Animal Models for Panic Disorder

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Abstract

Panic disorder (PD) is characterized by recurrent and unexpected panic attacks associated with behavioral changes and/or persistent anxiety due to the attacks. The development of behavioral models in animals is important for the understanding of the psychobiological and behavioral bases of PD. The present article reviews the main models used in the current literature. The elevated T-maze, used in rats, presents good predictive validity, but its face validity has been questioned. Models using electrical stimulation of the periaqueductal gray present good face validity, but lesser construct validity. Models relying on predator exposure present good predictive and construct validity. These three approaches seek coherence with theories on PD as a way to increase its translational potential; thus, while the elevated T-maze is supported by the Deakin/Graeff theory, the mouse defense test battery relies on the concept of defensive

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distance, and periaqueductal gray stimulation is based on the functional neuroanatomy of PD. Moreover, to higher or lower degree the three models are supported by an "etho-experimental" approach, with careful observation of animal behavior as a way of discriminating different defensive strategies that model different aspects of anxiety, fear, and panic. Finally, an alternative/complementary model is proposed that uses zebrafish alarm reaction to study this disorder.

Keywords: Panic disorder; Animal experimentation; Defensive behavior (Animal); Escape behavior (Animal)

1 Introduction

Panic disorder (PD) is characterized by recurrent and unexpected panic attacks that are associated with behavioral changes and/or persistent anticipatory anxiety due to these attacks (American Psychiatric Association, 2013). Panic attacks occur spontaneously, but there is evidence that, for the majority of patients, moderate phobic or hypochondriac symptoms precede the attacks (Craske, Miller, Rotunda, & Barlow, 1990; Fava, Grandi, & Canestrari, 1988); it was proposed, then, that panic attacks are "unpredictable" rather than "unexpected" (Barlow, 2002). Besides symptom phenomenology, PD differs from other anxiety disorders due to its particular pharmacological response (Donald F Klein, 1964, 1981) and, partially, due to the independent structure of genetic and environmental risk factors (Gratacòs et al., 2007).

From a neuroanatomical point of view, PD presents important relationships with circuits underlying fear, including different amygdala nuclei, insular cortex, anterior cingulate cortex, and periaqueductal gray area (PAG) (Graeff & Del-Ben, 2008; Lovick, 2000). The mechanisms associated with unconditional and conditional responses compose a fight/flight/freeze or aversive brain system that is represented by the medial hypothalamus, several amygdalar

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subnuclei, and the dorsal periaqueductal gray area (dPAG) (Brandão, Anseloni, Pandóssio, De Araújo, & Castilho, 1999; Canteras, 2002; Cezario, Ribeiro-Barbosa, Baldo, & Canteras, 2008; Gross & Canteras, 2012; LeDoux, 2003a; LeDoux & Pine, 2016; Sukikara, Mota-ortiz, Baldo, Felício, & Canteras, 2006). The electrical stimulation of these areas elicits a response pattern that characterize what is usually termed a defense reaction, and usually accompanies aversive subjective states characteristic of fear and anxiety (Brandão, Troncoso, Silva, & Huston, 2003). The functioning of this aversive brain system can also be related to panic attacks. The electrical stimulation of dPAG elicits behaviours similar to the defense reaction as well as ubjective and neurovegetative phenomena that resemble a panic attack not only in experimental animals, but also in humans (Graeff & Del-Ben, 2008; Graeff, Silveira, Nogueira, Audi, & Oliveira, 1993; Jenck, Moreau, & Martin, 1995; Lovick, 2000).

An important concept that organizes theories derived from animal models is that of defensive direction and defensive distance (or predatory imminence continuum) (Deakin & Graeff, 1991; Fanselow & Lester, 1988; McNaughton & Corr, 2004; Perusini & Fanselow, 2015). In general, it can be said that the more caudal portions of the system (especially the PAG) directly and immediately control defensive responses when the defensive distance is very small (proximal threat). As the distance increases, more complex defensive strategies emerge, and those responses are controlled by progressively more rostral structures, with the cingulate cortex representing the most distal threat. Defensive avoidance (“fear”) is controlled by the amygdala and the anterior cingulate (Dresler et al., 2013; McNaughton & Corr, 2004). Defensive approach (“anxiety”) occurs when a strong drive conflicts with avoidance, triggering risk assessment behavior; these patterns are controlled by septo-hippocampal system and posterior cingulate (R. J. Blanchard & Blanchard, 1988; Deakin & Graeff, 1991; Gray & McNaughton, 2000; LeDoux, 1998a).

