Journal Club

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Stress-Induced Increases in Locus Coeruleus Norepinephrine Underlie Extinction Learning Deficits

Ellen Rodberg
Department of Biology, Neuroscience and Behavior Program, University of Massachusetts Amherst, Amherst, Massachusetts 01003
Review of Giustino et al.

Fear triggers behavioral responses that help us avoid imminent threats. When stimuli or environmental contexts no longer pose a risk, we typically learn new behaviors and extinguish fear responses. Thus, both fear learning and extinction are important processes that help us navigate our environment to respond optimally to the world around us: fear learning allows us to stay safe when danger is present, but hyperactive, overgeneralized, or persistent fear associations can manifest as phobias or post-traumatic stress disorder (PTSD). To rewire the connections so that a stimulus no longer evokes unwarranted fear, clinicians often use exposure-based cognitive-behavioral therapy. Researchers use fear conditioning and extinction paradigms as useful models to understand the neural underpinnings of these learning processes that will ultimately lead to therapeutic interventions for pathologic fear.

In experimental settings, repeated pairings of an innocuous conditioned stimulus (CS), such as a light or tone, with a noxious unconditioned stimulus (US), such as a shock, produce associative learning that links the CS and US, so the CS begins to evoke fear responses. Extinction of conditioned fear responses is an active learning process in which, after several presentations of the CS without the US, the animals learn that the CS no longer predicts the US. This learning is modulated by context and time, and involves developing a new association that competes with the original conditioned fear association.

Studies characterizing the neural circuits required for fear and extinction learning have identified the amygdala, prefrontal cortex, and the hippocampus as major nodes. The basolateral amygdala (BLA) along with the medial prefrontal cortex, specifically the infralimbic cortex (IL), are integral to the acquisition and expression of fear conditioning and extinction (McGaugh, 2004; Corcoran and Quirk, 2009; Giustino and Maren, 2018), while the hippocampus processes contextual information to modulate fear conditioning and extinction retrieval (Corcoran and Quirk, 2009; Giustino and Maren, 2018). Initial fear learning involves BLA excitation in response to a US paired with a CS, which leads to strengthened fear associations in BLA so that the presentation of a CS alone results in BLA excitation. After CS presentation, BLA excitation results in fear expression via excitatory input to the central nucleus of the amygdala (CeA), the output nucleus of the amygdala that facilitates fear behavior. Fear extinction involves an inhibitory circuit where new learned associations drive IL inputs to BLA and intercalated cells in the amygdala, which inhibit the CeA, resulting in the suppression of a fear response (Sotres-Bayon and Quirk, 2010). The reciprocal connections among BLA, IL, and the CeA that are responsible for initial fear conditioning and extinction compete to either express or inhibit the learned fear response.

Underlying stress drives activity in neuromodulatory centers and impairs new learning, including extinction learning (Joëls et al., 2006; Maren and Holmes, 2016). The US in fear learning is a stressor, and, because of this, extinction performed close to the time of fear learning will be performed in a physiologically stressed state. Thus, when extinction training occurs immediately after fear conditioning, extinction is impaired, a phenomenon termed immediate extinction deficit (Maren, 2014). Stressed states impact learning in part by modifying neuromodulatory inputs to relevant circuitry. Norepinephrine (NE) is one neuromodulator that is increased after stressful stimuli, and it has been implicated in many stress-induced learning deficits (Morilak et al., 2005). The amygdala receives dense NE innervation from several regions including the locus Coeruleus (LC) (Asan, 1998). The LC–NE system is important for arousal, attention, and stress responsivity among other things (for review, see Berridge and Waterhouse, 2003), and projections from LC to amygdala have been implicated in anxiety- and fear-related behaviors (McCall et al., 2017).

Previous work demonstrated that systemic and intra-BLA, but not intra-IL antagonism of β-adrenergic receptors with propranolol before extinction training promoted extinction learning and

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Correspondence should be addressed to Ellen Rodberg at erodberg@umass.edu.
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prevented an immediate extinction deficit (Fitzgerald et al., 2015; Giustino et al., 2017), suggesting that NE acting on the BLA via β-adrenergic receptors can promote stress-induced immediate extinction deficits. Much remains unknown about these extinction deficits, including the origin of the NE that drives them and the precise circuit on which it acts, but one likely contributor is dysregulation within corticolimbic circuits, leading to prioritization of fear expression over learning new stimulus associations.

In a recent article published in *The Journal of Neuroscience*, Giustino et al. (2020) used fear conditioning in concert with pharmacology, electrophysiology, and cell type-specific chemogenetics to investigate whether NE originating from the brainstem LC drives amygdala hyperactivity and stress-induced immediate extinction deficit.

Giustino et al. (2020) showed that fear conditioning (using a tone followed by footshock) produced prolonged increases in spontaneous firing in BLA in addition to canonical freezing behavior. They confirmed that systemic injection of the β-adrenergic antagonist propranolol mitigated increases in freezing, and they demonstrated that propranolol reduced the magnitude of footshock-enhanced BLA firing. To determine whether NE release from the LC can facilitate stress-related increases in BLA activity and fear-related behaviors, Giustino et al. (2020) chemogenetically excited LC–NE neurons before administering a reduced stress fear-conditioning paradigm involving a low-intensity footshock. In this low-intensity paradigm, control rats showed transient freezing responses and modest BLA activation, whereas rats with LC–NE activation exhibited persistent freezing behavior and greater BLA excitation during conditioning. These findings demonstrate that increased LC–NE activity reduced the threshold for noxious stimuli to evoke fear-related responses behaviorally and physiologically in relevant fear circuits.

