European Journal of Pharmacology ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

# From neural to genetic substrates of panic disorder: Insights from human and mouse studies

Mónica Santos [a,b,c,d], Davide D'Amico [a,b,c,e], Mara Dierssen [a,b,c,*]

[a] Cellular & Systems Neurobiology, Systems Biology Program, Center for Genomic Regulation (CRG), E-08003 Barcelona, Spain
[b] Universitat Pompeu Fabra (UPF), E-08003 Barcelona, Spain
[c] CIBER de Enfermedades Raras (CIBERER), E-08003 Barcelona, Spain
[d] Institute of Biology, Otto-von-Guericke University, 39120 Magdeburg, Germany
[e] ZeClinics SL, E-08001 Barcelona, Spain

ARTICLE INFO

*Article history:*
Received 3 January 2015
Received in revised form
15 January 2015
Accepted 12 March 2015

*Keywords:*
Fear circuit
Anxiety
Cognition
TrkC
TgNTRK3

ABSTRACT

Fear is an ancestral emotion, an intrinsic defensive response present in every organism. Although fear is an evolutionarily advantageous emotion, under certain pathologies such as panic disorder it might become exaggerated and non-adaptive. Clinical and preclinical work pinpoints that changes in cognitive processes, such as perception and interpretation of environmental stimuli that rely on brain regions responsible for high-level function, are essential for the development of fear-related disorders. This review focuses on the involvement of cognitive function to fear circuitry disorders. Moreover, we address how animal models are contributing to understand the involvement of human candidate genes to pathological fear and helping achieve progress in this field. Multidisciplinary approaches that integrate human genetic findings with state of the art genetic mouse models will allow to elucidate the mechanisms underlying pathology and to develop new strategies for therapeutic targeting.



## 1. Introduction

The emotion we call fear is a response to an actual threat or to a potentially dangerous event in our life, which biological function is conferring protection from physical or emotional harm. This protective role makes fear essential for the survival, and thus, it has a strong influence on our daily life. While fear to certain kinds of stimuli is innately hardwired (*e.g.* a loud noise in newborns), fear to other stimuli can also be rapidly and lastingly learned, allowing adaptation to new or changing environmental situations.

However, if innate fear is excessive and persistent, or when learned fear is induced by events that represent no real threat for the individual it turns in a pathological condition. Such abnormalities in the detection, interpretation and response of fear underlie many forms of psychopathology that are categorized under the broad umbrella of anxiety disorders. The common feature of anxiety disorders is excessive, irrational fear and avoidance of real or imagined anxiety triggers (DSM-5, 2013). Thus, the brain fear circuitry is assumed to be the substrate to both anxiety and fear-related disorders. However, while some fear-related disorders are acquired non-associatively, others arise as a consequence of impaired associative learning (Bracha, 2006; Kendler et al., 2002). In most phobias, fear is an innate response and represents primitive fears possibly relying on phylogenetically older brain structures. Other fear disorders, such as panic disorder (PD), are associated with disturbances of cognitive processes in regions involved in high-level brain function together with the involvement of the autonomic system.

PD is a classical fear-related model. Diagnostic criteria include recurrent panic attacks as well as persistent worry or concern of having more attacks, which may ultimately produce significant changes in behavior (DSM-5, 2013; Fava and Morton, 2009). This review aims at exploring the emotional-cognitive integration underlying pathological fear. Specifically, we focus on the neural substrates that participate in cognitive dysfunction in PD, and the genetic factors that might contribute to the development of the cognitive component of the disorder. Further, we review the work in genetic mouse models with altered fear.

## 2. An overview on the neuroanatomical landscape of PD

The Gorman et al. (2000) neuroanatomical hypothesis that has dominated the field of fear-related disorders during the last decade proposed that a network of brain regions contribute to

* Corresponding author at: Center for Genomic Regulation (CRG), Barcelona Biomedical Research Park, C/ Dr. Aiguader 88, 08003 Barcelona, Spain. Tel.: +34 933160140.
*E-mail addresses:* monica.santos@ovgu.de (M. Santos), davide.damico@zeclinics.com (D. D'Amico), mara.dierssen@crg.es (M. Dierssen).

http://dx.doi.org/10.1016/j.ejphar.2015.03.039
0014-2999/

different PD categorical domains. These include (i) loci in the brainstem and the hypothalamus involved in panic attacks and stress response; (ii) the limbic system with a role in anticipatory anxiety and heightened fear, and (iii) the prefrontal cortex that participates in phobic avoidance and emotion regulation. Although some aspects have been refined, PD brain imaging studies have corroborated the involvement of cortical and limbic structures, reinforcing the role of these regions in its pathophysiology (Table 1).

Structures such as the amygdala and the hippocampus, both limbic structures residing deep inside the medial temporal lobe, or cortical regions in the prefrontal lobe, such as the anterior cingulate cortex, orbitofrontal cortex and insula, have been consistently reported as altered across different studies (Table 1). These studies suggest that the "cross-talk" between emotional (limbic) and cognitive control (prefrontal cortex) brain regions plays a key role in PD, an established notion in anxiety disorders which we will discuss here for PD to explain how some of these brain regions regulate learned fear.