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2. Animal models for panic disorder

The development of animal models for panic disorder is needed to establish the psychological and biological bases of this disorder, as well as to allow the development of novel therapeutics. Differently from models for generalized anxiety – that present a myriad of formats and exploit a diversity of different behaviors (Belzung & Lemoine, 2011; Griebel & Holmes, 2013) –, few paradigms explicitly model PD. The distinction between models for PD and models for generalized anxiety is based mainly on reference to the theoretical constructs that support the models (McNaughton & Zangrossi Jr, 2008): while tests for generalized anxiety use situations that impose approach/avoidance conflicts to animals – generating behavioral inhibition and risk assessment responses –, models that are relevant for PD are based on defensive responses elicited by aversive stimuli or by the stimulation of specific brain regions (Moreira et al., 2013). In rodents, four main strategies are used to model panic attacks and/or PD: conditional fear responses (fear conditioning), mouse defense test battery, and electrical stimulation of the periaqueductal gray and/or medial hypothalamus (Moreira et al., 2013).

2.1. Fear conditioning

Conditional fear responses have been widely used to establish fear-like responses and to elucidate the neural bases of these responses (LeDoux, 1998b, 2003b; Schafe, Nader, Blair, & LeDoux, 2001). In fear conditioning, animals learn to associate a previously neutral stimulus (CS) with an aversive unconditioned stimulus (US, such as an electrical shock (Pavlov, 1927). In cued fear conditioning, the CS is explicit and reliably predicts the US, acting as a cue or signal; in contextual fear conditioning, animals display a defensive reaction (usually freezing) when introduced to a context (e.g., apparatus or chamber) in which they have been previously

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exposed to the US, and therefore the context represents a complex CS (Davis, Walker, Miles, & Grillon, 2009). Typically, rats and mice display a initial activity burst to the footshock followed by freezing (LeDoux, 1998b); reexposure to the CS in the absence of the US triggers memory retrieval, which initiates reconsolidation and extinction processes.

While fear conditioning is adaptive, and although conditioning accounts of panic disorder have been widely criticized, this process is an integral part of contemporary learning explanations of PD that attempt to incorporate some complexities of contemporary learning theory . Moreover, these learning accounts of PD provide a compelling explanation for the persistence of PD, in spite of not always being able to explain the etiology of these disorders. Bouton et al. (2001) have argued that panic attacks are salient aversive USs during which feeligs of anxiety and fear can be associated with external and/or interoceptive cues that are present during the attacks; subsequent exposure to these cues can trigger feelings of anticipatory anxiety, which in its turn can exacerbate the next panic attack.

Enhanced fear conditioning has been associated with gene variants in rodent orthologues of human PD risk genes. For example, *Tmem132d* has been recently found to be associated in murine fear conditioning in a quantitative trait loci panel (Knoll, Halladay, Holmes, & Levitt, 2016), and female mice with humanized COMT Val158Met polymorphism show reduced contextual fear, increased cued fear, and reduced extinction recall (Risbrough, Ji, Hauger, & Zhou, 2014). Both genes have variants which increase the risk for PD in humans (Howe et al., 2015). A role for serotonin (5-HT) has also been suggested in conditional fear processes (Homberg, 2012): acute treatment with selective 5-HT reuptake inhibitors (SSRIs) facilitates fear conditioning, reduces contextual fear, and increases cued fear; 5-HT_{1A} receptors inhibit the acquisition and expression of contextual fear; 5-HT_{2A} receptors facilitate the consolidation of cued and contextual fear, while 5-HT_{2C} receptors inhibit the retrieval of cued fear. Therefore,

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5-HT appears to exert opposite effects on contextual and cued fear (Homberg, 2012). While consistent with the idea that contextual fear is more related to anxiety than fear, these results are actually opposed to what is observed in other models (Table 1).