Next, to investigate the contribution of LC–NE to the immediate extinction deficit, Giustino et al. (2020) moved animals to an extinction session, immediately after chemogenetic LC–NE stimulation and low-intensity fear learning. Animals that underwent LC–NE activation before fear learning, and immediate extinction showed increased levels of freezing when presented with the CS 2 d later. This suggested that activation of LC–NE can augment stress-related circuitry, producing an immediate extinction deficit after presentation of a weak shock stimulus that does not intrinsically generate stress-induced extinction deficits.

Last, to determine whether β-receptors in the BLA mediated the effect of LC–NE activation on fear learning and immediate extinction deficits, Giustino et al. (2020) used intra-BLA propranolol in combination with chemogenetic activation of LC–NE neurons. Animals received propranolol and LC activation sequentially before undergoing fear conditioning and immediate extinction. Animals with LC–NE activation exhibited an immediate extinction deficit and showed high freezing behavior (replicating the results from the previous experiment). Notably, intra-BLA propranolol prevented the LC-driven immediate extinction deficit, and those animals exhibited normal extinction. Together, these results support the hypothesis that NE released from LC terminals acts on β-adrenergic receptors in the BLA to increase BLA activity and produce deficits in extinction learning. Furthermore, these results indicate that LC–NE projections to BLA promote extinction learning deficits similar to those seen with stress-related impairment of extinction learning, identifying a circuit underlying this behavior.

More broadly, the results of the study by Giustino et al. (2020) contribute to a growing body of evidence that shows LC release of NE can alter behavioral responses in stressful conditions (Arnsten, 2015; Giustino and Maren, 2018). Here, stressful conditions and increased LC–NE in the BLA resulted in BLA excitability and subcortical control of behavior at the expense of cortical IL control of behavior. The actions of LC–NE in the BLA resulted in enhanced expression of reactive fear behavior and deficits in extinction learning. The effectiveness of a learned fear association is modulated by factors like stress and, in the case of the immediate extinction deficit, relies on LC–NE tone and receptor targeting in subcortical limbic areas (Giustino and Maren, 2018; Likhtik and Johansen, 2019).

This research adds to our understanding of the circuit underlying fear extinction and learning. The results from this study and previous studies from the Maren laboratory have found that systemic and intra-BLA, but not intra-IL, propranolol prevent immediate extinction deficit (Fitzgerald et al., 2015; Giustino et al., 2017). This suggests that NE action on β-adrenergic receptors in BLA impairs extinction learning by maintaining fear associations instead of acquiring a new extinction association. In this previous work, the authors proposed that, in addition to the competition between BLA excitation and IL-mediated inhibition of fear expression, the BLA may also inhibit the IL to further increase the expression of fear behavior and strengthen the fear association. Previous research has shown that glutamatergic projection neurons in the BLA modulate inhibitory interneurons in mPFC (McGarry and Carter, 2016). Together, a circuit explanation for impairment in extinction learning is created: stress-induced NE released from the LC increases BLA firing, which in turn inhibits IL by activating GABAergic microcircuits in mPFC, thereby reducing IL control of extinction behavior, while simultaneously increasing the excitatory BLA input to CeA, resulting in fear expression. The net result is that mPFC goes offline and, under sustained BLA activity, the expression of the initial learned fear response is continued at the expense of a new learned association.

Translationally, these insights are important for designing pharmacological and cognitive-behavioral therapies to treat fear and anxiety disorders such as PTSD. The immediate extinction deficit is relevant to PTSD and other phobias, which are hallmarked by an inability to extinguish or learn new associations between innocuous stimuli and past trauma-evoked fear (Pitman et al., 2012). Along with extinction learning deficits, patients with PTSD exhibit multiple disruptions in neural signaling, including elevated NE levels and hyperactive amygdala function (Pitman et al., 2012; Giustino et al., 2016). Acute stressful events may reduce the efficacy of extinction learning; however, this can be rescued with noradrenergic antagonists such as propranolol. Propranolol has previously been implicated as a potential treatment for PTSD (Giustino et al., 2016; Brunet et al., 2018), and Giustino et al. (2020) provide evidence for the neural mechanisms underlying the ability of propranolol to reduce fear associations.

An important area that remains to be investigated is the potential influence of sex differences in the proposed circuit and susceptibility to stress-related extinction differences. Giustino et al. (2020) included male rats in their experiments, but it is known that there are sex differences in the prevalence of stress-related psychiatric disorders like PTSD (Kessler et al., 1995; Kokras and Dalla, 2014; Bangasser et al., 2016). There are also sex differences in the morphology of LC and its response to stress: females exhibit greater responses to stress and negative stimuli (Pinos et al., 2001; Bangasser et al., 2016). In the future,

work including females may uncover whether sex differences in the regulation of extinction circuits and immediate extinction deficit contribute to the increased prevalence of stress-related disorders in females.

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