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Review

Bridging the Gap: Towards a cell-type specific understanding of neural circuits underlying fear behaviors

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ABSTRACT

Fear and anxiety-related disorders are remarkably common and debilitating, and are often characterized by dysregulated fear responses. Rodent models of fear learning and memory have taken great strides towards elucidating the specific neuronal circuitries underlying the learning of fear responses. The present review addresses recent research utilizing optogenetic approaches to parse circuitries underlying fear behaviors. It also highlights the powerful advances made when optogenetic techniques are utilized in a genetically defined, cell-type specific, manner. The application of next-generation genetic and sequencing approaches in a cell-type specific context will be essential for a mechanistic understanding of the neural circuitry underlying fear behavior and for the rational design of targeted, circuit specific, pharmacologic interventions for the treatment and prevention of fear-related disorders.

0. Introduction

Disorders whose major symptoms relate to the dysregulation of fear responses are usually characterized by over-generalization of fear and inability to extinguish fearful responses. Such dysregulation leads to a pathological expression of fear behaviors that can be quite debilitating, leading to a range of intrusive, hyperarousal, avoidance, cognitive, and depression symptoms. The treatment of fear-related disorders often involves cognitive-behavioral therapies, in particular exposure therapy, which mirrors behavioral extinction processes used in rodent models, relying on the repeated and non-reinforced presentation of cues previously associated with noxious stimulus.

Advances in cognitive-behavioral therapy approaches targeting traumatic memories have been made using cognitive enhancers, for example by targeting emotion-related synaptic plasticity via the NMDA, Dopamine, and Cannabinoid receptors (Singewald, Schmuckermair, Whittle, Holmes, & Ressler, 2015). Pharmacological interventions may be used to generally enhance plasticity within neural circuitry including that responsible for behavioral extinction. Across several fear- and anxiety-related disorders, the administration of cognitive enhancers, such as D-cycloserine, in conjunction with exposure-based psychotherapy has been shown to enhance the beneficial effects of behavioral therapy sessions in a rapid and long-lasting manner (Rodrigues et al., 2014; Singewald et al., 2015). Despite these advances, insufficient knowledge of the underlying molecular and cellular mechanisms mediating fear acquisition, expression, and extinction continues to limit the *specificity* and *effectiveness* of further therapeutic breakthroughs. Therefore, a greater understanding of the neural circuitry mediating fear processing will catalyze further progress in the development of more selective treatments for fear- and anxiety-related disorders.

In this review, we will begin by discussing the understanding of the circuitry governing the acquisition and extinction of classically conditioned fear behaviors. We will continue by discussing the advent of optogenetic approaches and the contributions this technique has made to our knowledge of fear circuits. We will discuss the use of genetic techniques to determine which and how cell populations are recruited into memory traces. With a special focus on studies that involve behavioral manipulations, we will examine recent advances in the manipulation of identified cellular

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sub-populations housed within canonical fear and emotional learning related circuitries. Finally, we will provide a brief review of methods for cell-type specific isolation of RNA for sequencing.

As the basic neural circuitry governing fear behaviors continues to be elucidated at a rapid pace, it is necessary to act prospectively by applying these findings towards the discovery of applicable treatments for patients suffering from fear and anxiety related disorders. By uncovering cell-type specific markers for neural circuitry governing fear and anxiety behaviors in rodent models modern researchers have an opportunity to concurrently open avenues for more targeted pharmacological therapies in humans. Cell type specific markers may be conserved across species and targeting these convergences will maximize translational value of discoveries. This review is meant to highlight the need for further cell-type specific approaches in order to make rapid progress towards more selective and targetable pharmacological treatments of fear-related disorders in humans.

1. Background on circuitry and fear

1.1. Pavlovian conditioning

Pavlovian fear conditioning is a popular and powerful technique for studying learning and memory in animal models. This is primarily due to it being a rapidly acquired behavior with consistent and easily measured behavioral outputs that rely on a well-characterized core neural circuit. Fear conditioning, also discussed as threat conditioning (LeDoux, 2014), occurs through the pairing of an initially innocuous conditioned stimulus (CS, e.g., an auditory tone during auditory fear conditioning or the context of training during contextual fear conditioning) with an aversive unconditioned stimulus (US, e.g., a mild foot shock). Following several CS-US pairings, the subject will exhibit fear response behaviors or conditioned responses (CRs) to presentations of the CS alone. The most common fear responses investigated are freezing (the cessation of all non-homeostatic movement) and fear potentiated startle (FPS, in which the amplitude of an animals' startle to a noise burst is potentiated upon combined presentation of the CS and noise burst) (Blanchard & Blanchard, 1969; Fanselow, 1980).

