Central amygdala projections to lateral hypothalamus mediate stress-induced alterations in alcohol-related behavior

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Background: In humans, avoidance coping in response to traumatic stress is associated with post-traumatic stress disorder (PTSD) and increased alcohol consumption. Using rats, our lab has shown that predator odor ("traumatic") stress produces persistent avoidance of stress-paired stimuli in a subset of subjects, termed 'Avoiders', mirroring avoidance PTSD symptomatology in humans. Interestingly, Avoider rats display long-lasting increases in alcohol self-administration following stress exposure, a phenomenon that is absent in stress-exposed Non-Avoiders, and that recapitulates findings in humans. Our published work show that Avoider rats have more c-Fos$^+$ cells and greater corticotropin-releasing factor (CRF) immunoreactivity in the central amygdala (CeA) after stress, compared to Non-Avoiders and unstressed Controls, and that intra-CeA antagonism of CRF_1 receptors rescues stress-induced avoidance behavior and escalation of alcohol self-administration in Avoider rats. Here, we sought to test the role of downstream circuits, specifically CeA outputs to the lateral hypothalamus (LH), in supporting an Avoider phenotype.

Methods: CRF_1-expressing CeA projections to LH were identified using a combination of retrograde tracing and RNAscope *in situ* hybridization. Synaptic connectivity between CeA terminals in LH and LH neurons was tested using a combination of *ex vivo* optogenetics and electrophysiology. Stress-induced activation of CeA-to-LH neurons was quantified using a combination of retrograde tracing and c-Fos immunohistochemistry. Intrinsic properties and synaptic transmission of CeA-to-LH neurons following stress were measured using slice electrophysiology. We also tested the effects of CeA-to-LH circuit manipulation on behavior using *in vivo* chemogenetics.

Results: We found that a population of CeA CRF_1^+ cells project to the LH, a brain region that modulates motivated behaviors, and that CeA-to-LH projections form functional GABAergic synapses with LH neurons. Following predator odor stress, Avoider rats had more c-Fos$^+$ CeA-to-LH cells, including CeA-to-LH cells that express CRF_1 receptors, than Non-Avoiders. CeA-to-LH neurons from Avoider rats also had greater intrinsic excitability than neurons from Non-Avoiders. Chemogenetic inhibition of CeA-to-LH neurons attenuated avoidance behavior in Avoider rats, and stimulation of CeA-to-LH neurons supported avoidance behavior in stress-naïve rats.

Discussion: Collectively, these results suggest that stress-induced activation of CeA-to-LH neurons, including those that express CRF_1 receptors, supports an Avoider phenotype. Current work is focused on testing the role of CRF_1^+ CeA-to-LH neurons, as well as the role of distinct LH circuits, in mediating 1) stress-induced avoidance behavior, 2) escalation of alcohol self-administration, and 3) tolerance to alcohol aversion.

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