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Insights into the Neurobiology of Anxiety and a Potential Target for Pharmacotherapy

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Review of Ahrens et al.

Exposure to a stressful event activates noradrenergic neurons of the locus ceruleus, and noradrenaline is released near cells of the limbic system, including the hippocampus and amygdala, which express α- and β-adrenergic receptors (Krugers et al., 2012; Szabadi, 2013). After a delay, corticotropin-releasing hormone (CRH) is released from neurons in the hypothalamic median eminence and acts on CRH receptors in the pituitary leading to corticosteroid release in the periphery (Joëls and Baram, 2009; Krugers et al., 2012). CRH receptors were previously thought to be only important in regulating endocrine responses (Bruchas et al., 2010). Now, several studies have demonstrated that CRH receptors are widely distributed in the brain, including the extended amygdala complex, suggesting that CRH interacts with several other neurotransmitters to mediate the stress response centrally (Joëls and Baram, 2009). The extended amygdala complex contains key regions, including the CeA and the BNST. Previous studies have suggested that the CeA and the BNST have distinct roles, mediating phasic and sustained (anxiety-like) fear, respectively (Davis et al., 2010). However, other studies have suggested that these brain regions orchestrate fear and anxiety states together. For example, a recent fMRI study found that activity in both the amygdala and the BNST is altered in people with generalized anxiety disorder (Yassa et al., 2012). Coordinated responses in CeA and BNST would not be surprising given that the CeA sends strong inhibitory projections to the BNST (Dong et al., 2001; Oler et al., 2017).

Ahrens et al. (2018) recently investigated the relationship between the lateral division of the CeA (CeL) and the BNST at the molecular level. Because somatostatin-expressing (SOM^+) cells in the CeL are essential for the acquisition and recall of fearful memories, as well as defensive behaviors (Yu et al., 2016), Ahrens et al. (2018) explored whether SOM^+ CeL neurons alter activity in the BNST, and whether changing the relationship between these brain regions produces anxiety-related behaviors. Previous experiments demonstrated that global deletion of the tyrosine kinase ErbB4 from SOM^+ cells in mice produced an anxiety-like phenotype as assessed by the open field test and the elevated plus maze test (Ahrens et al., 2015). In the open field test, ErbB4-deficient mice showed reduced time spent in the open arms and a reduced number of entries into the open arms (Ahrens et al., 2018, their Fig. 1*A*). In the elevated plus maze test, ErbB4-deficient mice displayed reduced time spent in the center and a reduced number of entries into the center (Ahrens et al., 2018, their Fig. 1*B*). Deleting ErbB4 selectively in SOM^+ CeL neurons also produced an anxiety-like phenotype. Additionally, ErbB4 deletion potentiated excitatory synaptic transmission onto SOM^+ CeL neurons by increasing the frequency of miniature EPSCs (mEPSCs) (Ahrens et al., 2018, their Fig. 3*B*,*C*). To determine whether increasing excitatory input to SOM^+ CeL neuron was responsible for the increase in anxiety-like phenotype in ErbB4 mutants, Ahrens et al. (2018) expressed a mutant form of the glutamate ionotropic receptor AMPA subunit 2 (GLUA2) selectively in these neurons in otherwise wild-type mice. This replicated the anxiety-like phenotype, indicating that hyperactive SOM^+ CeL neurons are sufficient to produce anxiety-like behaviors.

The CeL is functionally connected to the BNST (Dong et al., 2001; Oler et al., 2017), and retrograde tracing performed by Ahrens et al. (2018) confirmed that SOM^+ CeL neurons innervate SOM^+ and SOM^- neurons in the dorsal BNST (dBNST) (Ahrens et al., 2018, their Fig. 5*A*–*E*). Despite the increased excitatory drive to SOM^+ CeL neurons with inhibitory projections to the dBNST, the fre-

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quency of spontaneous IPSCs (sIPSCs) onto SOM$^+$ dBNST neurons was reduced in ErbB4-mutant mice. Because SOM$^+$ neurons in the amygdala typically release the inhibitory neurotransmitter GABA (McDonald and Mascagni, 2002; Saha et al., 2002), it is paradoxical that increasing excitatory drive to SOM$^+$ CeL neurons leads to disinhibition of SOM$^+$ dBNST neurons. There are two possible explanations for this finding. First, SOM$^+$ CeL neurons might reduce GABAergic transmission onto SOM$^+$ dBNST indirectly, via other unknown neuronal populations. But this was previously ruled out (Li et al., 2012). Alternatively, as Ahrens et al. (2018) suggest, SOM$^+$ CeL neurons corelease a neuromodulator that regulates inhibitory neurotransmission. In support of the latter hypothesis, RNA sequencing of SOM$^+$ CeL neurons revealed that they express high levels of prodynorphin RNA, a gene encoding a precursor of the endogenous κ-receptor agonist dynorphin. To determine whether enhanced dynorphin release mediated the reduction of inhibitory neurotransmission onto SOM$^+$ BNST neurons, Ahrens et al. (2018) treated dBNST slices with norbinaltorphimine (norBNI), a selective κ-opioid receptor antagonist; this treatment restored inhibitory synaptic transmission onto SOM$^+$ dBNST neurons in ErbB4 mutants, but not in wild-type mice. These data suggest that hyperactive SOM$^+$ CeL neurons disinhibit SOM$^+$ dBNST neurons by increasing dynorphin signaling at the synapse.