In addition to measures of freezing and fear potentiated startle, there are a multitude of tests to parsimoniously examine an animal's motivational state. Briefly, in contrast to freezing or startle responses, tests demanding an active or passive avoidance response require an additional instrumental learning procedure to either perform or inhibit performance of an action such as shuttling in order to avoid a shock (Curzon, Rustay, & Browman, 2009; Picciotto & Wickman, 1998; Sousa, Almeida, & Wotjak, 2006). These learning paradigms utilize additional important circuitries and may provide further insights into the etiologies of fear related disorders (Izquierdo & Medina, 1997). The present review will focus primarily upon conditioned fear responses such as freezing and FPS following either the acquisition or extinction of fear; however, understanding the neural substrates governing additional motivated behaviors is likewise important for understanding the spectrum of fear-related processes.

Notably, fear responses are adaptive only when the CS clearly predicts the US. When these stimuli are no longer paired, such as during extinction (when the CS is repeatedly presented without any US reinforcement), a subject will learn that the CS is no longer predictive of the US, and CRs will decrease. Importantly, extinction is generally considered to be a new learning event that modulates rather than modifies the original learned fear association; for an excellent discussion of extinction see: Myers and Davis (2007). In this review, we refer to 'fear conditioning' or training as the period when CS – US pairings are presented; 'fear extinction' as a period when multiple or continuous CS presentations occur in the absence of the US, resulting in a decrement in CRs; 'fear expression' refers to eliciting CRs to a CS; and 'extinction expression' refers to the testing for suppression of CRs to a CS after extinction learning.

1.2. Fear learning: Basic circuitry and key players

The circuitry attributed to controlling elements of fear conditioning is ever expanding and we will discuss several additional areas in the course of this review; however, the core 'canonical' circuitry remains well understood and centers on the core amygdala nuclei. For recent in-depth reviews of the current understanding of the neural circuitries governing fear and anxiety see: Duvarci & Pare, 2014; Ehrlich et al., 2009; Myers & Davis, 2007; Pape & Pare, 2010; Pare, Quirk, & Ledoux, 2004. The core nuclei within the amygdala consist of the lateral (LA), basolateral (BA), and central (CeA) amygdala, which may be subdivided into the dorsolateral LA (LAdl), ventromedial LA (LAvm), ventrolateral LA (LAvl), anterior BA (BAa), posterior BA (BAp), central or capsular CeA (CeC), lateral CeA (CeL), and medial CeA (CeM). These nuclei may be even further subdivided. In the present review, the basolateral complex (BA + LA) will be abbreviated BLA.

Experimentally, dissections of CeC/CeL/CeM and LA/BA circuitries often fail to sufficiently discriminate between nuclei for a number of reasons, foremost due to their small sizes and close proximity. Specifically the CeC and the CeL tend to be conflated and the anterior aspect of the BAa is usually treated as representative of the whole BA or BLA. These, previously unavoidable, imprecisions may need to be corrected in time as more rigorous descriptions of micro-circuitries are performed. Furthermore, molecularly determined cell-type specific identification will lead to more powerful approaches to understanding microcircuit function in the future.

In the case of auditory fear conditioning (in which an auditory tone CS is paired with the US), salient information regarding the CS and US converge on the LA. Auditory information flows into the LA from the secondary auditory cortex (AuV) and auditory thalamus: medial geniculate nucleus/posterior intralaminar nucleus (MGn/PIN) (LeDoux, Ruggiero, & Reis, 1985; Linke, Braune, & Schwegler, 2000). Information regarding the US is communicated via the somatosensory cortex, somatosensory thalamus and periaqueductal gray (PAG) (McDonald, 1998; LeDoux, Farb, & Ruggiero, 1990). The LA integrates the information regarding both the tone and shock, and is a major site of learning related plasticity (Muller, Corodimas, Fridel, & LeDoux, 1997). Projections from the LA can modulate CeA activity directly or indirectly through projections to the BA. Additional inhibitory controls come from the intercalated cell nuclei (ITC). The ITC are made up of islands of GABAergic neurons surrounding the BLA. ITC nuclei receive strong inputs from the LA and BA and may receive additional inputs from extrinsic regions such as the medial prefrontal cortex (mPFC) (Giustino & Maren, 2015; Sierra-Mercado, Padilla-Coreano, & Quirk, 2011). ITC nuclei act as regulators of information flow between the BLA and CeA by providing feed-forward inhibition to multiple nuclei of the CeA (Blaesse et al., 2015; Brigman et al., 2010; Busti et al., 2011; Ehrlich et al., 2009; Giustino & Maren, 2015; Likhtik, Popa, Apergis-Schoute, Fidacaro, & Pare, 2008; Marcellino et al., 2012; Millhouse, 1986; Palomares-Castillo et al., 2012). Interestingly, the dorsal ITC (ITCd) receive inputs from LA neurons and provide feed-forward inhibition of the CeL, while more ventral medial ITCs receive input from BA neurons and inhibit CeM populations (Pare & Duvarci, 2012). The CeM is generally regarded as the main output station of the amygdala on account of its projections to the brain stem effector regions of fear behaviors such as the PAG, lateral hypothalamus and paraventricular nucleus of the thalamus (PVT) (Campeau & Davis, 1995; Repa