Cocaine-mediated promoter DNA methylation of microRNA-124 promotes microglial activation through TLR4 signaling

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Neuroinflammation plays a critical role in the development of reward-related behavior in cocaine self-administration rodents. Cocaine, one of most commonly abused drugs, has been shown to activate microglia both *in vitro* and *in vivo*. Detailed molecular mechanisms underlying cocaine-mediated microglial activation remain poorly understood. MicroRNAs (miRs), belonging to a class of small non-coding RNA superfamily have been shown to modulate the activation status of microglia. MiR-124, one of the microglia-enriched miRs functions as an anti-inflammatory regulator that maintains microglia in a quiescent state. To date, the possible effects of cocaine on microglial miR-124 levels and the associated underlying mechanisms have not been explored before. In the current study, we demonstrated that cocaine exposure decreased miR-124 levels in microglia *in vitro*, and is critical for activation of TLR4 signaling. These findings were further validated *in vivo;* wherein we demonstrated decreased the abundance of miR-124 in the purified microglia isolated from cocaine-administered mice brains compared with cells from saline administered animals. Molecular mechanisms underlying these effects involved cocaine-mediated increased mRNA and protein expression of DNMTs in microglia. Consistently, cocaine substantially increased the promoter DNA methylation levels of miR-124 precursors (pri-miR-124-1, and -2) but not that of pri-miR-124-3 both *in vitro* and *in vivo*. Krüpple-like factor 4 (KLF4), was identified as a novel substrate of miR-124. Besides KLF4, miR-124 also modulated the levels of several other proteins including TLR4, MyD88, TRAF6, and IRAK1. Reciprocally, overexpression of miR-124 in microglia blocked cocaine-mediated activation both *in vitro* and *in vivo*. In summary, our findings demonstrate a yet unexplored mechanism for cocaine-mediated activation of microglia via down-regulation of miR-124 (through promoter DNA hypermethylation), leading ultimately to increased TLR4 activation. Our results thus implicate that epigenetic modulation of miR-124 could be considered as a potential therapeutic approach to ameliorate microglial activation and possibly, the development of cocaine addiction. **Support: DA043138, DA033150, DA035203, DA040397, DA041751, MH106425, DA043164, DA036157**

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