Integrative synaptomics in heavy drinking macaques identifies cross-species proteogenetic targets for treating excessive drug-seeking

Patrick J. Mulholland, PhD

Medical University of South Carolina, Departments of Neuroscience and Psychiatry & Behavioral Sciences, Charleston Alcohol Research Center, 67 President Street, Charleston, SC 29425

Substance use disorders are chronic relapsing brain diseases characterized by cognitive impairments, the inability to regulate drug intake, and neuropathological changes in the prefrontal cortex (PfC). The orbitofrontal cortex (OfC) is a critical cortical sub-region that controls learning, decision-making, and prediction of reward outcomes, and OfC dysfunction contributes to executive cognitive function deficits in addicts. In this study, we describe maladaptive neuroadaptations in OfC pyramidal neurons in heavy drinking macaques. Whole-cell electrophysiological recordings of deep-layer pyramidal neurons demonstrated that long-term drinking produced aberrant homeostatic intrinsic excitability and glutamatergic synaptic adaptations. Profiling of the OfC synaptome identified drinking-induced changes in synaptic proteins controlling glutamate release and glutamatergic signaling. An exploratory analysis of the synaptomics data identified 13 proteins that best discriminate between heavy and low drinking monkeys. Our bioinformatics analysis shows that genes encoding the top proteins (*ADD1*, *ADD3*, *C2CD2L*, *DIRAS2*, *PYCR2*) are located within published human and rodent QTLs for alcohol consumption and preference. In addition to alcohol, these five genes are found in multiple drug-related QTLs for at least two additional illicit drugs. Exploration of PfC transcript levels in BXD recombinant inbred strains of mice revealed significant correlations with alcohol consumption (*Add1*, *Add3*, *C2cd2l, Diras2*, *Pycr2*) and intravenous cocaine self-administration (*Add1*, *Add3*, *C2cd2l*). We identified that *C2cd2l* is associated with strong cis-acting expression QTLs in brain regions within the addiction circuitry, and BXD strains that inherited the *D* allele at the *C2cd2l* interval drank markedly less alcohol than strains that inherited the *B* allele. Our bioinformatics analysis also revealed that four of these genes (*Add1*, *Add3*, *Diras2*, *Pycr2*) are differentially expressed in postmortem brain tissue from alcohol, heroin, or cocaine addicts. In summary, we describe functional neuroadaptations in OfC pyramidal neuron synapses from heavy drinking monkeys. Our integrative synaptomics and bioinformatics analyses identified several synaptic proteins with strong cross-species genetic links to intake of and behaviors related to multiple abused drugs. Further preclinical and clinical studies are necessary to determine if these novel genes/proteins have substantial potential as proteogenetic targets for the treatment of substance use disorders.