Critical neuropsychobiological analysis of panic attack- and anticipatory anxiety-like behaviors in rodents confronted with snakes in polygonal arenas and complex labyrinths: a comparison to the elevated plus- and T-maze behavioral tests

Norberto C. Coimbra, Tatiana Paschoalin-Maurin, Gabriel S. Bassi, Alexandre Kanashiro, Audrey F. Biagioni, Tatiana T. Felippotti, Daoud H. Elias-Filho, Joyce Mendes-Gomes, Jade P. Cysne-Coimbra, Rafael C. Almada, Bruno Lobão-Soares

1Laboratório de Neuroanatomia e Neuropsicobiologia, Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, SP, Brazil. 2Instituto de Neurociências e Comportamento (INeC), Ribeirão Preto, SP, Brazil. 3Núcleo de Pesquisa em Neurobiologia das Emoções (NAP-USP-NuPNE), FMRP, USP, Ribeirão Preto, SP, Brazil. 4Departamento de Biofísica e Farmacologia, Universidade Federal do Rio Grande do Norte (UFRN), Natal, RN, Brazil.

Objective: To compare prey and snake paradigms performed in complex environments to the elevated plus-maze (EPM) and T-maze (ETM) tests for the study of panic attack- and anticipatory anxiety-like behaviors in rodents.

Methods: PubMed was reviewed in search of articles focusing on the plus maze test, EPM, and ETM, as well as on defensive behaviors displayed by threatened rodents. In addition, the authors’ research with polygonal arenas and complex labyrinth (designed by the first author for confrontation between snakes and small rodents) was examined.

Results: The EPM and ETM tests evoke anxiety/fear-related defensive responses that are pharmacologically validated, whereas the confrontation between rodents and snakes in polygonal arenas with or without shelters or in the complex labyrinth offers ethological conditions for studying more complex defensive behaviors and the effects of anxiolytic and panicolytic drugs. Prey vs. predator paradigms also allow discrimination between non-oriented and oriented escape behavior.

Conclusions: Both EPM and ETM simple labyrinths are excellent apparatuses for the study of anxiety- and instinctive fear-related responses, respectively. The confrontation between rodents and snakes in polygonal arenas, however, offers a more ethological environment for addressing both unconditioned and conditioned fear-induced behaviors and the effects of anxiolytic and panicolytic drugs.

Keywords: Innate fear; panic attacks; prey versus snakes paradigms; polygonal arenas for snakes; elevated plus-maze test; elevated T-maze test

Introduction

Several studies have focused on the morphological and physiological bases of unconditioned and conditioned fear-related responses resulting from dysfunction of brain systems, anxiety, and panic disorder. There are robust data on the use of simple apparatuses, such as the elevated plus maze (EPM) and T-maze (ETM) tests, for the study of anxiety and other innate fear-related diseases. These devices have been submitted to extensive ethological and pharmacological validation and are considered useful for the study of anxiolytic and panicolytic drugs. For example, the EPM and ETM tests have been used to compare the effects of anxiolytic/panicolytic drugs delivered via systemic injection or microinjection into specific encephalic structures, thereby facilitating the study of the neuroanatomical basis of fear- and anxiety-induced reactions and the establishment of correlations between behavioral responses and different stress-related models.

More recently, prey-versus-predator paradigms have also been used to investigate innate and conditioned fear-related reactions and the effects of drugs that act in the neural substrates of aversive stimulus-induced emotional responses. As an example, in our laboratory, different species of rodents were confronted with venomous or constrictor snakes in a complex labyrinth or in polygonal arenas. These prey-versus-predator paradigms also seem to be a good experimental tool for studying innate fear-induced behavioral responses elicited in a more ethological situation of threat, as well as the effects of new potential panicolytic drugs. In this
sense, the aim of the present article was to discuss the literature regarding the classical EPM and ETM tests, the current knowledge about snake-based prey-versus-predator interaction approaches to anxiety, fear, and panic-related behaviors, and our 10-year experience with snake versus predator interactions.

Methods

We reviewed PubMed in search of articles focusing on the plus maze test, EPM, and ETM, as well as on defensive behaviors displayed by rodents. We also included in this review selected chapters from classical books about anxiety and defensive behavior in rodents and humans and about anxiety treatment or neuropsychopharmacology of mental diseases.7,29-33

For the critical review of the experience of our team, we examined a set of six papers,22-27 two doctoral theses,34,35 nine congress abstracts,28,34,36-43 and one book chapter44 based on prey-versus-predator paradigms using wild snakes. All these experiments were performed in the Ophidiarium at the Laboratório de Neuroanatomia e Neuropsicobiologia – Faculdade de Medicina de Ribeirão Preto – Universidade de São Paulo (LNN-FMRP-USP)/Instituto de Neurociências e Comportamento (INeC) using polygonal arenas and a complex labyrinth for confrontation between venomous and non-venomous snakes and small rodents, designed by Prof. N.C. Coimbra (first author) and ethologically validated from 2007 to 2013.24-27 These apparatuses, which are licensed by the Brazilian government (IBAMA Committee; processes 3543.6986/2012-SP and 3543.6984/2012-SP) and by the São Paulo state government (SMA/DeFau 15.335/2012; MEDUSA Project, SISBIO processes 41435-1, 41435-2 and 41435-3), have been used since then in undergraduate medical courses, graduate programs, and in original scientific studies. New data produced during a laboratory demonstration of the model to undergraduate and graduate medical students are also discussed.

Results

Elevated classical labyrinths to record anxiety and fear-related behaviors – EPM and ETM tests

Elevated labyrinths such as the EPM and the ETM are simple experimental apparatuses with closed and/or open arms assembled 50 cm above the laboratory floor. They are used to study the natural aversion of rodents to high and open areas.