Notably, the authors recapitulated their findings in the acute stress model. If an acute stressor affects CeL and dBNST neurons, such as ErbB4 deletion, then these data may provide insight about the neurobiology of anxiety. Administering uncontrolled and unpredictable foot shocks increased anxiety-like behaviors in wild-type mice compared with mice that did not receive foot shocks. Subsequent electrophysiological recordings revealed an increase in mEPSCs onto SOM$^+$ CeL neurons and a reduction of sIPSCs onto SOM$^+$ dBNST neurons. Furthermore, inhibitory synaptic transmission onto dBNST neurons was restored after exposure of acute dBNST slices to norBNI. Importantly, norBNI infusion into the dBNST of behaving ErbB4 mutant mice reduced anxiety-like behaviors. The behavioral effects of norBNI infusion into the dBNST of acutely stressed wild-type mice was not reported, however. Therefore, selective κ-opioid receptor antagonism may not be anxiolytic in intact mice limiting the translational potential of the results.

Dynorphin is intimately connected to the stress response. Following acute stress or intracerebroventricular injection of CRH, phosphorylation of κ-opioid receptors, an indicator of agonist binding to the κ-opioid receptor, increased in several brain regions, including the extended amygdala (Land et al., 2008). Furthermore, intra-amygdala injection of CRH increased extracellular dynorphin concentration in the CeA as measured by microdialysis in rats (Lam and Gianoulakis, 2011). Therefore, it is not surprising that activation of the hypothalamo-pituitary-adrenal axis via acute uncontrolled foot-shocks increased dynorphin release onto SOM$^+$ dBNST neurons (Ahrens et al., 2018, their Fig. 9*C*,*E*). A surge in CRH after an acute stressor may lead to the activation of CRH receptors on putative SOM$^+$ CeL neurons (Asok et al., 2018), subsequent dynorphin release and κ-opioid receptor activation. The κ-opioid receptor is coupled to a G-inhibitory protein, and activation of the receptor may hyperpolarize neurons via increasing potassium conductance and/or decreasing calcium conductance (Bruchas et al., 2010). However, in the present study, Ahrens et al. (2018) reported that κ-opioid receptor antagonism restored sIPSCs onto dBNST neurons 24 h after foot-shocks, suggesting that the expression and activation of κ-opioid receptors on postsynaptic dBNST neurons are unlikely. Indeed, previous research has demonstrated that the ability of κ-opioid receptors to inhibit GABA release is dependent on ERK signaling activated presynaptically in CeA neurons (Li et al., 2012). Together, the above evidence suggests that SOM$^+$ CeL neurons regulate GABAergic tone onto the dBNST through the activation of presynaptic κ-opioid receptors. To relate these findings more closely to anxiety states in humans, future studies should assess the consequences of chronically elevated dynorphin signaling at the CeL-dBNST synapse. An intense or repeated stressor may increase dynorphin signaling to the point of desensitizing and/or internalizing κ-opioid receptors. κ-Opioid receptor desensitization has previously been shown to depend on G-protein receptor kinase phosphorylation of the agonist-bound form, elicited by dynorphin binding (Al-Hasani and Bruchas, 2011). Indeed, prolonged κ-opioid receptor activation by the κ-opioid receptor agonist U50,488 resulted in increased receptor phosphorylation and analgesic tolerance *in vivo* (McLaughlin et al., 2004). Thus, κ-opioid receptor desensitization could disrupt CeL-dBNST functional homeostasis and contribute to the manifestation of chronic anxiety-like states.

The experiments by Ahrens et al. (2018) have provided evidence for a novel therapeutic target in the treatment of anxiety disorders. Indeed, dysregulated κ-opioid signaling has been implicated in psychiatric disorders, such as addiction and depression (Bruchas et al., 2010). Moreover, administration of buprenorphine (a partial μ-opioid receptor agonist and κ-receptor antagonist) together with samidorphan (a μ-opioid receptor antagonist) significantly decreased depressive symptoms in patients with treatment-resistant major depressive disorder (Fava et al., 2016). Furthermore, Almatroudi et al. (2018) showed that BU10119, a short-acting μ-/κ-opioid receptor antagonist, relieved anxiety-like symptoms in mice. Whereas BU10119 significantly decreased the latency to drink in a novelty-induced hypophagia task, it had no effect on two other tasks that are thought to measure anxiety-like symptoms: the elevated plus maze task and the light-dark exploration test (Dulawa and Hen, 2005; Griebel and Holmes, 2013), possibly because these tests were not sufficiently stressful (Almatroudi et al., 2018). Nonetheless, the study by Ahrens et al. (2018) provides evidence that increased dynorphin release caused by dysregulation of the extended amygdala may be involved in the pathophysiology of anxiety disorders. Future studies should explore the efficacy of κ-opioid receptor antagonists in relieving anxiety, using intense acute or chronic stress models and anxiety-sensitive assays in wild-type rodents. If successful, blockade of κ-opioid receptors could be developed as a potential pharmacotherapy in the treatment of anxiety disorders in humans.

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