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Variant Enhancer Loci (VEL) due to Opioid Overdose Impact Forebrain Neuronal Function

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Opioid overdose death is a global crisis accounting for 80% of the 600,000 drug related deaths worldwide in 2019. The lack of mechanistic understanding of the effects of genetic and epigenetic alterations linked to opioid overdose is a major roadblock in developing new therapeutic measures. We previously reported epigenetic variation linked to opioid overdose in the dorsolateral prefrontal cortex. We identified individual-specific hypoacetylation events, termed VELs that distinguished opioid overdose cases from control. Using 3D chromatin interaction datasets, we found VELs frequently converged on five genes- *ASTN2*, *DUSP4*, *ENOX1*, *GABBR2* and *KCNMA1*. Here, we utilized gene knocked down in forebrain neurons derived from iPSCs to evaluate the functional impact of these genes. While these five genes are reported to have diverse functions, we found knock-down of all fives targets to result in differential expression of genes involved in insulin signaling pathway. Additionally, we observed a significant reduction in axonal length in the knockdown of ASTN2 and DUSP4. We evaluated whether this change in morphology could be attributed to insulin signaling by exposing forebrain neurons to insulin inhibitor and activator (insulin). Interestingly, we also observed decrease in axonal length in cells exposed to insulin receptor inhibitor. We found this phenotype to be rescued when we exposed ASTN2 and DUSP4 gene knock downs to insulin. Collectively, these results suggest there may be additional functional overlap in genes linked to opioid overdose via epigenetic dysregulation and suggests insulin signaling disruption in the forebrain as a potential contributor to opioid overdose.