The EPM test is a prototypic anxiety model, and one of the most used ethological tools for measuring and manipulating anxiety in rodents.7,45 Despite the controversies surrounding the EPM, including the intensity of the anxiogenic stimuli to which the tested animal is submitted,36 it is a useful tool for verifying the behavioral effects of anxiolytic and anxiogenic drugs.2,10

Different species of rodents that vary in size and height can be tested in the EPM, a structure elevated 50 cm from the floor, made of wood or plexiglass and consisting of two open arms placed perpendicularly to two closed high-walled arms of the same size. Some authors claim that replacing the classic opaque walls in the two closed high-walled arms with transparent walls increases the sensitivity of the behavioral effects of anxiolytic drugs.47-49

In the clear model, each animal is placed in the center of the EPM, generally facing one of the open arms, and is recorded for 5 or 10 min of free exploration. The primary indexes of anxiety-like behavior in the EPM comprise spatiotemporal measures, e.g., the number of entries in the closed arms and the percentage of entries and time spent in the open arms, two significant behavioral parameters related to locomotion and anxiety/fear behaviors in rodents.1,3,7,50,51 However, as in any other animal model of anxiety, the precision of the EPM depends on many factors, since anti-anxiety effects may have baseline levels of anxiety that are too low or too high to be detected.7 To avoid these limitations, researchers1,3,46 have highlighted the necessity of improving the sensitivity, reliability, and ecological validity of the test by focusing on what the animal actually does in the maze, e.g., aspects of defensive behaviors and the location within the maze where these behaviors occur.1,3,7,52,53 These complementary parameters are sometimes called “risk assessment behaviors” and visually show the type of behavioral strategy the animal actually exhibits in a dangerous situation (Table 1). The biological function of these behaviors is to gather information regarding the potential threat from the environment.30,54

However, as there are different parameters of risk assessment behaviors, it is not necessarily clear what each behavior tells us about the elicited anxiety-like behavior in the EPM test. Cruz et al.3 and Rodgers & Johnson9 elegantly answer this question by classifying each behavior: (Table 1).

Table 1: Description of the complementary behaviors exhibited by rodents in the EPM

| Behavioral parameter | Description |
|----------------------|-------------|
| Scanning             | Looking over the edge of one of the open arms with scanning movement in any direction. |
| Head dipping         | Downward visual screening movement at the edge of the open arm. |
| End-arm exploration  | The animal reaches the end of the open arm and dips its head. |
| Stretch-attend posture (SAP) | Forward elongation of the body and retreat to the original position when the animal is standing still or moving slowly forward. |
| Flat-back approach   | Forward elongation of the body with forward movement by slowly pulling the hind body. |
| Rearing              | Bipedal posture supported by the hind paws. |
| Peeping out          | Projection of the head and shoulders from the closed arms toward the central part of the EPM. |
| Grooming             | The four paws are maintained inside the closed arm. |
| Immobility           | Cleaning or scratching of the fur, nose, ears and whiskers using the paws or tongue. Complete stillness of the animal (resembles freezing behavior). |

Rev Bras Psiquiatr. 2017;39(1)
the parameters into factors representing different emotional dimensions. Cruz et al.\textsuperscript{3} classified these complementary parameters into four distinct factors: anxiety, motor activity, decision-making, and displacement behavior. The behavioral measures of scanning, head dipping, end of arm exploration, and the classic ethological factors (number of entries into the open and closed arms and time spent inside these arms) are strongly correlated with anxiety; rearing, number of entries into the closed arms, and total number of entries into the arms are correlated with motor activity; time spent in the central square of the maze is correlated with decision-making behavior; and grooming is related to displacement behavior. To this behavioral analysis, Rodgers & Johnson\textsuperscript{8} have added three factors: a) stretch-attend posture (SAP), a primary index of learning about a potentially dangerous environment, together with sniffing or investigation; b) exploration, which consists of head-dipping at the edge of the open arm and is considered a primary index of exploration, with high sensitivity for testing anti-anxiety and pro-anxiety drugs\textsuperscript{56}; and c) vertical activity, which consists of rearing and grooming (two behaviors that are negatively correlated with each other). It is important to highlight that not only has “factor analysis” been used for identifying the relationship between specific test indexes and factors/dimensions, such as anxiety and locomotor activity, it is also employed to assess whether different animal models can be used to measure the same type of anxiety.\textsuperscript{8}

In 1991, Deakin & Graeff\textsuperscript{57} proposed a model to separate conditioned fear, which is related to generalized anxiety disorders (GAD), from unconditioned fear, which is related to panic disorders (PD). In that new model, the T-maze test, was adapted from the EPM test by blocking the entrance to one of the closed arms.\textsuperscript{58,59} In the ETM, the rat may perform one of two tasks: inhibitory avoidance (conditioned fear) and one-way escape (unconditioned fear).

In the first task, the rat is placed at the end of the enclosed arm so that it can only see the open arms if it positions its head beyond the end of the closed arm. Because the open arm seems to represent an aversive experience for rodents, it evokes inhibitory avoidance, i.e. - if this task is repeated, the latency to leave the closed arm will increase over the trials. Three consecutive trials, with 30-s inter- and intra-trial intervals, are recorded when the animal exits the arm with all four paws during each task.

In the second task, the rat is placed at the end of the open arms so it can move towards the closed arm, probably performing an escape response. It is important to highlight that while the latencies to leave the enclosed arm increase over the trials, reflecting habituation to the maze environment, exploratory activity, and learning, the latencies to leave the open arms (escape task) do not change due to a persistent aversive motivation.\textsuperscript{4,10,58} This situation is not affected by the administration of diazepam, a classic anxiolytic drug.\textsuperscript{58}

The ETM test elicits the activation of different brain areas related to memory formation (hippocampus)\textsuperscript{60} and to fear/anxiety responses, as revealed by Fos protein immunolabeling in different brain areas.\textsuperscript{60,61} These findings corroborate behavioral results, and encephalic activation was found to differ during escape and avoidance tasks. Neural activation in the basolateral amygdaloid nucleus and in the dorsal periaqueductal gray matter was observed during escape, whereas enhanced activity in the amygdaloid nucleus, anterior hypothalamic nucleus and the median raphe nucleus was observed in the avoidance task. Both tasks, however, activated common structures, such as the paraventricular nucleus of the thalamus and the dorsomedial hypothalamic nucleus.\textsuperscript{61} These structures are related to the elaboration of anxiety and innate fear.\textsuperscript{52-67}

Whilst the ETM seems to be a reliable tool for studying the GAD and PD-like behaviors in rats, experiments with mice showed different results. Jardim et al.\textsuperscript{68} demonstrated that the time spent to escape from the open arm (escape behavior) was similar to the latency to leave the closed arm (avoidance behavior) in the first trial. Furthermore, the latencies for the mouse leaving the closed arm were high and did not differ significantly between the three consecutive trials. Jardim et al.\textsuperscript{69} have asserted that “it does not seem to be possible to separate conditioned and unconditioned fear in the elevated T-maze to mice.”

Nevertheless, Carvalho-Netto & Nunes-de-Souza\textsuperscript{2} showed that mice did acquire inhibitory avoidance in the ETM. When the number of exposures to the avoidance trials was increased from three to five, the latency to leave the closed arm increased statistically from baseline. However, even with five trials, the latency to leave the open arm in the escape trial did not change, as verified in rats.\textsuperscript{10} Furthermore, they showed that mice in an ETM with transparent walls have a lower avoidance baseline latency and a lower escape latency than those submitted to an ETM with opaque walls, suggesting that the apparatus with transparent walls is more useful for studying avoidance and escape behaviors in mice.

