

Neuronal enriched RNA-Binding Protein HuD and microRNA miR-495 oppositely regulate cocaine induced addiction-related gene expression and place preference behavior

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Post-transcriptional regulation (PTR) plays an important role in nervous system development and function, however, very little is known about its role in substance use disorders. RNA binding proteins (RBPs) provide one such regulatory mechanism in PTR. HuD is a neuronal specific RBP that associates with the 3'UTR of specific mRNAs containing AU-rich instability elements (AREs). Through association with these regions, HuD stabilizes and protects mRNA transcripts from degradation. microRNAs similarly target specific mRNAs by association with the 3'UTR. In opposition to HuD, microRNAs aided by the RNA-inducible silencing complex cause the translational repression or degradation of target mRNA. We found that HuD and miR-495 target a similar sequence in a set of mRNAs which have been implicated in addiction (addiction-related genes; ARGs), including CaMKII $\alpha$ , Bdnf, Mef2c, and Arc. Additionally, HuD itself is found in the Knowledgebase of Addiction related genes (KARG) further suggesting it may play a role in this disorder. Finally, many of these shared targets have been found to play a role in conditioned place preference (CPP). Since HuD and miR-495 have opposite effects on mRNA stability, regulation of HuD or miR-495 could oppositely alter CPP associated target mRNA leading to changes in CPP behavior. First, we assessed the expression of HuD, miR-495, and its targets after the acquisition of cocaine CPP (15 mg/kg) in male C57 mice. miR-495 was significantly decreased within the Nucleus Accumbens (NAc) and Dorsomedial striatum (Dms) but not the Dorsolateral striatum (Dls). Conversely, HuD protein was significantly increased following this same regional pattern. Additionally, CaMKII $\alpha$  and BDNF mRNA and protein was increased in a similar fashion. To isolate the contribution of HuD to this behavior, we used mice overexpressing neuronal specific HuD (HuD OE). HuD OE mice spend significantly more time in the cocaine paired chamber compared to WT littermates. Additionally, NAc isolated from these animals showed significantly increased Bdnf and CaMKII $\alpha$  protein. Next, we infused miR-495 within the NAcsh to isolate its role in CPP. LV-GFP and LV-miR-495 animals both acquired CPP robustly but no significant differences were found between the two. To test the role of NAcSh miR-495 in other stages of CPP, we extinguished the place preference found previously. LV-miR-495 treated animals showed significantly decreased CPP compared to LV-GFP animals on Day 3. This suggests that miR-495 and HuD are involved in drug-induced gene expression and addiction-like behavior.

These two molecules may also play more important roles in drug abstinence related behaviors such as extinction.