The Role of ALPHA5 Single Nucleotide Polymorphism on Nicotine Dependence

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Nicotinic acetylcholine receptors, or nAChRs, form the basis of nicotine addiction. The ALPHA4BETA2ALPHA5 nAChR is the focus of this study. When an individual uses, either a cigarette or an e-cigarette, nicotine is released, rapidly moving from the bloodstream to the brain. There it binds to the nAChRs and results in the opening of the receptor, sodium, and calcium enter into the receptor and potassium exits. The effect of calcium entering releases the neurotransmitter dopamine. Once this transmitter is released the user experiences a pleasurable feeling, which reinforces continued use of the substance to achieve more pleasure, thereby strengthening the addiction. This process is known as the “Dopamine Reward Pathway.” Thus, defining the nicotinic receptor subunit composition in vivo remains a critical issue to be addressed in characterizing the mechanisms underlying addiction. Two tests were conducted to help both characterize and reverse the role of the ALPHA5 receptors. Electrophysiology allowed me to distinguish between the ALPHA5(D) and ALPHA5(N) nAChR subunit variants through ACh stimulated whole cell patch current. After measuring the desensitization rate of both, I was lead to two hypotheses. The new tool that was used to help with the knockdown of the ALPHA4BETA2ALPHA5 nAChR is the gene editing tool called CRISPR-Cas9. After finding the correct antibody, I was able to with the help of the pX330 DNA plasmid knock-down the ALPHA4BETA2ALPHA5 nAChR. And to help visualize the knockdown, western blots were performed, allowing me to identify the presence of specific proteins.

Awards Won:
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