anxious is proportional to the ability in displaying active, adequate, defensive responses and to deal effectively with danger. Biologically, orientation, preparation and defense reaction are natural steps of an adaptive response to stimuli that puts at risk the organism survival. On the other hand, uncoordinated, explosive responses accompanied by sensation of imminent death are not adaptive strategies to cope with danger.

Recently, Walker and Carrive (2003) proposed that the freezing induced by strong contextual fear conditioning is made up of two components: a phasic initial stage of a hyporeactive-hypotensive immobility pattern and a delayed stage of a rapid hyperreactive immobility pattern when the animal is tense and ready for action but temporarily immobilized. The phasic component seems to be related to "anxiety-like states" and the second component seems to be linked to "fear-like states". As we can see in Figure 4, comparable levels of freezing exist during the fear-potentiated and fear-depressed startle in non pre-exposed and pre-exposed animals, respectively, submitted to the contextual fear procedure. From what has been discussed so far, the first kind of response may also be considered phasic and anxiolytic-sensitive and the second one tonic and anxiolytic-insensitive. Thus, two pathways should subserve each component of this process.

To comply with this dual process activated by fear stimuli an interesting hypothesis has been put forward by Walker and Carrive (2003). These authors imagined the possibility that the brake on motor function was only partial, that is, it only affects the phasic component of activity but not the tonic component that is what generates the movement but not the increase in muscle tone necessary for the execution of movement. They made an analogy with the brake pedal of a racing car before the start of a race. Pressing the brake pedal immobilizes the car but does not stop the engine running; in fact the driver may also press the accelerator pedal at the same time to obtain a greater acceleration at the start of the race. The two pedals, the accelerator and the brake, can be seen as the two pathways described above, and in these conditions; the freezing rat is like the racing car about to surge ahead, aroused,

tense and ready for action, but temporarily immobilized. The freezing can be seen as made up of two components: the immobility, which is mediated by the vPAG and the tense posture, which is the unopposed tonic component of the motor activation, mediated by the arousal pathway. Freezing is lost after vPAG blockade simply because the immobility brake is released and the tonic component becomes integrated with movement. This explanation is attractive because regards freezing as part of a broader readjustment of the somatomotor system.

The reason why animals are not active during activation of vPAG despite being aroused is because vPAG impose immobility and inhibits the effects of arousal on activity. The blockade of vPAG results in loss of immobility releasing the effect of arousal on activity. Indeed, we have shown that vPAG lesions cause an increase in the locomotor activity of the animals in an open field (Vianna et al., 2001). If this is the case it is hypothesized the existence of two parallel pathways with opposing influences on motor expression (Walker and Carrive, 2003). One pathway would deal with the arousing properties of the stimulus it would turn on motor systems and induce activity. The other pathway would deal with some of the aversive properties of the context including its inescapability and it would turn off motor systems, that is, it would impose immobility by acting as a brake on the other pathway. According to this model, the two pathways would be activated at the same time and competing against each other to set up the appropriate motor response. The neural substrates of the brain aversion system responsible for this component of the defense reaction could be the medial hypothalamus. When by any reason the escape is not possible and the defensive preparedness for flight, concomitant with the feeling of danger, is "thwarted" the emotional state of the animal change abruptly. This emotional change also reflects a shift in the neural mechanisms that will command this new defense reaction. Response may go to a non-directed desperate flight, fight or freeze-collapse. In other words, when the normal orientation and defensive escape resources have failed to resolve the situation, the defense mechanisms switch to non-directed flight, fight, freezing, or collapse, which has been linked to the dorsal aspects of PAG.

As a working hypothesis, then, it could be advanced that past experience with stressors also leads to an emotional shift switching the neural circuits responsible for the production of the defensive response to an aversive context. Panic is the secondary emotional anxiety state that is evoked when the preparatory orientation processes of danger-orientation and preparedness to flee are not successful. Disturbances or thwarting the normal organization of defense responses or the ability to integrate and orchestrate the whole pattern of a defense reaction may result in freezing and explosive escape responses, which are the corollary of the panic attacks.

FINAL COMMENTS

The defense reaction displayed by animals is a good model of anxiety because its roots in man may be found in the defensive behaviors of the animals when they face dangerous situations. Anxiety has often been linked to the physiology and experience of these defensive reactions. In general, when an animal is attacked by a predator it will first attempt to escape through directed-oriented running. However, if the escaping animal is cornered so that escape is diminished, it may run in an uncoordinated and disorganizaed manner, without a directed orientation, or it may attempt to fight. Some animals may abruptly appear to go dead at the moment of physical contact, often before injury is actually inflicted. Autonomic physiology also changes accordingly. The animal is in fact highly activated internally, even though visible movement is almost nonexistent. Prey animals are immobilized in a sustained pattern of neuromuscular activity and high autonomic and brain wave activity. Sympathetic and parasympathetic responses are also concurrently activated, like brake and accelerator, working against each other.