Graeff et al.\textsuperscript{4} showed that in rats, anxiolytic and anxiogenic compounds increased and decreased, respectively, the latency for the avoidance task in the ETM. Neuropeptides, psychostimulants, phenylethylamine hallucinogens, and a monoamine oxidase inhibitor A were ineffective, though the avoidance task was impaired by diazepam, buspirone, and ipsapirone, which are three well-known compounds that ameliorate GAD.\textsuperscript{31} Furthermore, Carvalho-Netto & Nunes-de-Souza\textsuperscript{2} performed the same experiments with similar compounds in mice (five trials). A summary of drugs with pharmacological actions in rodents submitted to the ETM test is shown in Table 2.

Confrontation between rodents and serpents as a model of generalized anxiety and panic attacks

This may be the first laboratory approach based on snake and rodent interaction in enriched experimental environments, addressing the effect of limbic system activation in a threatening situation that elicits both the predatory and antipredatory ethological repertoires during pharmacological testing of new drugs with potential antiaversive effects.\textsuperscript{26,27}

Wild snakes have been used as aversive stimuli to either small non-human primates,\textsuperscript{59-71} apes,\textsuperscript{75-76} and rodents.\textsuperscript{79,80}
Recent reports using the apparatuses designed by the first author to contain complex labyrinths and polygonal arenas\textsuperscript{24,64} have shown that the rodent versus snake (both venomous and non-venomous) paradigms are useful for conducting ethological and neuropharmacological experiments.\textsuperscript{22-27,34,36-39}

**Table 2** Summary of the elevated T-maze test pharmacological validation

| Compound      | Drug action                  | Passive avoidance | Escape |
|---------------|------------------------------|------------------|--------|
| 8-OH-DPAT     | 5-HT\textsubscript{1A} agonist | \(?\)             | \(?\)  |
| Buspirone     | 5-HT\textsubscript{1A} partial agonist | \(+)\             | \(0\)  |
| Caffeine      | Anxiogenic compound (psychomotor stimulant) | \(0\)             | \(0\)  |
| Clomipramine  | 5-HT\textsubscript{2A} antagonist; 5-HT uptake blocker | \(-\)             | \(0\)  |
| D,L-amphetamine | Psychomotor stimulant    | \(0\)             | \(0\)  |
| d-Fenfluramine| 5-HT releaser               | \(-\)             | \(0\)  |
| Diazepam      | Benzodiazepine agonist      | \(0\)             | \(0\)  |
| DOI           | 5-HT\textsubscript{2A/2C} agonist | \(0\)             | \(0\)  |
| FG 7142       | Benzodiazepine inverse agonist | \(-\)             | \(0\)  |
| Flumazenil    | 5-HT\textsubscript{1A} full agonist | \(0\)             | \(0\)  |
| Haloperidol   | Neuroleptic                 | \(0\)             | \(0\)  |
| Imipramine    | 5-HT/NA reuptake blocker    | \(-\)             | \(0\)  |
| Ipsapirone    | 5-HT\textsubscript{1A} partial agonist | \(+\)             | \(0\)  |
| mCPP          | 5-HT\textsubscript{2B/2C} agonist | \(-\)             | \(0\)  |
| Mocllobemide  | Monoamine oxidase A inhibitor | \(-\)             | \(0\)  |
| Ritalserin    | 5-HT\textsubscript{2A/2C} antagonist | \(+\)             | \(0\)  |
| SB 206464A    | 5-HT\textsubscript{2B/2C} antagonist | \(+\)             | \(0\)  |
| SER 082       | 5-HT\textsubscript{2B/2C} antagonist | \(+\)             | \(0\)  |
| SR 46349B     | 5-HT\textsubscript{2A} antagonist | \(+\)             | \(0\)  |
| TFPP          | 5-HT\textsubscript{2B/2C} agonist | \(-\)             | \(0\)  |
| Yohimbine     | Alpha\textsubscript{2} noradrenergic receptor antagonist | \(-\)             | \(0\)  |

5-HT = serotonin.

**Simple and complex polygonal arenas for snakes**

The polygonal arena consists of a semi-transparent acrylic enclosure (154 × 72 × 64 cm). The inner walls are covered with a reflective film that provides 80% light reflection, and consequently minimal visual contact by the prey and predator with the surrounding experimental area. A green fluorescent line (4.2 mm width; Pritt mark-it) is used to divide the arena into 20 equal rectangles to facilitate analysis of locomotion. The acrylic base of the arena is placed over a rectangular stainless steel platform, and the whole apparatus is placed on a granite surface (2 × 85 × 170 cm) positioned 83 cm above the floor to minimize vibratory stimuli. It is important to highlight that when the burrow is present in the arena, the behaviors may be proportionally recorded in relation to the time spent by each animal inside and outside the burrow through a behavioral index (BI = \([100 \times \text{number of behavioral responses}]/\text{time in seconds spent outside or inside the burrow}\))\textsuperscript{26,27} Moreover, the duration of each behavioral response may be expressed as the percentage of a given behavioral response duration displayed outside or inside the burrow, considering as 100% the total time of the behavioral test.\textsuperscript{26} In an attempt to compare the defensive behaviors of mice exposed to the polygonal arena without (Figure 1) or with a small burrow present in one of its angular extremities (Figure 2), it was observed that mice confronted with venomous snakes, such as the South American coral snake *Micrurus lemniscatus carvalhoi* (Figure 1A and C), the *Crotalus durissus terrificus* (Figure 1B, Figure 2A, B, and C) or the *Bothrops alternatus* (Figure 2D and E),...
displayed expressive anxiety-related defensive responses, such as alertness (Figure 1A, Figure 2A), risk assessment (Figure 2C), and inhibitory avoidance (Figure 2E), panic-related defensive responses, such as freezing (Figure 1C, Figure 2B), and both oriented (Figure 1B) and non-oriented (Figure 2D) escape behavior. Interestingly, even during imminent risk of death, some mice closely interacted with the predator (Figure 2C). In this experiment, the survival rate of mice confronted by snakes was 83.30%, as shown in Table 3. This high rate of survival may have resulted from the presence of the burrow inside the polygonal arena, or may have even been related to the huge size of the simple polygonal arena. However, depending on the genus of the snakes used, the survival rate can be as high as 100% even when the polygonal arena has no burrow (Table 3). This survival rate has been observed in confrontations between golden hamsters and South American coral snakes (the highly venomous *Micrurus frontalis* and the non-venomous *Oxyrhopus guibei*).34

Polygonal arenas with burrows can also potentially be used as a model of post-traumatic stress disorder (PTSD). Experiments focusing on exposure to a live snake and re-exposure to the contextual arena are being conducted with mice and rainbow Boidae constrictor snakes in the LNN-FMRP-USP/INeC Ophidiarium to address this possibility in an investigation of comorbidity between PTSD and chronic pain.28

**Complex arenas and labyrinth for snakes**

Complex polygonal arenas containing natural rocks and artificial shelters for rodents and natural branches for

---

**Figure 1** Innate fear-induced defensive responses evoked by *Mesocricetus auratus* confronted with the South American coral snake *Micrurus lemniscatus carvalhoi* (A and C), and the South American rattlesnake *Crotalus durissus terrificus* (B) for 5 min in a polygonal arena without a burrow. Anxiety/fear-related response: alertness (A) elicited in the presence of the coral snake. Panic attack-like responses: oriented escape (B) and freezing (C) elicited in the presence of each venomous snake.

