NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION

RESEARCH ARTICLE

T. Paschoalin-Maurin et al. / Neuroscience xxx (2017) xxx–xxx

The Rodent-versus-wild Snake Paradigm as a Model for Studying Anxiety- and Panic-like Behaviors: Face, Construct and Predictive Validities

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Abstract—Using an innovative approach to study the neural bases of psychiatric disorders, this study investigated the behavioral, morphological and pharmacological bases of panic attack-induced responses in a prey-versus-coral snake paradigm. *Mesocricetus auratus* was chronically treated with intraperitoneal administration of the selective serotonin uptake inhibitor paroxetine or the gamma aminobutyric acid (GABA)/benzodiazepine receptor agonist alprazolam at three different doses and were then confronted with a venomous coral snake (*Micrurus frontalis*, Reptilia, Colubridae). The threatened rodents exhibited defensive attention, flat back approaches, defensive immobility, and escape defensive responses in the presence of the venomous snake, followed by increases in Fos protein in limbic structure neurons. Chronic administration of both paroxetine and alprazolam decreased these responses with morphological correlates between the panicolytic effect of both drugs administered at the highest dose and decreases in Fos protein-immunolabeled perikarya found in the amygdaloid complex, hypothalamus and periaqueductal gray matter columns, which are structures that make up the encephalic aversion system. These findings provide face, construct and predictive validities of this new experimental model of anxiety- and panic attack-like behavioral responses displayed by threatened prey confronted with venomous coral snakes.

Key words: paroxetine, alprazolam, rodent-versus-snake paradigm, defensive behavior, venomous coral snakes, *Micrurus frontalis*.

INTRODUCTION

Several attempts to correlate experimental models in rodents with some pathological anxiety state, such as generalized anxiety disorder (GAD) and panic disorder syndrome (PD), have been undertaken in Bard's (1928) and Hess and Brüger's (1943) studies. There is evidence that instinctive stimuli signaling danger induce defensive reactions and activate encephalic circuits that are responsible in animal tests for generating and elaborating aversive states (Adams, 1979; Blanchard and Blanchard, 1988), which have been interpreted as a motivational state of fear in human beings (Nashold et al., 1969; Wilent et al., 2010).

Predator silhouettes, emotional expressions indicating rage and imminent attack, odors or sounds, threatening postures of a potential predator, and any other factor

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Abbreviations: ANOVA, analysis of variance; DAB, 3,3′-diaminobenzidine; GAD, generalized anxiety disorder; PD, panic disorder; PMd, dorsal pre-mammillary; PVNp, posterior periventricular.

https://doi.org/10.1016/j.neuroscience.2017.11.031
0306-4522/

that might indicate the occurrence of noxious or painful stimuli have been frequently found in rodent-versus-snake paradigms (Guimarães-Costa et al., 2007; Almada and Coimbra, 2015; Coimbra et al., 2017a,b). In threatening situations, rodents can use sonorous clues to evaluate dangerous situations in their territory (Owings et al., 2002). Ground squirrels threatened by *Crotalus viridis oreganus* distinguished between rattlesnakes close to their shelters and those situated further away, and they clearly associated rattling sounds with the predator, proving to be able of assessing determinants of snake dangerousness based on acoustic cues (Swaisgood et al., 1999a,b).

Consequently, the defensive antipredatory behavioral repertory can be displayed as inhibitory avoidance and defensive attention (Coimbra et al., 2017a,b), defensive immobility (Uribe-Mariño et al., 2012), escape behavior (Twardowschy et al., 2013) and fear-induced antinociception (Coimbra et al., 2006, 2017a; Biagioni et al., 2013, 2016a; de Oliveira et al., 2017). In cases from which it is impossible to escape, some rodents exhibit tonic immobility (Leite-Panissi et al., 2003), or there might be elicited a set of threatening postures and vocalizations that can be followed by an attack (Blanchard and Blanchard, 1988).