	SSRIs (acute)	**5-HT$_{1A}$R**	**5-HT$_{2A}$R**	**5-HT$_{2C}$R**
Cued fear	↑	?	↑	↓
Contextual fear	↓	↓	↑	?
ETM escape	↓	0	0	0
MDTB	0	0	0	0
PAG stimulation	↓	↓ (local injection)	↓ (local injection)	0 (local injection)
Zebrafish alarm reaction	↓	0	?	?

Table 1 – Effect of serotonergic drugs on putative models of fear and panic-like behavior. Abbreviations: ETM: Elevated T-mazed; MDTB: mouse defense test battery; PAG: periaqueductal gray area; SSRIs: selective serotonin reuptake inhibitors.

2.2. Elevated T-maze

The elevated T-maze (ETM) is derived from the behavioral model for anxiety that is most widely used contemporaneously – the elevated plus-maze –, and is explicitly based on the Deakin/Greaff hypothesis on the role of 5-HT on defensive behavior (Graeff, Ferreira Netto, & Zangrossi, 1998; Guimarães, Carobrez, & Graeff, 2008; Zangrossi Jr & Graeff, 2014). The

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ETM attempts to explicitly separate defensive behaviors that are evoked by motivational conflict (i.e., behavioral inhibition and risk assessment) from behaviors associated with fear states (i.e., escape). The apparatus consists in two open arms and one closed arm in a perpendicular configuration, elevated 50 cm from the floor (Viana, Tomaz, & Graeff, 1994). In a ETM session, the rat executes two tasks: inhibitory avoidance and escape. First, the animal is transferred to the end of the closed arm, and the latency to leave this arm is measured; after 30 s intervals, the task is repeated twice, and increased latency to leave the closed arm is interpreted as the acquisition of inhibitory avoidance (Graeff et al., 1998). After this IA task, the animal is positioned at the end of one of the open arms to measure the escape response (Viana et al., 1994). These two responses – IA and escape – recruit different neuronal circuits (Leite Silveira, Zangrossi, De Barros Viana, Silveira, & Graeff, 2001) and present opposite 5-HTergic modulation (Graeff, Viana, & Mora, 1996; Sena et al., 2003; Zangrossi Jr & Graeff, 2014; Zangrossi Jr et al., 2001; Table 1). IA is interpreted as modeling anxiety-like responses, while escape is interpreted as modeling panic attacks (Graeff et al., 1998). Moreover, the model appears to present good predictive validity for anxiolytic and panicolytic drugs (Graeff et al., 1998). An important critique to the model, however, is its low face validity: the association of escape from the open arm to panic attacks is tenuous, given that the escape observed in the ETM is slow, directed, and controlled – very different from escape responses which are observed in other models (Moreira et al., 2013).

2.3. Mouse defense test battery

The mouse defense test battery (MDTB) evaluates the behavior of mice exposed to a natural predator, the rat, at different defensive distances (D. C. Blanchard, Griebel, & Blanchard, 2001; Yang et al., 2004). The mouse is positioned in an oval runway, and an

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anesthetized rat is manipulated by the experimenter so that its approaches the mouse at a fixed velocity; when the rat approaches the mouse by about 1 m, the mouse usually starts running, attempting to escape the aversive stimulus This model explicitly assumes defensive distance (or predatory imminence continuum) – the psychological distance from the threat (R. J. Blanchard & Blanchard, 1988; Fanselow & Lester, 1988) – as a central variable in decision making by the mouse. The model presents excellent predictive validity, since acute treatment with triazolobenzodiazepines and chronic treatment with imipramine, fluoxetine, and moclobemide decrease escape responses, while acute treatment with imipramine and fluoxetine or with panicogenic agents increase escape (D. C. Blanchard et al., 2001). Moreover, facilitating 5-HTergic neurotransmission in the periaqueductal gray inhibits escape in the MDTB (Pobbe, Zangrossi Jr, Blanchard, & Blanchard, 2011). The model presents as advantages the high ethological relevance, because it uses escape responses in an ecologically valid context and avoids using artificial stimuli such as electric shocks; moreover, the model allows the evaluation of two distinct responses – approach or escape the stimulus – that are predictive of anxiolytic or panicolytic effects, respectively (Moreira et al., 2013).

