

## Cell subtype transcriptional regulation of mitochondrial dynamics in cocaine action

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Many studies demonstrate altered brain energy homeostasis in cocaine abuse. However, the effect of cocaine on mitochondria, the fundamental component of energy homeostasis, has not been well examined. We sought to examine mitochondrial (mito)-nuclear genes, molecules that perform mitochondrial function or transcriptional regulation but are transcribed in the nucleus, after repeated cocaine since a subset of the mito-nuclear genes have promoter binding sites for the transcription factor, Egr3. We previously found Egr3 to play a dynamic role in cocaine action through nucleus accumbens medium spiny neurons (MSNs), D1-MSNs and D2-MSNs. These Egr3 mito-nuclear target genes include the mitochondrial outer membrane protein, Tomm20; the mitochondrial fission molecule, Drp1; the mito-nuclear gene transcriptional coactivator, Pgc1 $\alpha$ ; mito-nuclear gene transcription factors, Nrf1 and Nrf2; the catalytic subunit of the mitochondrial DNA polymerase, Poly; and mitochondrial genome transcription factors, TFAM and Tfb1. We performed chromatin immunoprecipitation (ChIP) using an Egr3 antibody on NAc tissue from mice that received repeated cocaine. Egr3 binding was significantly enriched on promoters for Drp1, Nrf2, Pgc1 $\alpha$ , and Poly in NAc of the cocaine group compared to the saline group. Additionally, AAV viral overexpression of Egr3 in D1-MSNs increased gene

expression of Egr3 target genes Drp1, Nrf1, Pgc1 $\alpha$ , Pol $\gamma$ , and Tfam. Using the RiboTag approach, we observe an upregulation of ribosome-associated mRNA of Drp1, Pgc1 $\alpha$  and Tfam in D1-MSNs while mRNA of these genes is reduced in D2-MSNs after repeated cocaine. Additionally we found that mRNA of many of these genes is increased in the NAc after cocaine self-administration and in postmortem NAc of cocaine dependent individuals. Next, to determine if the change in gene expression of these mito-nuclear genes has implications on mitochondria, we examined mitochondrial dynamics in MSN subtypes after cocaine self administration. We observed an enhanced frequency of smaller mitochondria in D1-MSN dendrites, implicating enhanced fission in these neurons, after cocaine self-administration. We next used a small molecule inhibitor of mitochondrial fission, Mdivi-1, which blocks the activated form of Drp1. Mdivi-1 treatment blocked seeking after cocaine conditioned place preference and blocked the expression of cocaine locomotor sensitization. Finally, genetic overexpression of a fission promoting Drp1 variant, selectively in D1-MSNs, enhanced cocaine seeking after abstinence from cocaine self administration. These findings demonstrate a novel role for the underlying molecular mechanisms that mediate mitochondrial dynamics in MSN subtypes in cocaine action.