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**"Orexin Modulation of Alcohol Brain Reward and Stress System Interactions"**

Orexin neuropeptides have been implicated with modulating hunger, circadian rhythm, and reward systems in the human body. Their involvement with reward systems may offer potential clarity in piecing together the uncertain development of alcohol dependence in humans. Regarding brain circuits, the ventral tegmental area (VTA) is well characterized in its mesolimbic reward circuit with the nucleus accumbens. However, it has distinct projections onto central amygdala (CeA) neurons that are not well characterized. These are speculated to play a role in the negative affect associated with alcohol withdrawal in humans with Alcohol Use Disorder (AUD). We have previously demonstrated activation of the VTA-CeA circuit in alcohol dependent mice and rats during withdrawal, raising the possibility that this circuit plays a role in withdrawal-associated behavior. The mechanism by which the VTA-CeA circuit becomes activated is unknown. Orexin-mediated disinhibition has been demonstrated in VTA neurons of unknown projection target, and orexin plasma concentrations rise during alcohol withdrawal in alcohol dependent humans. Therefore, in this study we explored whether intra-VTA orexin is necessary and sufficient for inducing increased anxiety-like behavior associated with alcohol withdrawal and attempted to identify specific neuronal targets in the CeA. We hypothesized that intra-VTA orexin would be necessary and sufficient to increase anxiety-like behavior in male and female rats and that this occurs via activation of the VTA-CeA circuit.

Adult Long-Evans rats (8 male and 8 female) were used in this study. Rats were injected with orexin A (50 nM) or vehicle (aCSF) using guide cannulae positioned above the VTA, then allowed to explore an elevated plus maze (EPM) apparatus. Orexin administration significantly reduced the time spent in the open arm of the EPM, suggesting that intra-VTA orexin elicits an anxiety-like phenotype. We then performed *in situ* hybridization experiments to assess whether intra-VTA orexin administration is associated with neuronal activation in the CeA. Rats were sacrificed 30 min after intra-VTA orexin (50 nM) or aCSF administration, and CeA-containing sections were collected. The early immediate gene *c-Fos* was used to gauge neuronal activity while expression of dopamine receptor D1 (*DRD1*) and cocaine and amphetamine regulated transcript (*CART*) genes were used to identify potential co-expressors of the activated neurons. We found no significant difference in the total number of *c-Fos*+ neurons in the CeA of orexin-treated animals compared to controls, and co-expression patterns between *c-Fos*+, *DRD1*+, and *CART*+ were similar between the two groups. These data indicate that, while intra-VTA orexin is sufficient to produce an anxiety-like phenotype in rats, this is not associated with activation of CeA neurons. Future research will assess activation of other downstream VTA targets and use alternate approaches to evaluate the VTA-CeA circuit more directly.