**Figure 2** Instinctive fear-induced defensive responses evoked by *Mus musculus* confronted with the South American Viperidae snakes *Crotalus durissus terrificus* (A, B, C) and *Bothrops alternatus* (D and E) for 5 min in a polygonal arena with a burrow. Anxiety/fear-related response: alertness (A) elicited in the presence of the rattlesnake, and inhibitory avoidance (E, e') displayed in the presence of *Bothrops alternatus*. Panic attack-like responses: freezing (B) displayed by prey threatened by a rattlesnake, and non-oriented escape (D) displayed by prey threatened by *Bothrops alternatus* venomous snake.

constrictor snakes can be considered as a more ethologically acceptable alternative to study the defensive repertoires of different species of rodents confronted with venomous and *Boidae* snakes. This approach can be useful to investigate feeding preferences considering the different sizes of laboratory animals. Despite the high frequency of interaction between gerbils and *Boidae* snakes, no feeding preference was observed when the constrictor snakes *Boa constrictor amarali* and...
Epicrates cenchria crassus were confronted with Mus musculus, Meriones unguiculatus, Cavia porcellus, or Rattus norvegicus.43

The complex labyrinth for the confrontation between snakes and rodents, which was designed by the first author in 2000, was ethologically validated by Guimarães-Costa et al.24 This apparatus consists of a transparent acrylic enclosure containing a small polygonal arena contiguous with a complex maze. The gallery walls are made of black acrylic. The arena measures 38.5 m², and the remaining labyrinth measures 15 cm in height and 6.92 m in length. The whole apparatus containing the complex labyrinth and arena measures 140 × 70 × 15 cm. The top of the labyrinth and the arena contain 84 circular holes (1.5 cm in diameter). The floor of the complex labyrinth is made of a clear crystal acrylic plaque (140 × 70 cm) that is placed on another metallic plaque made of 1-mm-wide stainless steel with the same dimensions. The arena is divided by 0.4 cm green fluorescent lines into 20 equal rectangles (27.7 × 17.2 cm each). It is important to highlight that even wild constrictor snakes as heavy as 2,500 g can invade the galleries during hunting behavior, which increases the panic attack-like behaviors of prey animals.24 The confrontation between rodents and snakes can occur in both divisions of the complex labyrinth, i.e., within the arena or inside the galleries.

According to Guimarães-Costa et al.,24 Mongolian gerbils display the best exploratory response in this apparatus when compared to Wistar rats and golden hamsters. Gerbils explore the whole arena and the galleries of the complex labyrinth for 5 min, displaying anxiety-related behaviors (such alertness, flat back approach, and stretch attend posture) and panic-attack-related responses. Similar behavioral reactions can be evoked by mice confronted with constrictor snakes inside the complex labyrinth, as shown in Figures 3 and 4. In this case, the survival rate was 100% (Table 3). The advantages of the complex labyrinth over other types of labyrinths include the possibility of studying aversive memory-related responses and innate fear-related behavior as well as the increased chances of survival of threatened rodents. The disadvantage of this apparatus is the difficulty of interaction between prey and predator, considering the species of rodents and snakes used in each experiment. Some species of rodents, including Mongolian gerbils and mice, can explore the entire apparatus in five minutes, whereas rats and guinea pigs commonly show a delay in finding all gallery exits. To minimize this delay and reduce the species-specific differences in time spent exploring the maze, all rodents are habituated to the complex labyrinth for at least 3 days before the experiments.

Similar patterns of instinctive fear-induced defensive behavior were displayed by mice confronted with three different species of non-venomous rainbow Boidae constrictor snakes: Boa constrictor constrictor (Figure 3), Epicrates cenchria assissi (Figure 4A, B, C, and D), and Epicrates cenchria cenchria (Figure 4E and F). In fact, mice displayed anxiety-related behaviors, such as alertness (Figure 3A, Figure 4B and E), inhibitory avoidance (Figure 3C, Figure 4B), flat back approach and stretch attend posture (Figure 3E, Figure 4C), and panic-related behavior, such as freezing (Figure 3B, Figure 4A and F) and escape behavior (Figure 3D). Despite the galleries, prey and predator interacted closely, as shown in Figure 3E and F, and in Figure 4D.

Table 4 summarizes the main characteristics of the EPM, T-maze, and rodent vs. snake models.

Neural substrates involved in threatened prey animals

Many studies using models of confrontation between rodents and a predator consider explosive escape behavior to be a consequence of periaqueductal gray matter (PAG) neuron activation.84,85 In fact, the dorsomedial, dorsolateral, and lateral columns of the PAG in Syrian hamsters confronted with coral snakes show several Fos protein-labeled neurons.40 This finding corroborates previous reports showing that rats exposed to a natural predator (cat) express Fos-labeled neurons in the dorsal and ventral columns of the PAG.54 These PAG columns are involved in the organization of behavioral and physiological responses that are crucial for the survival of threatened animals based on whether the dangerous situation is distal or proximal.29,86 In addition, the interaction between the intramesencephalic endogenous opioid peptide-mediated pathways and the GABAergic nigrotectal inputs seems to be critically involved.
in both the modulation of defensive responses organized by the dorsal midbrain and the defensive responses evoked in prey versus wild snakes paradigms. However, the confrontation between Syrian hamsters and coral snakes showed neuronal activation also in the hamsters’ corpora quadrigemina, medial hypothalamus and amygdaloid complex. In fact, other encephalic regions are also activated during similar critical situations. For example, the amygdaloid complex is recruited in both unconditioned and conditioned fear-induced responses. Nevertheless, the posterior basomedial amygdaloid nucleus is particularly responsive to cat odor and seems to be involved in the identification of pheromone cues from predator odors.

