Title: **Epigenetics of Drug Sensitization: MicroRNA and HDAC5 expression in Amphetamine-induced Neural Remodeling**

Short title: **Target-guided Delivery and MRI Quantitation in vivo**

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**Abstract**

Our **aim** is to apply our in vivo molecular contrast-enhanced (MCE) magnetic resonance imaging (MRI) to investigate neural network remodeling after amphetamine exposure in preclinical models of mice.

**Background**: Methamphetamine induces oxidative stress, neurogenesis, re-organization of neural cells in striatum and subventricular zone and behavioral modifications. Histone deacetylases (HDACs) act to remodel chromatin for gene expression, playing a key role in memory, learning and neural developmental processes. Changes in HDAC5 activity have mechanistic role in epigenetics and have been proposed to be involved drug sensitization of aduult animals; however, to date, evidence to support adult brain remodeling after drug addiction has been insufficient. We have reported neural remoding in progenitor cells that express Nestin and NeuN in pericytes during cerebral damage repair with or without therapy uisng human granulocyte-colony stimulating factor (Fig 1), which is known to promote neural network remodeling after stroke. Our **hypothesis** is that cerebral regions with elevated HDAC5 expression after exposure to amphetamine may be involved in neural network remodeling. Our approach is to use in vivo molecular contrast-enhanced (MCE) magnetic resonance imaging (MRI) and the results are validated using ex vivo technologies including modified in situ hybridization, RT-PCR, immunhistochemistry, and transmision electron microscopy.

**Methods and Results** We emplyed the binding of miR2861 to HDAC5 mRNA and developed MR contrast using miR2861 mimics for HDAC5 expression in a preclinical model for amphetamine sensitization. We labeled miD2861 with superparamagnetic iron oxide (SPION) for MCE-MRI and we found elevated frequency of signal reduction in MRI postively proportional to HDAC5 mRNA copy number estimated by TaqMan Analysis. Therefore SPION-miD2861 targets HDAC5 mRNA with percision hybridization. We identified elevation of HDAC5 mRNA in the lateral septum and nucleus accubens of mice in chronic exposure paradigm (Fig 2A). These region of interest (ROI) were found to express neuroprogenitor cells that co-expressed neurN (neuronal biomarker) and glial fibrillary acidic protein (GFAP, glial biomarker) in the same cells, some of them are found in micro-vessels (Fig 2C). In mouse brain treated saline, we found glial cells do not express NeuN and have long axons (Fig 2B); Progenitor cells in the LSD (Fig 2D) appears to have the appearance that were identified in stem cells in treatment model of cerebral ischemia known to be involved in neural remodeling (Fig 1, boxes).

**Conclusions**: We conclude that miD2681 targets HDAC5 expression in cells that undergo active repair/remodeling after neural damage by chronic exposure to amphetamine. MEC-MRI is a tool to identify such ROI in vivo with precision similar to that of RT-PCR. The precise delivery of miD2861 may therefore serve as a vehicle for the delivery of nanoparticles for research that identifies developmental precesses in adult animals and assists therapeutic purposes.

**Key words:** amphetamine, MCE MRI, drugs of abuse, microglia, miRNA, molecular imaging, neuroal remodeling, precision nanoparticles.

Figure 1 The presence of NeuN (green) in capillary is elevated in mice with gene therapy using hG-CSF after cerebral ischemia (BCAO model). (reported in Liu et al., FASB J, 2015).

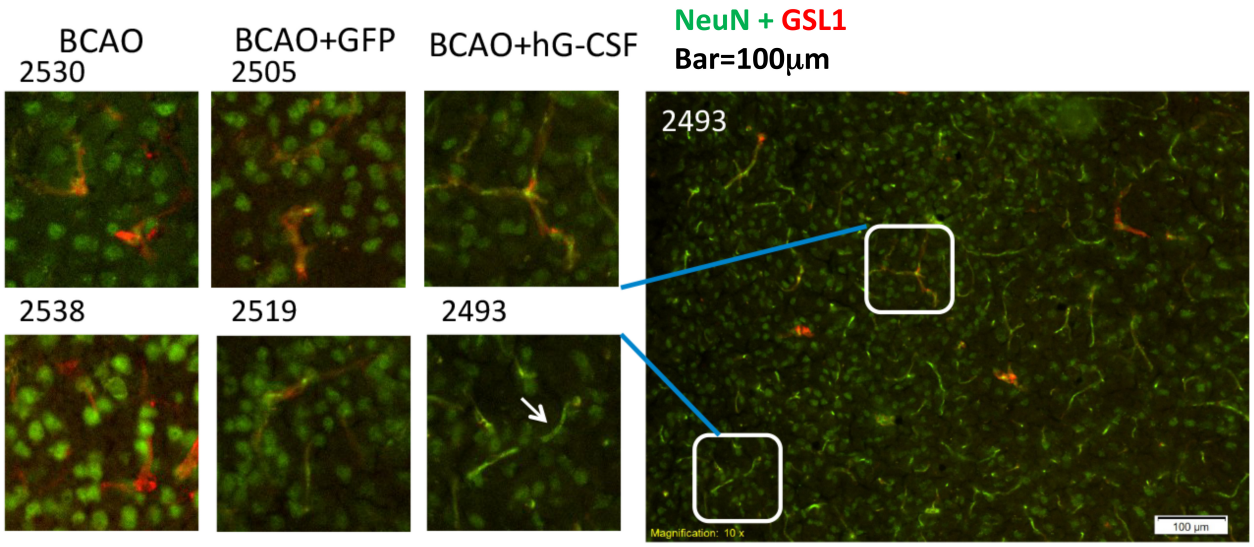


Figure 2. Chronic expousre to amphetamine induced neuroprogenitor cells in ROI identified for elevated HDAC5 mRNA expression using SPION-miD2861 and MCE-MRI (Fig 2A). We validate neuroprogenitor cells using co-expresion of NeuN and GFAP (Fig 2C & 2D). In mature adult brain GFAP (red) and NeuN (green) do no co-expressed in neural cells (Fig 2B).