Neurobiological mechanisms of hnRNP H1 in methamphetamine addictive behaviors

Neema Yazdani[1,2], Eric R. Reed[5], Qiu T. Ruan[2], Michael Chau[1], Farzad Mortazavi[3], Douglas Rosene[3], W. Evan Johnson[4], Camron D. Bryant[1]

[1] Laboratory of Addiction Genetics, Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, 72 E. Concord St, L-606C, Boston, MA 02118

[2] NIGMS Ph.D. Program in Biomolecular Pharmacology, Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA 02118

[3] Department of Anatomy & Neurobiology, Boston University School of Medicine, 72 E. Concord Street, L-1004, Boston, MA 02118

[4] Department of Medicine, Division of Computational Biomedicine, Boston University School of Medicine, 72 E. Concord Street, E-645, Boston, MA 02118, [5] Ph.D. Program in Bioinformatics, Boston University, 44 Cummington Mall, Boston, MA 02215

BU TTPAS Trans-disciplinary Training Program in Addiction Science

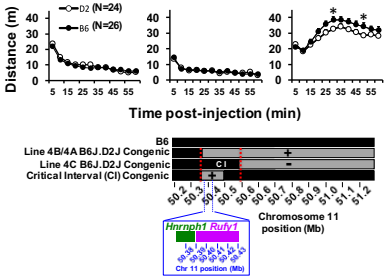
ABSTRACT

Using fine mapping and gene editing, we recently identified Hnrnph1 (heterogeneous nuclear ribonucleoprotein H1) as a quantitative trait gene for the locomotor stimulant properties of methamphetamine (MA) (PLOS Genetics, 1(12):e1005713). To extend the contribution of Hnrnph1 to MA reward, we conducted conditioned place preference (CPP) at multiple doses, which consisted of one day of baseline preference assessment, four alternating saline and MA training days on blunt or sharp floor contexts, two consolidation days, and assessment of MA drug preference on Day 8. According to Day 8 vs. Day 1, Hnrnph1$^{+/-}$ mice were significantly less sensitive to the rewarding properties of MA at 0.5 mg/kg (i.p.). Transcriptome analysis of Hnrnph1$^{+/-}$ striatal tissue (100 bp paired-end sequencing) followed by Ingenuity Pathway Analysis (IPA) revealed perturbations in EIF2 signaling (global protein translation), PKA signaling, and axon guidance signaling in neurons, among others. Spliceome analysis followed by IPA revealed perturbations in GABA receptor, glutamate receptor and alpha-adrenergic signaling. Diseases and biofunctions analysis for both datasets ranked neurological disorders as a top category which clustered genes into functional annotation groups including "movement disorders", "disorder of basal ganglia", and "degeneration of neurons." Currently, we are conducting a thorough immunohistochemical (IHC) assessment of hnRNP H throughout adult WT B6 and Hnrnph1$^{+/-}$ brains. Preliminary results illustrate pan-neuronal expression of hnRNP H and exclusion in glia. There was also a trend for an increase in striatal glia in Hnrnph1$^{+/-}$ brains. Thus far, we hypothesize hnRNP H1 to be vital in the development and function of midbrain dopaminergic neurons and glia, which is necessary for the rewarding and stimulant properties of psychostimulants.

BACKGROUND

LOCOMOTOR ACTIVITY PROTOCOL

DAY 1	DAY 2	DAY 3
Saline	Saline	2 mg/kg Meth

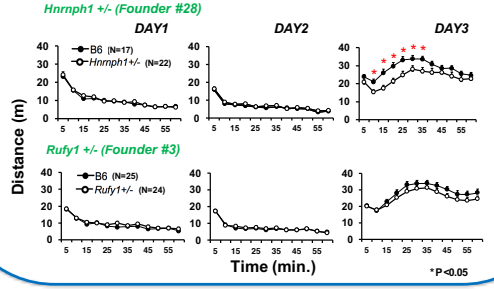


- Using fine-mapping in B6xD2 congenic lines, *Hnrnph1* and *Rufy1* were identified as candidate quantitative trait genes for day 3 methamphetamine (MA) sensitivity (Yazdani et al., *PLOS Genetics*, 2015)
- (+) → Displays reduced MA sensitivity
- (-) → Does not display phenotype

Left TAL Effector DBD — FokI Cleavage zone — Right TAL Effector DBD

WT *Hnrnph1*: CG*TGGTGAAGGTCCGGGGC*TTGCCCTGGTCCTGC*TCCGCGGATGAAGTGCA*GC
Founder #22: CG*TGGTGAAGGTCCGGGGC*T----------TGC*TCCGCGGATGAAGTGCA*GC
Founder #28: CG*TGGTGAAGGTCCGGGGC*TT----------*GCGGATGAAGTGCA*GC

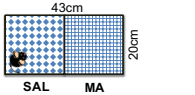
WT *Rufy1*: CGTGACCTAACAACATGGCCGCCCGCGGAGAATGCCGCCGGGCGGGGCAGGATT
Founder #3: CGTGACCTAACAACATGGCCGCCCGC----------GGCGGGGCAGGATT