The Fos immunoreactivity study showed that the main encephalic structures recruited during a prey versus predator confrontation are situated in the hypothalamus and involve the recruitment of the anterior hypothalamus, the dorsomedial division of ventromedial hypothalamic nucleus, and mainly the dorsal premammillary nucleus. Previous reports have shown that these nuclei are critically involved in the organization of innate defensive responses; this proposition is based on Fos immunoreactivity in rats exposed to natural predators, such as cats and snakes. Other studies using electric or chemical stimulation of the hypothalamic defense system are in agreement with the findings using prey versus predator confrontations, and both these experimental models of panic attacks result in oriented escape reactions.

It is possible that the Fos-labeled neurons in the dorsal midbrain and the hypothalamic nuclei of rodents exposed to a live predator, to the odor of their skin, or to their excrements are indicative of the involvement of these structures in the elaboration of innate fear-induced behaviors, such as defensive alertness, defensive immobility and escape behavior. These behaviors are commonly displayed by rodents confronted with venomous and constrictor snakes and other natural predators.

Discussion

There still are questions regarding how strong EPM-evoked behaviors corroborate anxiety disorders. Studies have shown that exposure to the EPM increases the expression of Fos protein in encephalic areas related to the organization of defensive behaviors, which include the amygdaloid complex, the hippocampal formation, the midbrain periaqueductal gray matter, the hypothalamus, and the prefrontal brain areas. Fos protein expression is linked to a general increase in cell metabolism, which is differentially activated in specific brain areas according to the presented stimulus. For example, anxiogenic stimuli such as those present in the EPM open arms can activate neurons from these brain areas related to anxiety or fear responses. These areas are also activated when the animal is exposed to a predator, aversive ultrasonic vocalisations, and the systemic administration of anxiogenic drugs. Corroborating these data, the diverse components of defensive behaviors with distinct brain areas that coordinate the

Table 3 Offensive/defensive responses of Crotalus durissus terrificus and Bothrops alternatus snakes in Coimbra polygonal arenas and complex labyrinth during a 5-min exposure to mice

| Response            | Polygonal arenas | Complex labyrinth |
|---------------------|------------------|-------------------|
|                     | C. durissus terrificus | B. alternatus | C. durissus terrificus | B. alternatus |
| Threatening posture | 2                | 3                | 3                | 1              |
| Defensive attack    | 0                | 0                | 0                | 0              |
| Offensive attack    | 0                | 0                | 0                | 0              |
| Predation           | 1                | 1                | 0                | 0              |
| Rodents survival (%)| 83.3             | 83.3             | 100              | 100            |
behavioral strategies when a threat is present have been classified. A potential threat elicits risk assessment and behavioral inhibition, which are related to the activation of the posterior cingulate cortex and septo-hippocampal system and results in anxiety. This same potential threat can also elicit avoidance, activating the anterior cingulate cortex and amygdaloid complex, resulting in anxiety. Complementarily, distal threats elicit freezing behavior via the activation of the ventral columns of periaqueductal gray matter, resulting in instinctive fear. Finally, a proximal threat elicits freezing or fight or flight behavior via the activation of dorsal columns of the periaqueductal gray matter, resulting in a generalized panic reaction.

Interestingly, the EPM test can predict the elicitation of specific behaviors depending on the stress suffered by the animal. Some behavioral parameters evoked in the EPM are related to increased hormonal components of stress response or psychiatric disorders, such as high serum levels of steroid hormones. In patients, anxiety-related symptoms are correlated with an unbalance of steroid secretion within the hypothalamic-pituitary-adrenal axis.31,33 Corroborating these studies, increased levels of plasmatic corticosterone in rodents have been associated with higher frequencies of anxiety-related SAP in the EPM test.102-104 Additionally, chronic administration of anabolic androgenic steroids increases the number of rearing response and decreases SAP frequencies without affecting the time spent and frequency of open-arm entries.105

Another defensive reaction generally observed in rodents exposed to the EPM is the antinociception induced by instinctive fear, as evaluated by the tail-flick test.106,107 This reaction has a clear adaptive value because it permits the animal to exhibit other defensive behaviors even though an injury has occurred, consequently increasing its chances of survival in a dangerous situation, and can be also elicited by electrical and chemical stimulation of diencephalic66-98,108 and mesencephalic81,109,110 structures. Nevertheless, this defensive reaction was not observed in mice submitted to the formalin pain test and exposed to the EPM13,17,111-113 Therefore, the authors who performed those experiments decided to use the open elevated plus maze, which has the same dimension of the standard EPM but is comprised of four open arms. Using this apparatus, they verified antinociception of high magnitude. The open-arms EPM seemed to be a good model for studying fear-induced antinociception; this modified apparatus has also been behaviorally and pharmacologically validated.114

Experimental evidence from the 1970s, 1980s, and early 1990s suggests that serotonin (5-HT) facilitates punished behavior by recruiting the amygdaloid complex activity (resulting in anxiety) and inhibits escape behavior by acting in the dorsal periaqueductal gray matter (the unbalance of which causes panic).115,116 This led Deakin & Graeff97 to propose that 5-HT neurons mediate avoidance behaviors while simultaneously inhibiting escape behaviors.1 Because of its direct implications for anxiety and panic, serotonin and its actions in the amygdaloid complex or the periaqueductal gray matter have been extensively studied in rodent models of anxiety, such as the EPM. These exhaustive investigations have helped establish the EPM test as a reliable experimental tool for studying anxiety in rodents.65,117-121 Notwithstanding the high reliability of the EPM for measuring anxiety-like behaviors in rodents,122 this test has been criticized as a mixed model of anxiety that combines an avoidance behavior with an escape behavior: in the avoidance behavior (a conditioned-like behavior or anticipatory anxiety-related response), the animal is in the...
closed arms and avoids the open arms, whereas in the escape (panic-like) behavior, the animal is in the open arms and draws back to the closed arms in search of a protected environment. Thus, exposure to the open and closed arms may elicit different types of defensive behaviors. Considering these psychobiological characteristics of the EPM test, this type of mixed model might not be very appropriate for studying the effects of all anxiolytic compounds, or it may need to be used with caution if a unique behavioral task is used to evaluate anxiety.

Prey versus predator paradigms are excellent approaches for studying innate fear-related behaviors, not only because these paradigms allow us to focus on the effects of a discrete intervention in a given structure of the limbic system or a functionally related structure, but also because they are useful to study the integrated activation of the limbic system in a threatening situation. Although much research now focuses on more invasive methods, such as the use of electrically and chemically restricted brain stimulation, to clarify the involvement of a given brain structure in the organization of defensive behavioral responses, the prey-versus-predator paradigm is still useful for studying the activity of the brain aversion system activity, testing new drugs with potential applications in neuropsychiatry, or for assessing the behavioral phenotypes of genetically-modified mice.