There are many forms of anxiety. In animal models of generalized anxiety or "signal anxiety", preparedness to flee, preparatory processes of danger-orientation and responses that avoid or terminate the aversive stimulus are well-succeeded. Therefore, where escape or avoidance is possible, the organism responds with an active pattern of coping. On the other hand, in animal models of anxiety in its panic form fear stimuli that originate active but incomplete patterns of defensive response ensure emotional states of different nature. There is a continuous experience of danger, running and escape, often uncoordinated and explosive. So, a shift in the emotional states may occur depending on the intensity of the threatening situation or danger stimuli. As a working hypothesis, it is advanced that past experience with stressors may lead to this emotional shift. In this case, active forms of defensive response are aborted and incomplete and an emotional state of different nature ensues. Therefore, response to past experience plays a significant role in threatening situations in that it may thwart instinctive orientation, organized motor patterns for preparing adequate defensive behaviors.

REFERENCES

Antelman SM, Cunnick JE, Lysle DT, Caggiula AR, Knopf S, Kocan DJ, Rabin BS, Edwards DJ. Immobilization 12 days (but not one hour) earlier enhanced 2-deoxy-D-glucose induced immunosuppression; evidence for stressor – induced time dependent sensitization of the immune system. *Prog Neuropsychopharmacol Biol Psychiatr 14*:579-590, 1990.

Anseloni VZ, Messias G, Brandão ML. Behavioral and pharmacological validation of the elevated plus maze constructed with transparent walls. *Braz J Med Biol Res, 28*: 597-601, 1995.

Blanchard, D. C.; Blanchard, R. J. Crouching as an index of fear. *J. Comp. Physiol. Psychol. 67*:370–375; 1969.

Blanchard, D. C.; Blanchard, R. J. Innate and conditioned reactions to threat in rats with amygdaloid lesions. *J. Comp. Physiol. Psychol. 81*:281–290; 1972.

Brandão ML, DiScala G, Bouchet MJ, Schmitt P. Escape behavior produced by the blockade of glutamic acid decarboxilase (GAD) in mesencephalic central gray or medial hypothalamus. *Pharmacol Biochem Behav 24*:497-502, 1986.

Brandão ML, Anseloni VZ, Pandóssio JE, De Araújo JE, Castilho VM. Neurochemical mechanisms of the defensive behavior in the dorsal midbrain. *Neurosci Biobehav Rev 23*: 863-875, 1999.

Brandão ML, Cardoso SH, Melo LL, Motta V, Coimbra NC. Neural substrate of defensive behavior in the midbrain tectum. *Neurosci Biobehav Rev 18*, 339-346, 1994.

Caeser M, Ostwald J, Pilz PKD. Startle responses measured in muscles innervated by facial and trigeminal nerves show commom modulation. *Behav Neurosci 103*:1075-1081, 1989.

Caggiula AR, Antelman SM, Aul E, Knopf S, Edwards DJ. prior stress attenuates the analgesic response but sensitizes the corticosterone and cortical dopamine response to stress 10 days later. *Psychopharmacology 99*:223-237, 1989.

Canteras NS, Goto M. Fos-like immunoreactivity in the periaqueductal gray of rats exposed to a natural predator. *Neuroreport, 10*: 413-418, 1999.

Carrive P, Leung P, Harris J, Paxinos G. Conditioned fear to context is associated with increased fos expression in the caudal ventrolateral region of the midbrain periaqueductal gray. *Neuroscience 78*(1): 165-177, 1997.

Cruz-Morales SE, Santos NR, Brandão ML. One-trial tolerance to midazolam is due to enhancement of fear and reduction of anxiolytic-sensitive behaviors in the elevated plus-maze retest in the rat. *Pharmacol Biochem Behav 72*:973-978, 2002

Davis M, Astrachan DI. Conditioned fear and startle magnitude: Effects of different footshock and backshock intensities used in training. *J Exp Psychol: Anim Behav Proc 4*:95-103, 1978.

Fanselow, M.S. Neural organization of the defensive behavior system responsible for fear. *Psychonom Bull Rev 1*:429-438, 1994.

Fendt M, Koch M, Schnitzler H-U. Lesions of the central gray block the sensitization of the acoustic startle response in rats. *Brain Res 661*:163–173, 1994.

Frankland P W, Yeomans JS. Fear-potentiated startle and electrically evoked startle mediated by synapses in rostrolateral midbrain. *Behav Neurosci 109*:669–680, 1995.

Graeff FG (1994). Neuroanatomy and neurotransmitter regulation of defensive behaviors and related emotions in mammals. *Braz J Med Biol Res 27*: 811-829.

Graeff FG. Brain defense systems and anxiety. In: Burrows GD, Roth M, Noyers Jr (eds), *Handbook of Anxiety*. Amsterdam: Elsevier Science Publishers, pp. 307-354, 1990.