Consistent findings have been reported since the work of Blanchard and Blanchard (Blanchard and Blanchard, 1988), based on threatening stimulus-induced interspecific confrontations in attempts to better understand these behavioral responses (Guimarães-Costa et al., 2007; Almada and Coimbra, 2015). According to this point of view, elucidating the neural bases of innate fear could be achieved by anti-predatory responses induced by a potential natural predator (Canteras et al., 1997; Comoli et al., 2003; Coimbra et al., 2017a). Several studies using ethological approaches have shown the neural substrates and neurochemical bases of instinctive stimuli signaling danger (Blanchard et al., 1990; Griebel et al., 1996). Furthermore, our research team showed that different species of rodents, such as *Rattus norvegicus* (Coimbra et al., 2017a), *Mus musculus* (Lobão-Soares et al., 2008; Uribe-Mariño et al., 2012; Twardowschy et al., 2013; Almada and Coimbra, 2015; Almada et al., 2015) and *Meriones unguiculatus* (Guimarães-Costa et al., 2007), displayed defensive responses when threatened by wild snakes. Although there have been several reports of rodent exposure to cats (Martinez et al., 2011), cat odor (do Monte et al., 2008; Souza and Carobrez, 2016), fox odor (Vincenz et al., 2017), and even wild snakes (Swaisgood et al., 1999a,b; Uribe-Mariño et al., 2012; Almada and Coimbra, 2015), few experiments have shown the neural bases of the antipredatory responses displayed by threatened rodents, and the majority of these reports have related experiments using rats confronted with cats/cat odor or fox odor (Blanchard et al., 2005; de Lima et al., 2017; Vincenz et al., 2017). However, there has been a lack of morphological and pharmacological evidence in terms of the validity of the experimental model using wild snakes as a threatening stimulus in a controlled, dangerous environment. The advantages of using venomous coral snakes in this model, is the exploratory behavior displayed by these snakes in the polygonal arena that enhances the probability of prey-versus-snake encounters, as well as the great (100%) survival index of threatened laboratory animals (Coimbra et al., 2017a).

In this sense, GAD has been related to defensive behaviors displayed in response to potential, as well as in real but distant, threats, which consist of approach-avoidance conflict eliciting inhibitory behaviors and defensive attention. Conversely, models of PD consider the freezing and escape responses induced by a real and proximal threat related to panic attacks (Shekhar, 1994; Blanchard et al., 2001; Borelli et al., 2004; Ribeiro et al., 2005; Castellan-Baldan et al., 2006). We used, in the current work, the venomous coral snake *M. frontalis* in an attempt to provide threatening stimuli to Syrian hamsters, aimed at the activation of as many aversion-related structures of the limbic system as possible, resulting in the organization of defensive behavioral responses, which are suggestive of innate fear and are correlated with a motivational state of fear in humans, as described above.

Thus, the purpose of the present work was to provide validation of a new model of panic attack using a combination of ethological, morphological and pharmacological methodologies. These approaches allowed for the careful investigation of the neural substrates and the role played by serotonin- and GABA/benzodiazepine-mediated systems in the modulation of instinctive fear displayed by rodents in a threatening situation.

EXPERIMENTAL PROCEDURES

Animals

Male Syrian hamsters (*Mesocricetus auratus,* Rodentia, Cricetidae) weighing 100–150 g (n = 6–8 per group) from the animal house of the School of Medicine of Ribeirão Preto of the University of São Paulo (FMRP-USP) were used in the present work as prey. The Syrian hamsters (n = 76) were kept (four in a cage) in an experimental environment (48 h prior to the experiments), with free access to food and water under a light/dark cycle of 12/12 h (lights on from 7 a.m. to 7 p.m.) at a room temperature of 23 ± 1 °C. Females were excluded from this experiment to avoid the effects of different levels of estrogen and progesterone in each estrous cycle on behavioral responses elicited in the presence of coral snakes and to keep the total number of laboratory animals confronted by venomous snakes as small as possible.

As a source of threatening stimuli, we used wild male venomous coral snakes (*Micrurus frontalis, Reptilia, Colubridae*) weighing 200–150 g. The coral snakes (n = 3) were collected from Brazilian rainforests and were maintained in an ophidiarium. The ophidiarium is a naturally lit compartment with calcareous rocks, tropical plants, and artificial thermal caves and burrows. The venomous coral snakes used in these experiments are commonly found in Brazilian rainforests, which consist of a natural source of visual and olfactory stimuli with

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