In conclusion, simple labyrinths such as the elevated plus maze and elevated T-maze are excellent apparatuses for the study of anxiety- and instinctive fear-related responses, respectively. Both apparatuses have been sufficiently validated in both behavioral and pharmacological terms. The confrontation between rodents and snakes in polygonal arenas, however, offers a more ethological environment for addressing both unconditioned and conditioned fear-based behaviors and the effects of anxiolytic and panicolytic drugs. More specifically, in the prey-predator confrontation approach, the possibility of testing both anxiety and fear or combined panic-related behaviors allows for a more complete approach to new drug or rodent phenotype testing, considering the diverse aversive stimuli in the rodent ethogram.

Acknowledgements

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (grant 2012/03798-0), Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (CNPq) (grant 470119/2004-7), Fundação de Apoio ao Ensino, Pesquisa e Assistência do HC-FM/USP (FAPEA) (grants 1291/97, 355/2000, 68/2001, and 15/2003), and a research grant from the Pro-Rectory of Universidade de São Paulo (USP) (IPAQ2012; NAP-USP-NuPNE-156). The authors thank Daoud Hibrahim Elias-Filho for technical support. JPC-C is an under-graduate student from the Neurosciences for Kids Program of LNN-FMRP-USP and the Caraggavio Workshop (Project MEDUSA-LNN-FMRP-USP/INeC Ophidiaram). NCC is a researcher (level 1A) from CNPq (processes 301905/2010-0 and 301341/2015-0). DHEF received a technician’s scholarship from FAPESP (TT-2, process 02/01497-1) and was the recipient of scholarships sponsored by CNPq (processes 501858/2005-9, 500896/ 2008-9, and 505461/2010-2).

Disclosure

The authors report no conflicts of interest.

References

1. Carobrez AP, Bertoglio LJ. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neurosci Biobehav Rev. 2005;29:1193-205.
2. Carvalho-Netto EF, Nunes-de-Souza RL. Use of the elevated T-maze to study anxiety in mice. Behav Brain Res. 2004;148:119-32.
3. Cruz AP, Frei F, Graeff FG. Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol Biochem Behav. 1994;49:171-6.
4. Graeff FG, Netto CF, Zangrossi H Jr. The elevated T-maze as an experimental model of anxiety. Neurosci Biobehav Rev. 1998;23:237-46.
5. Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl). 1987;92:180-5.
6. Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods. 1985;14:149-67.
7. Rodgers RJ, Cole JC. The elevated plus-maze: pharmacology, methodology and ethology. In: Cooper SJ, Hendrie CA, editors. Ethology and psychopharmacology. Chichester: John Wiley & Sons; 1994. p. 9-44.
8. Rodgers RJ, Johnson NJ. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol Biochem Behav. 1995;52:297-303.
9. Treit D, Menard J, Royan C. Anxiogenic stimuli in the elevated plus-maze. Pharmacol Biochem Behav. 1993;44:463-9.
10. Zangrossi H Jr, Graeff FG. Behavioral validation of the elevated T-maze, a new model of anxiety. Brain Res Bull. 1997;44:1-5.
11. File SE, Zangrossi H Jr. “One-trial tolerance” to the anxiolytic actions of benzodiazepines in the elevated plus-maze, or the development of a phobic state? Psychopharmacology (Berl). 1993;110:240-44.
12. Guimarães FS, Beijamini V, Moreira FA, Aguiar DC, de Lucca AC. Role of nitric oxide in brain regions related to defensive reactions. Neurosci Biobehav Rev. 2005;29:1313-22.
13. Mendes-Gomes J, Nunes-de-Souza RL. Concurrent nociceptive stimulation impairs the anxiolytic effect of midazolam injected into the periaqueductal gray in mice. Brain Res. 2005;1047:97-104.
14. Roncon CM, Biedorf C, Santana RG, Zangrossi H Jr, Graeff FG, Audi EA. The panicolytic-like effect of flusoxetine in the elevated T-maze is mediated by serotonin-induced activation of endogenous opioids in the dorsal periaqueductal grey. J Psychopharmacol. 2012;26:525-31.
15. de Paula Soares V, Campos AC, Bortoli VC, Zangrossi H Jr, Guimarães FS, Zuardi AW. Intra-dorsal periaqueductal gray administration of cannabidiol blocks panic-like response by activating 5-HT1A receptors. Behav Brain Res. 2010;213:225-9.
16. Zangrossi H Jr, Leite JR, Graeff FG. Anxiolytic effect of carbamazepine in the elevated plus-maze: possible role of adenosine. Psychopharmacology (Berl). 1992;106:85-9.
17. Mendes-Gomes J, Nunes-de-Souza RL. Anxiolytic-like effects produced by bilateral lesion of the periaqueductal gray in mice: influence of concurrent nociceptive stimulation. Behav Brain Res. 2009;203:180-7.
18. Santos P, Bittencourt AS, Schenberg LC, Carobrez AP. Elevated T-maze evaluation of anxiety and memory effects of NMDA/glycine-B site ligands injected into the dorsal periaqueductal gray matter and the superior colliculus of rats. Neuropharmacology. 2006;51:203-12.
19. Adamiec R, Fougeron R. Left and right: CRF receptor blockade prevents initiation and consolidation of stress effects on affect in the predator stress model of PTSD. Int J Neuropsychopharmacol. 2010;13:747-57.
20 Calabrese EJ. An assessment of anxiolytic drug screening tests: hermetic dose response predominant. Crit Rev Toxicol. 2008;38:489-542.

21 Zangrossi H Jr, File SE. Behavioral consequences in animal tests of anxiety and exploration of exposure to cat odor. Brain Res Bull. 1992;29:381-8.

22 Almada RC, Coimbra NC. Recruitment of striatonigral disinhibitory and nigro-territorial inhibitory GABAergic pathways during the organization of defensive behavior by mice in a dangerous environment with the venomous snake Bothrops alternatus (Reptilia, Viperidae). Synapse. 2015;69:299-313.

23 Almada RC, Roncon CM, Elias-Filho DH, Coimbra NC. Endocannabinoid signaling mechanisms in the substantia nigra pars reticulata modulate GABAergic nigrostriatal pathways in mice threatened by urutu-cruzeiro venomous pit viper. Neuroscience. 2015;303:503-14.

24 Guimarães-Costa R, Guimarães-Costa MB, Pippa-Gadioli L, Weltson A, Uibial WA, Paschoalin-Maurin T, et al. Innate defensive behavior and panic-like reactions evoked by rodents during aggressive encounters with Brazilian constrictor snakes in a complex labyrinth: behavioral validation of a new model to study affective and agonistic reactions in a prey versus predator paradigm. J Neurosci Methods. 2007;165:25-37.

