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Functional studies of genes identified in human GWAS of smoking behaviors (Clu and Akt2)

Recent work has implicated a role for astrocyte function in nicotine neurobiology. To identify astrocyte-expressed genes relevant to nicotine use in humans, we used TWAS results from recent GWAS on smoking behaviors along with astrocyte expression data to identify genes of interest (GOI) and assess potential astrocyte involvement in nicotine responses. Using a CRISPR knockout approach to knock down GOI expression, we are screening 25-50 GWAS-identified genes using area analysis. We are assessing the role of the GOI on astrocyte size and morphology in primary mouse astrocytes following nicotine treatment. We are currently investigating the role of Clusterin (Clu) in nicotine-induced astrocyte morphology and reward behavior. In addition to emerging as a strong candidate from our screen, this gene also emerged as differentially expressed using spatial transcriptomics in the nucleus accumbens. Mouse astrocytes were assessed in primary cell culture exposed to nicotine (100 μ m) and in the adult hippocampus after subcutaneous nicotine injection (0.35 mg/kg) using immunohistochemical staining for area and Sholl analysis of tissue to examine the effects of Clu knockout. Clu loss reduced astrocyte morphological responses to nicotine in vitro and in vivo. Preliminary data suggest that Clu knockout mice exhibit place aversion to nicotine ($p = 0.07$). We are also using live imaging to study the dynamic nature of astrocytic activation. We used an Adeno-Associated Virus (AAV) to transfect astrocytes with mCherry for visualization. We found differences in the instantaneous and average speed of astrocytes, suggesting that nicotine affects astrocytic motility. This strategy will be applied to study the effects of a previously studied gene, Akt2, and Clu, on motility in response to nicotine. These results enable the identification of potential novel drug targets and will improve the current understanding of the astrocytic response to nicotine. Supported by R21/R33 DA055781 (Ehringer, Hoeffler, Stitzel; MPIs); T32 DA017637 (MEB)

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