2.4. Electrical stimulation of the dlPAG

The electrical stimulation of the dorsolateral periaqueductal grey (dPAG) evokes abrupt escape and/or freezing behavior and cardiovascular responses that is reminiscent of a panic attack (Beckett & Marsden, 1995; Brandão, Zanoveli, Ruiz-Martinez, Oliveira, & Landeira-Fernandez, 2008; Schenberg, 2010; Schenberg et al., 2014). Currents with different amplitude, or different concentrations of glutamatergic or nitrergic drugs, are needed to induce freezing and escape (running and jumping) responses (de Oliveira, Del Bel, & Guimarães, 2001; Schenberg, Bittencourt, Sudré, & Vargas, 2002; Vianna, Graeff, Brandão, & Landeira-

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Fernandez, 2001). Usually, lower concentrations or currents elicit alertness and escape, while higher concentrations or currents elicit a sequence that is characterized by alertness, freezing, and escape behavior interspersed with periods of tense immobility (Brandão et al., 2005, 2003, 2008). Similarly, disinhibiting the medial hypothalamus – a diencephalic structure that composes, along with the periaqueductal gray, the encephalic circuits which mediate fear responses and panic attacks (Canteras, 2002) – with GABAergic antagonists promote escape responses, hypertension, and tachycardia (DiMicco, Samuels, Zaretskaia, & Zaretsky, 2002; Johnson, Lowry, Truitt, & Shekhar, 2008; Johnson, Truitt, Fitz, Lowry, & Shekhar, 2008). Both models are based on observations on the effects of electrically stimulating these regions in humans, placing its construct validity in the similarity of neuronal circuits (Moreira et al., 2013); these models also appear to present a good predictive validity, since panicolytic, but not anxiolytic drugs, alter behavior after stimulation (Johnson, Lowry, et al., 2008; Schenberg et al., 2002). However, from an etiological point of view, the induction of responses by direct stimulation of brain regions has a tenuous relation to spontaneous panic attacks observed in humans.

In addition to freezing that is observed during dPAG stimulation, post-stimulation freezing is also observed (Brandão et al., 2008). Interrupting the electrical stimulation of the dPAG at the escape threshold elicits this freezing behavior, that is accompanied by activation of the laterodorsal nucleus of the thalamus, suggesting transfer of information to more rostral structures (Brandão et al., 2008). This led Brandão et al. (2008) to speculate that post-stimulation freezing is more related to risk assessment, as the animal freezes in order to better accumulate information on threat levels (Hagenaars, Oitzl, & Roelofs, 2014); as a result, while stimulation-elicited freezing should be treated as a model for a panic attack, post-stimulation freezing would be a better model for anticipatory anxiety in PD (Brandão et al., 2008).

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2.5. Summary

All four approaches are heavily based on construct validity, looking for coherence with theories on PD as a way to elevate the translational potential of the model; as such, while the ETM is grounded on the Deakin/Graeff theory, the MDTB is grounded mainly on the concept of defensive distance. Moreover, to a greater or lesser degree, the four models are sustained by an "etho-experimental" approach (R. J. Blanchard & Blanchard, 1988), based on the careful observation of animal behavior as a way to discriminate between different defensive strategies that model different aspects of anxiety, fear, and panic. The four approaches present important advantages and disadvantages in modeling PD. Given the complexity of PD, it is to be expected that the use of a single model will not mimic all its aspects, withal considering the limitations related to the subjective aspects of the disorder (LeDoux & Pine, 2016; Pine & LeDoux, 2017). Using different animal models can mimic different aspects of the disorder, allowing better elucidation of the behavioral and neuropathological underpinnings of the disease. Ideally, various animal models should be used to study the same disorder, including the use of different species (de Mooij-van Malsen, Vinkers, Peterse, Olivier, & Kas, 2011; Kalueff, Ren-Patterson, LaPorte, & Murphy, 2008; Kas, Gelegen, Schalkwyk, & Collier, 2009; van der Staay, 2006).