Gray A, McNaughton N. Fundamentals of the septo-hippocampal system. In: *The Neuropsychology of Anxiety: An enquiry into the functions of septo-hippocampal system*. Gray JA, McNaughton N (eds).: 2nd edition. Oxford: Oxford University Press, 2000, pp 204-232.

Irwin J, Ahluwalia P, Anisman H. Sensitization of norepinephrine activity following acute and chronic footshock. *Brain Res. 379*:98-103, 1986.

Kim, J.J. and Fanselow, M.S. 1992. Modality-specific retrograde amnesia of fear. *Science 256*: 675-677, 1992.

Kim SD, Rivers S, Bevins RA, Ayres JB. Conditioned stimulus determinants of conditioned response form in Pavlovian fear conditioning. *J Exp Psychol 22*:87-104, 1996.

Klemm WR. Historical and introductory perspectives on brainstem-mediated behaviors. In: Klemm WR, Vertes RP (eds). *Brainstem mechanisms of behavior*. New York: John Wiley & Sons, Inc., pp. 3-32, 1990.

Lamprea MR, Cardenas FP, Vianna DM, Castilho VM, Cruz-Morales SE, Brandão ML. The distribution of fos immunoreactivity in rat brain following freezing and escape responses elicited by electrical stimulation of the inferior colliculus. *Brain Res. 950(1-2)*:186-94, 2002

LeDoux JE. Emotion: clues from the brain. *Ann. Rev. Psychol. 46*:209-235, 1995.

McNish KA, Gewirtz JC, Davis M. Evidence of contextual fear after lesions of the hippocampus: A disruption of freezing but not fear-potentiated startle. *J Neurosci 17*:9253-9360, 1997.

Molina VA, Heyser, CS, Spear LP. Chronic variable stress or chronic morphine facilitates immobility in a forced swim test: Reversal by naloxone. *Psychopharmacology 114*:433-440, 1994.

Pandóssio JE, Molina VA, Brandão ML. Prior electrical stimulation of the inferior colliculus sensitizes rats to the stress of the elevated plus-maze test. *Behav Brain Res 109*:19-25, 2000.

Phillips RG, LeDoux JE. Differential contributions of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci 106*:274-285, 1992.

Sandner G, Oberling P, Silveira MCL, Di Scala G, Rocha B, Bagri A., Deportere

R (1993) What brain structures are active during emotions? Effects of brain stimulation elicited aversion on c-fos immunoreactivity and behavior. *Behav Brain Res 58*:9-18.

Schmitt P, DiScala G, Brandão ML, Karli P. Behavioral effects of microinjections of SR95103, a new GABA-A antagonist, into medial hypothalamus or the mesencephalic central gray. *Europ J Pharmacol 117*:149-158, 1985.

Sigmundi RA, Bouton ME, Bolles RC. Conditioned freezing in the rat as a function of shock intensity and CS modality. *Bull Psychon Soc 15*:254-256, 1980.

Sigmundi RA, Bolles RC. CS modality, context conditioning and conditioned freezing. *Anim Learn Behav 11*:205-212, 1983.

Silva RCB, Cruz APM, Landeira-Fernandez J, Avanzi V, Brandão ML. Distinct contributions of median raphe nucleus to contextual fear conditioning and fear potentiated startle. *Neural Plasticity*, in press.

Van Dijken, HH, Van Der Heyden JAM, Mos J, Tilders FJH. Inescapable footshocks induce progressive and long-lasting behavioral changes in male rats. *Physiol Behav 51*:787-794, 1992.

Vianna DML, Landeira-Fernandez J, Brandão ML. Dorsolateral and ventral regions of the periaqueductal gray matter are involved in distinct types of fear. *Neurosci Biobehav Rev 25*: 711-719, 2001.

Walker P, Carrive P. Role of ventrolateral periaqueductal gray neurons in the behavioral and cardiovascular responses to contextual fear and poststress recovery. *Neuroscience 116*:897-912, 2003.

Walker DL, Cassella JV, Lee Y, De Lima TCM, Davis M. Opposing roles of the amygdala and dorsolateral periaqueductal gray in fear-potentiated startle. *Neurosci Biobehav Rev 21(6)* 743–753, 1997.

Watkins L, Sherwood A, Goldstein DS, Maixner W. Mechanisms underlying cardiovascular defense reaction evoked by dorsal periaqueductal gray stimulation. *Am J Physiol 265*:R1155–R1161; 1993.

Weiss JM, Goodman PA, Losito BG, Corrigan S, Charry JM, Bailey WM. Behavioral depression produced by an uncontrollable stressor: relationship to norepinephrine, dopamine and serotonin levels in various regions of the rat brain. *Brain Res Rev 3*:167-205, 1981.