## Shahrdad Lotfipour, PhD

Post-Translational Mechanisms of a 3' UTR Alpha6 Nicotinic Receptor Polymorphism in Adolescent Substance Use

This project offers a new avenue of research in neuroscience by evaluating non-coding regions in the genome using novel tools in chemical biology to study mechanisms of addiction. Regulation of drug-induced behaviors via translated small open reading frames (smORFs) has been overlooked in addiction neuroscience. We have identified a 3' untranslated region (UTR) single nucleotide polymorphism (SNP) in the alpha( $\alpha$ )6 nicotinic acetylcholine receptor (nAChR) subunit (encoded by the CHRNA6 gene) that appears to regulate nicotine addiction. Our data show that this SNP leads to a two-fold increase in nicotine seeking and substance use in rodents and humans, respectively. The results are unique as studies in the field thus far have focused on SNPs in the coding regions of the genome. Our laboratory has engineered a CHRNA6 3' UTRC123G SNP mutant rat line that replicates the genetic variation in humans in order to functionally validate the mechanisms of enhanced nicotine use. Using CRISPR-Cas9 genomic engineering, we have introduced the entire human 3' UTR, with the 'C' to 'G' single nucleotide change in the 123 position in Sprague Dawley rats ( $\alpha$ 6CC and  $\alpha$ 6GG-carriers), thereby enhancing the animal model's translational significance to humans. We will test the hypothesis that the  $\alpha 6$  3' UTR SNP regulates nicotine-induced behaviors via alterations in  $\alpha 6$  nAChR expression and/or other microprotein expression in ventral tegmental area (VTA) brain reward neurons. By providing a new conceptual framework for how 3' UTR genetic human polymorphisms regulate drug-induced behaviors, our approaches could uncover new proteins in addiction. Our studies may significantly influence the field, as we aim to discover targeted smORF microproteins and/or other 3' UTR regulatory mechanisms mediating sex- and genotype-dependent effects in addiction. The microproteins found to regulate genotype- and sex-dependent effects could then be targeted. Such work on the  $\alpha 6$  3' UTR SNP could lead to future high throughput screening strategies with the ability to translate genomic sequence into drug therapeutics for substance use cessation. Overall, our studies have potential impacts in nAChR biology, addiction neuroscience, and drug discovery. This is significant given the exponential increase in adolescent electronic nicotine and poly-drug use, necessitating improved prevention / intervention strategies. Our approach will be to use a novel viral method with CRISPR-Cas9 engineering to implement Targeted Knock-In with Two (TKIT) guides to tag the  $\alpha 6$  nAChR subunit (encoded by the CHRNA6 gene) and putative 3' UTR smORFs in our humanized rats. Parallel studies will use targeted mass spectrometry (MS) to acquire guantitative peptide data on smORF encoded microproteins. Our team is uniquely positioned for these studies given our expertise in behavior, genetics, smORFs, proteomics, drug discovery, chemistry, and MS. There is a vital need to understand the role of non-coding SNPs within nAChR genomic clusters and their function in substance use and related disorders. How a SNP in the 3' UTR of human CHRNA6 alters adolescent substance use is currently not known. Such studies could help delineate the mechanisms for the susceptibility to addiction/psychiatric disorders.