### 2.1. The amygdala

The amygdala is the core brain structure in rodent and human fear expression, acquisition and extinction (Herry et al., 2008; Hitchcock and Davis, 1986; Maren and Quirk, 2004; Sierra-Mercado et al., 2011) and has an important role in fear-related disorders. Structural magnetic resonance imaging (MRI) studies have consistently reported a reduction in amygdala volume in PD patients (Asami et al., 2009; Hayano et al., 2009; Massana et al., 2003b; Uchida et al., 2003). Nevertheless, the functional relevance of such MRI findings cannot be directly inferred, as the amygdala is a functionally heterogeneous structure, with central and basolateral nuclei composed mainly of excitatory neurons, and the intercalated cell cluster composed mainly of inhibitory neurons. However, functional MRI studies also showed abnormalities in the activation of the amygdala in PD (Chechko et al., 2009; Pillay et al., 2006, 2007; Tuescher et al., 2011). These patients show decreased amygdala activation on functional MRI while viewing fearful faces, when compared to a control population (Pillay et al., 2006). Instead, increased amygdala activation was found in emotional conflict paradigms such as the Stroop-like presentation of incongruent and congruent emotional stimuli (Chechko et al., 2009; Tuescher et al., 2011).

The relevance of decreased amygdala activation to fearful faces in the context of PD might relay on the hyperarousal presented by these patients. One of the clinical features in PD is the development of anticipatory anxiety, resulting in an increase in the basal activation of the amygdala and consequent reduced emotional response to fear-related stimuli, as reflected in amygdala activation levels.

Moreover, the cognitive-activation tasks here employed (fearful *vs.* happy *vs.* neutral faces or the Stroop paradigm) involve a differential engagement of prefrontal cortex regions, which might, in part, explain the apparent different findings. The increased amygdala activation in the Stroop paradigm might reflect abnormal cognitive processing of information at high-level centers.

Given the strong anatomical and functional connections between the amygdala and cortical centers, such as the cingulate cortex and the orbitofrontal cortex, incorrect processing of information at the later will have consequences in the recruitment of the amygdala, and therefore in fear expression (reviewed in Kim et al. (2012)).

### 2.2. Prefrontal brain regions

The ventromedial prefrontal cortex can modulate fear expression through descending projections to the amygdala. The human anterior cingulate cortex is functionally related with cognitive re/appraisal – dorsal anterior cingulate cortex – and emotional processing – ventral anterior cingulate cortex (Etkin et al., 2011). In MRI scans PD patients showed a reduction in the anterior cingulate cortex volume (Asami et al., 2008, 2009; Protopopescu et al., 2006; Uchida et al., 2008). In functional MRI studies further abnormalities have been reported. Specifically, PD patients show increased anterior cingulate cortex activation in response to happy faces, but reduced activation in response to fearful faces (Pillay et al., 2006, 2007). Moreover, in an instructed fear-conditioning paradigm, reduced activation to the "threat" and increased sensitivity to the "safe" conditions were found in the subgenual cingulate in PD patients, as compared to control subjects (Tuescher et al., 2011).

These results suggest that PD patients have a cognitive bias, meaning that they will attribute neutral/safe stimuli stronger than normal emotional valence. Chronic hyperarousal in PD could be responsible for a generalization in the activation of the anterior cingulate cortex in response to neutral/safe stimuli. In agreement, PD patients show a deficit in discrimination learning by presenting an enhanced startle potentiation response to the learned safety cue rather than abnormal reactivity to the danger cue (Lissek et al., 2009).

On the other hand, the hypoactive response of the anterior cingulate cortex when emotionally negative or conflictive information has to be processed (but see Maddock et al. (2003)) seems to translate into a disinhibition of the amygdala and increased fear expression in PD patients.

Another potential area with abnormal functioning in PD is the orbitofrontal cortex. The orbitofrontal cortex is a ventral prefrontal cortex brain region with direct reciprocal connections with the amygdala. Enhanced/generalized fear reactivity and impaired fear extinction after orbitofrontal cortex lesion have been reported in both rodents and nonhuman primates (Agustin-Pavon et al., 2012; Zelinski et al., 2010), suggesting that inputs from orbitofrontal cortex may be important for fear response suppression. In recent years, a number of studies that evaluated orbitofrontal cortex volume and function in PD patients reported abnormalities in this brain region (Kent et al., 2005; Lai and Wu, 2012; Protopopescu et al., 2006; Roppongi et al., 2010). In line with basic research results, MRI studies found a volume reduction of gray matter in orbitofrontal cortex of PD patients, when compared to healthy subjects. Kent et al. (2005) showed, by using positron emission tomography scans, that decreased baseline cerebral blood flow in orbitofrontal cortex predicts panic vulnerability and is negatively correlated with anxiety scores.

Finally the insular cortex, located at the center of the cerebral hemispheres, has connections with a wide range of brain regions and is considered an important site of multimodal convergence (reviewed in Nagai et al. (2007); see also Menon and Uddin (2010)). The insula is classically known for its role in the emotional experience derived from information about bodily states (Critchley et al., 2001; Damasio, 1996). Thus, misinterpretation of bodily sensations and/or biased cognitive evaluation of stimuli valence, which are hallmarks of PD, may result from ineffective salience processing in the insula. Critchley et al. (2004) reported that gray matter volume in the anterior insula positively correlates with interoceptive accuracy and subjective ratings of visceral awareness. However, MRI studies measuring gray matter volume of the insula in PD patients are not consistent (Asami et al., 2009; Lai and Wu, 2012; Uchida et al., 2008).

Overall, the above studies reinforce the concept that a medial frontal cortex network is an important, though still not well understood, contributor to the establishment of fear circuitry disorders.

Cognitive behavioral therapies targeting plasticity in cortical structures are being widely employed in the treatment of anxiety disorders though successful treatment does not reach all patients (Ganasen et al., 2010). In patients presenting an effective intervention, return of fear – relapse – presents a significant drawback

Please cite this article as: Santos, M., et al., From neural to genetic substrates of panic disorder: Insights from human and mouse studies. Eur J Pharmacol (2015), http://dx.doi.org/10.1016/j.ejphar.2015.03.039