Circuit-Specific CRISPR/Cas9 Gene Editing Reveals an Extended Amygdala Neuropeptide Receptor Signaling Mechanism Driving Alcohol Drinking, Anxiety, and Avoidance

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Alcohol use disorder is prevalent in the United States, where alcohol drinking imposes a massive burden on public health, including an annual death toll of ~90,000 people. Alcohol dependent individuals often achieve extreme intoxication through repeated cycles of binge drinking, and maintain high blood alcohol levels to avoid withdrawal symptoms appearing upon detoxification (including compulsive reward-seeking, irritability, anxiety, insomnia, and sleep fragmentation). Thus, major efforts are aimed at elucidating the physiological and genetic mechanisms within neural stress and sleep/wake arousal systems that will inform innovative new strategies for remedying the negative emotions and disrupted sleep patterns associated with alcohol misuse.

Negative affective states linked to addiction are thought to arise from dysregulated activity and detrimental neuroplasticity in limbic systems. In particular, the effects of stress on addiction are proposed to occur via long-lasting adaptations in reciprocally connected circuits of the hypothalamus and amygdala. Our experiments focused on lateral hypothalamus (LH) neurons containing the neuropeptide hypocretin (Hcrt; orexin), which are critical for stabilizing wakefulness and motivated behaviors. Our previous research identified dense connectivity between Hcrt-LH neurons and "extended amygdala" neurons of the bed nuclei of stria terminalis (BNST) containing the prototypical stress neuropeptide corticotropin-releasing factor (Crf). These prior studies characterized Hcrt-LH neurons and Crf-BNST neurons as tightly coupled nodes in a stress-promoting neurocircuit, suggesting their involvement in the dysregulated emotional states observed in addiction.

To study addiction, we utilized a well-established paradigm in which mice with unrestricted access to food and water voluntarily drink intoxicating levels of alcohol. We developed a custom CRISPR/Cas9 gene editing system, and disrupted the expression of the Hcrt type-1 receptor within specific cell types to test the impacts on excessive alcohol drinking. These experiments advanced our earlier findings by identifying the precise neuronal subpopulations and receptor signaling mechanisms by which LH and BNST circuits mobilize the behavioral processes and physiological adaptations underlying voluntary binge alcohol consumption. Additional studies of sucrose drinking, anxiety-like behavior, and approach/avoidance further defined the phenotypic characteristics associated with circuit-specific Hcrt receptor signaling events. Collectively, our findings established a framework in which chronic alcohol intake enhances negative emotional states through precisely defined neurocircuit connections and signaling mechanisms. These outcomes have considerable implications for the multifaceted challenges faced in the search for effective strategies to treat addiction.

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