25 Lobão-Soares B, Walz R, Prediger RD, Freitas RL, Calvo F, Bianchin MM, et al. Cellular prion protein modulates defensive and innate fear-induced behavior evoked in transgenic mice subjected to an agonistic encounter with the tropical coral snake Oxyrhopus guibei. Behav Brain Res. 2008;194:129-37.

26 Uribe-Maríno A, Franciso A, Castilbanchor-Urbina MA, Twardowschy A, Salgado-Rohner CJ, Crippa JA, et al. Anti-aversive effects of cannabinoids on innate fear-induced behaviors evoked by an ethological model of panic attacks based on a prey vs. the wild snake Epicrates cenchria cenchria confrontation paradigm. Neuropsychopharmacology. 2012;37:412-21.

27 Twardowschy A, Castilbanchor-Urbina MA, Uribe-Maríno A, Biagioni AF, Salgado-Rohner CJ, Crippa JA, et al. The role of 5-HT1A receptors in the ant-aversive effects of cannabinoids on panic attack-like behaviors evoked in the presence of the wild snake Epicrates cenchria cenchria. J Psychopharmacol. 2013;27:1149-59.

28 Mendes-Gomes J, Paschoalin-Maurin T, Freitas RL, Donaldson L, Lumb BM, Coimbra NC. Unconditioned fear induces antinociception in sham rats threatened by wild snakes but not in those with neuropathic pain. Eur Neuropsychopharmacol. 2014;24:S587.

29 Bandler R, Depaulis A. Midbrain periaqueductal gray control of defensive behavior in the cat and in the rat. In: Depaulis A, Bandler R, editors. The midbrain periaqueductal gray matter: functional, anatomical and neurochemical organization. New York: Plenum; 1991. p. 175-98.

30 Blanchard DC, Blanchard RJ, Rodgers RJ. Risk assessment and animal models of anxiety. In: Olivier B, Mos J, Slangen JL, editors. Animal models in psychopharmacology. Bale: Birkhäuser; 1995. p. 117-34.

31 Nutt DJ. Anxiety and its therapy: today and tomorrow. In: Briley M, File SE, editors. New concepts in anxiety. London: MacMillan; 1991. p. 1-12.

32 Uibial WA. Behavioural detection of anxiolytic action. In: Elliott JM, File SE, editors. Behavioural consequences in animal tests of anxiety and exploration of exposure to cat odor. Brain Res Bull. 1992;29:381-8.

33 Paschoalin-Maurin T, Coimbra NC. Chronic treatment with alprazolam decreases defensive reactions elicited by Golden hamsters in confrontation with venomous coral snakes. Eur Neuropsychopharmacol. 2006;16:S229.

34 Paschoalin-Maurin T, Coimbra NC. Chronic treatment with alprazolam decreases defensive reactions elicited by Golden hamsters in confrontation with Brazilian coral snakes. J Psychopharmacol. 2006;20(5):A15.

35 Paschoalin-Maurin T, Coimbra NC. Acute paroxetine or alprazolam attenuate defensive responses of hamsters confronted with venomous coral snake. Eur Neuropsychopharmacol. 2008;18: S222-3.

36 Paschoalin-Maurin T, Coimbra NC. Effect of chronic paroxetine or alprazolam on the increase of c-Fos expression in amygdaloid complex of golden hamsters confronted with South American venomous coral snake. Int J Psychopharmacol. 2008;69:269.

37 Weltson A, Pippa-Gadioli L, Koji-Narasaki F, Paschoalin-Maurin T, Del Bel EA, Coimbra NC. Neuroromorphologic evidence for c-Fos-immunoreactive neurons in forebrain, diencephalon and brainstem structures involved in the elaboration of panic and fear in golden hamster after aggressive encounter with South American coral snake. Int J Psychopharmacol. 2002;45:156-7.

38 Weltson A, Rocha MAJ, Coimbra NC. Antipanic-like effect of chronic treatment with clomipramine on fear-induced responses elicited by preys in aggressive confront with wild rattlesnakes. J Psychopharmacol. 2006;20:A16.

39 Weltson A, Coimbra NC. Chronic paroxetine and alprazolam use PAG columns as pharmacological targets. In: XIV World Congress of Psychiatry; 2008, Prague, Czech Republic. p. 1080-1.

40 Weltson A, Pippa-Gadioli L, Twardowschy A, Coimbra NC. Interacao entre diferentes especies de roedores e serpentes constrictoras brasileiras em um paradigma baseado no confronto entre presa e predador. In: Anais do XXV Encontro Anual de Ectologia; 2007. p. 299.

41 Weltson A, Pippa-Gadioli L, Twardowschy A, Coimbra NC. Mendes-Gomes J, D Silva JA, Dos Anjos-Garcia T, Ullah, Almada RC. New ethological and morphological perspectives for the investigation of panicoletic-like effect of cannabinoid. In: Preedy VR, editor. The Handbook of cannabis and related pathologies: biology, diagnosis, treatment, and pharmacology. Amsterdam: Elsevier; 2017. Chapter e14, p. e104-9.

42 Handlel SL, McBlane JW. An assessment of the elevated X-maze for studying anxiety and anxiety-modulating drugs. J Pharmacol Toxicol Methods. 1999;39:129-35.

43 Faller U, Gower AJ, Gobert J. Resistance of baseline activity in the elevated plus-maze to exogenous influences. Behav Pharmacol. 1993;12:3:135.

44 Anseloni VC, Motta V, Lima G, Brandão ML. Behavioral and pharmacological validation of the elevated plus maze constructed with transparent walls. Brain Res. 2000;877:207-10.

45 Anseloni VZ, Coimbra NC, Morato S, Brandão ML. A comparative study with two types of elevated plus-maze constructed with transparent walls. Brain Res. 2000;859:99-101.

46 Falter U, Gower AJ, Gobert J. Resistance of baseline activity in the elevated plus-maze to exogenous influences. Behav Pharmacol. 1993:12:3:135.

47 Anseloni VZ, Motta V, Lima G, Brandão ML. Behavioral and pharmacological validation of the elevated plus maze constructed with transparent walls. Brain Res. 1997;8:533-40.

48 Albrechet-Souza L, Oliveira AR, De Luca MC, Tomazini FM, Santos NR, Brandão ML. A comparative study with two types of elevated plus-maze (transparent vs. opaque walls) on the anxiolytic effects of midazolam, one-trial tolerance and fear-induced analgesia. Prog Neuropsychopharmacol Biol Psychiatry. 2005;29:571-9.