3. Complementary models: The alarm response of zebrafish

Aiming to enrich the breadth of etho-experimental approaches traditionally made with rodents, the use of non-mammalian species has been proposed (Maximino et al., 2015). Among these, zebrafish (*Danio rerio* Hamilton 1822), a cyprinid fish widely used in genetics and developmental biology, has gained traction as model in behavioral research (Gerlai, 2010; Kalueff et al., 2012; Norton & Bally-Cuif, 2010; Rinkwitz, Mourrain, & Becker, 2011; Shams,

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Rihel, Ortiz, & Gerlai, 2018). Importantly for the study of fear and panic is the range of defensive responses that are observed in this species, among which the alarm reaction is relatively well-characterized and relevant for modeling PD (Gerlai, 2010, 2013; Jesuthasan & Mathuru, 2008; Maximino et al., 2018; Stewart et al., 2015). This is a response that is initiated in the olfactory system by substances that are released by the damaged skin of conspecifics, and is characterized by dramatic and measurable changes in swimming patterns, as well as in well-defined physiological responses. The "alarm substance" produced by specialized skin cells ("club cells") and released after these cells are damaged is of unknown composition (Døving & Lastein, 2009; Jesuthasan & Mathuru, 2008), but it presents hypoxanthine 3-*N*-oxide and chondroitin fragments – substances which mimic part of the behavioral and physiological reactions (Mathuru et al., 2012; Parra, Adrian Jr, & Gerlai, 2009). During exposure to this substance, an increase in shoal cohesion (Speedie & Gerlai, 2008) and the initiation of patterns of erratic swimming followed by freezing (Mathuru et al., 2012; Speedie & Gerlai, 2008) is observed.

Differently from the behavior that is observed during exposure, behavior observed after exposure usually involves increased bottom dwelling associated with erratic swimming and freezing (Cachat et al., 2011; Egan et al., 2009), analgesia (Maximino, 2011), and increased dark preference associated with erratic swimming, freezing, and thigmotaxis (Maximino, Lima, Costa, Guedes, & Herculano, 2014). Moreover, the alarm substance also produces intense autonomic resposnes, with increased plasma levels of glucose, haemoglobin, norepinephrine, and epinephrine (Maximino et al., 2014), and a neuroendocrine stress response, with increased whole-body cortisol levels (Abreu, Giacomini, Koakoski, Piato, & Barcellos, 2017; Schirmer, Jesuthasan, & Mathuru, 2013). This array of behavioral and physiological adjustments simulate some important behavioral aspects and neurovegetative symptoms of panic attacks, lending

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significant face validity to the model. Moreover, the dissociation between responses produced during alarm substance exposure (erratic swimming and freezing, without increased bottom dwelling) and after exposure (increased bottom dwelling, erratic swimming, and freezing) is reminiscent of the distinction between freezing responses during and after the electrical stimulation of the dorsolateral column of the PAG in rats (Brandão et al., 2008), and could be further exploited as dependent variables in models for panic attacks and PD, respectively.

4. Conclusions

The animal models reviewed here present different advantages that can be exploited to discover the neurobiological and behavioral mechanisms associated with PD, as well as the specific disadvantages that suggest the use of complementary approaches. These models vary in terms of validity – for example, the face validity of the elevated T maze is lower than the other presented models, but its construct validity is higher than the models involving electrical or chemical stimulation.

Finally, the introduction of new models – including models with non-mammalian species, such as the use of alarm substance in *Danio rerio* – is an approach with great potential to amplify the field of investigation, testing novel psychobiological, behavioral, and developmental hypotheses on this disorder and associated factors. The field of animal modeling can only profit from the inclusion of these approaches in the roster of investigation strategies.

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