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Novel approach for the investigation of X-chromosome inactivation in mice exposed to addictive substances

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The contribution of X chromosome inactivation (XCI) and escape as an epigenetic mechanism in the determination of sex differences to substance use disorder is incompletely understood. Females have two X chromosomes (XX), and during X-chromosome inactivation (XCI), one X chromosome is randomly chosen to be transcriptionally silenced. However, some X-linked genes escape XCI and display biallelic gene expression. However, in females, it is not known which X linked genes escape XCI, and if so, what cells XCI escape occurs during exposure to addictive substances. We are therefore developing and optimizing a novel single cell RNA sequencing methodology and bioinformatic approach to determine the global molecular landscape of XCI in neuronal and non-neuronal cells exposed to cocaine or morphine. This approach utilizes F1 hybrid mice from *Mus musculus* and *Mus castaneus* crosses. Breeding these mice (C57BL/6 with CAST/EiJ) maximizes the level of allelic differences detectable by SNPs frequencies. Hybrid F1 mice were exposed to PBS, morphine or cocaine after which nuclei were prepared from the nucleus accumbens for a modified single nuclei next generation sequencing and analytical pipeline. Using this approach, we intend to identify genes that escape XCI, as well as the different cell populations that display bi-allelic expression of X-linked genes in brain regions during chronic cocaine and morphine exposure that potentially contribute to sex differences in addictive behavior.