49 Graeff FG, Almeida SS, Graeff EO, Hunziker MH. Behavioral effects of benzodiazepines on behavior and anxiety-modulating drugs. J Pharmacol Toxicol Methods. 1999;39:129-35.

50 Uibial WA, Rocha MAJ, Coimbra NC. Antipanic-like effect of chronic treatment with clomipramine on fear-induced responses elicited by preys in aggressive confront with wild rattlesnakes. J Psychopharmacol. 2006;20:A16.

51 Adamec RE, Shallow T. Lasting effects on rodent anxiety of a single hermetic dose response predominant. Crit Rev Toxicol. 2001;25:71-24.

52 Adamec RE, Shallow T. Lasting effects on rodent anxiety of a single hermetic dose response predominant. Crit Rev Toxicol. 2001;25:71-24.

53 Rodgers RJ, Blundell J, Collins A. Neural plasticity and stress induced changes in defense in the rat. Neurosci Biobehav Rev. 1999;23:155-65.

54 Rodgers RJ, Coimbra NC, Brandão ML. A comparative study of the effects of morphine in the dorsal periaqueductal gray and nucleus accumbens or rats submitted to the elevated plus-maze test. Exp Brain Res. 1999;129:260-8.

55 Anseloni VC, Coimbra NC, Morato S, Brandão ML. A comparative study of the effects of morphine in the dorsal periaqueductal gray and nucleus accumbens or rats submitted to the elevated plus-maze test. Exp Brain Res. 1999;129:260-8.
Borelli KG, Ferreira-Netto C, Coimbra NC, Brandão ML. Fos-like immunoreactivity in the brain induced by performance of avoidance or escape in the elevated T-maze. Behav Brain Res. 2001;126:13-21.

Biagioni AF, dos Anjos-Garcia T, Ullah F, Fischer IR, Falconi-Sobrinho LL, de Freitas RL, et al. Neuroethological validation of an experimental apparatus to evaluate oriented and non-oriented escape behaviour: Comparison between the polygonal arena with a burrow and the circular enclosure of an open-field test. Behav Brain Res. 2016;298:65-77.

Biagioni AF, dos Anjos-Garcia T, Ullah F, Fischer IR. Fos-like immunoreactivity in the dorsal periaqueductal gray matter in the organization of freezing or oriented and non-oriented escape emotional behaviors. Behav Brain Res. 2015;293:143-52.

Canteras NS, Ribeiro-Barbosa ER, Canteras NS. Predatory hunting and exposure to alive predator induce opposite patterns of Fos immunoreactivity in the PAG. Behav Brain Res. 2003;138:143-52.

Carneiro P, Leung P, Harris J, Paxino G. Conditioned fear to context is associated with increased Fos expression in the caudal ventrolateral region of the midbrain periaqueductal gray. Neuroscience. 1997;78:165-77.

Eichenberger GCD, Ribeiro SJ, Osaki MY, Maruoka YF, Resende GC, Castellan-Baldan L, et al. Neuroanatomical and pharmacological evidence for interaction between opioid and GABAergic neural pathways in the modulation of fear and defense elicited by electrical and chemical stimulation of the deep layers of the superior colliculus and dorsal periaqueductal gray matter. Neuropsychopharmacology. 2002;42:48-59.

Ohman A. Skin conductance responses to masked conditioned stimuli: phylogenetic/ontogenetic factors versus direction of threat? Biol Psychol. 2007;74:328-36.

Crake MG, Sipsas A. Animal phobias versus clастrophobias: exteroceptive versus interoceptive cues. Behav Res Ther. 1992;30:569-81.

Swaisgood RR, Owings DH, Rowe MP. Conflict and assessment in a predator-prey system: ground squirrels versus rattlesnakes. Anim Behav. 1999;57:1033-44.

Coimbra NC, de Oliveira R, Freitas RL, Ribeiro SJ, Borelli KG, Pacagnella RC, et al. Neuroanatomical approaches of the tectum-reticular pathways and immunohistochemical evidence for serotonin-positive perikarya on neuronal substrates of the superior colliculus and periaqueductal gray matter involved in the elaboration of the defensive behavior and fear-induced analgesia. Exp Neurol. 2006;197:93-112.

Silveira MC, Sandner G, Graeff FG. Induction of Fos immunoreactivity in the brain by exposure to the elevated plus-maze. Behav Brain Res. 1993;56:115-9.

Cook M, Mineka S. Observational conditioning of fear-relevant stimuli. J Abnorm Psychol. 2005;104:653-63.

Corrêa VM, Coimbra NC. Topographic and functional neuroanatomical study of opioid pathways in the mesencephalic tectum: effect of µ1- and κ-opioid receptor blockade on escape behaviour induced by electrical stimulation of the inferior colliculus. Brain Res. 2003;992:179-92.

Castellan-Baldan L, de Oliveira R, et al. Neuroanatomical and Neuropharmacological study of opioid pathways in the mesencephalic tectum of the defensive behavior and fear-induced analgesia. Exp Neurol. 2006;197:93-112.

Falk CR, Germain P, Offord DR, et al. The unmasking of conditioned fear in children with obsessive-compulsive disorder: a randomized clinical trial. J Clin Psychiatry. 2005;66:486-93.

Canteras NS, Chiavegatto S, Ribeiro do Valle LE, Swanson LW. Severe reduction of rat defensive behavior to a predator by destruction of hypothalamic chemical lesions. Brain Res Bull. 1997;44:297-305.

Canteras NS, Ribeiro-Barbosa ER, Comoli E. Tracing from the dorsal premammillary nucleus prosencephalic systems involved in the organization of innate fear response. Neurosci Biobehav Rev. 2001;25:661-8.
Biagioli AF, Silva JA, Coimbra NC. Panic-like defensive behavior but not fear-induced antinociception is differently organized by dorsomedial and posterior hypothalamic nuclei of Rattus norvegicus (Rodentia, Muridae). Braz J Med Biol Res. 2012;45:328-36.

Rojas-Ortiz YA, Rundle-Gonzalez V, Ramos-Rivera I, Jorge JC. Reis FM, Albrechet-Souza L, Franci CR, Brandão ML. Risk modulation of elevated plus-maze behavior after chronic exposure to the elevated plus-maze are sensitive to the anxiolytic-like effects of midazolam. Stress. 2012;15:318-28.

Carvalho MC, Albrechet-Souza L, Masson S, Brandão ML. Changes in the biogenic amine content of the prefrontal cortex, amygdala of rats exposed to the elevated plus-maze test. Braz J Med Biol Res. 2003;140:203-14.

Rev Bras Psiquiatr. 2017;39(1)