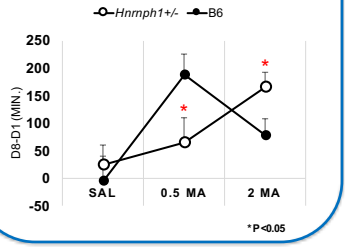
CONDITIONED REWARD

CONDITIONED PLACE PREFERENCE PROTOCOL

DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7	DAY8	DAY9
SAL	SAL	MA	SAL	MA	Home cage	Home cage	SAL	MA
OPEN	TRAIN	TRAIN	TRAIN	TRAIN			OPEN	OPEN



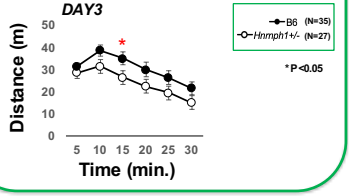
SAL (0)	0.5mg/kg MA	2mg/kg MA
B6 (N=23)	B6 (N=23)	B6 (N=19)
Hnrnph1+/- (N=24)	Hnrnph1+/- (N=22)	Hnrnph1+/- (N=16)



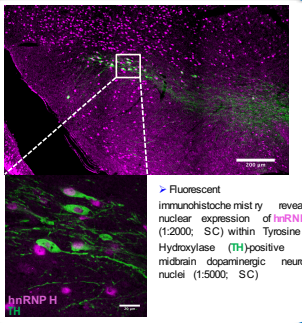
OPIOID SENSITIVITY

LOCOMOTOR ACTIVITY PROTOCOL

DAY 1	DAY 2	DAY 3
Saline	Saline	0.2 mg/kg FENT



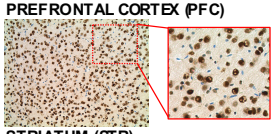
hnRNP H IN THE MIDBRAIN



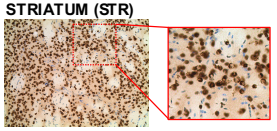
- Fluorescent immunohistochemistry reveals nuclear expression of hnRNP H (1:2000; SC) within Tyrosine Hydroxylase (TH)-positive midbrain dopaminergic neuron nuclei (1:5000; SC)

hnRNP H IN THE BRAIN

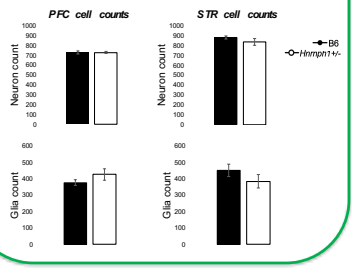
PREFRONTAL CORTEX (PFC)



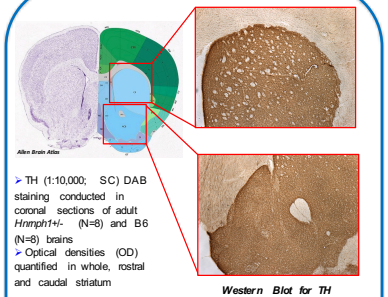
STRIATUM (STR)



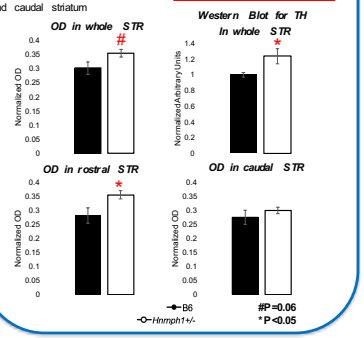
- 3,3'-Diaminobenzidine (DAB) staining for hnRNP H (1:50,000; Bethyl Labs) in coronal sections of adult *Hnrnph1+/-* (N=8) and B6 (N=8) brains
- Protein is expressed in most if not all neurons & absent in glia



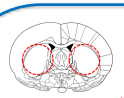
TYROSINE HYDROXYLASE



- TH (1:10,000; SC) DAB staining conducted in coronal sections of adult *Hnrnph1+/-* (N=8) and B6 (N=8) brains
- Optical densities (OD) quantified in whole, rostral and caudal striatum



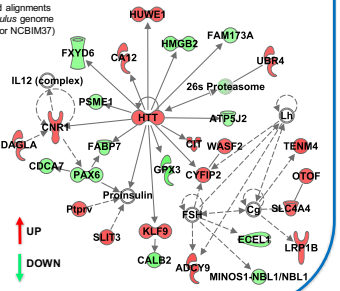
RNA-SEQ: PATHWAY ANALYSIS



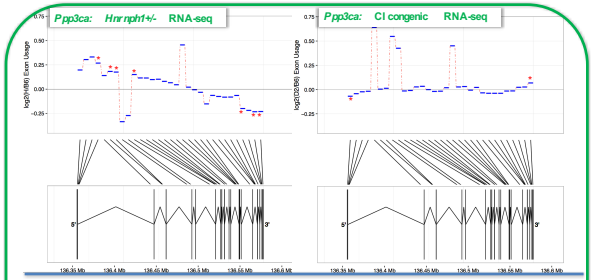
Hnrnph1+/- & C57BL/6J dissections → Striatum RNA extraction → Poly-A cDNA amplification & fragmentation → 100bp paired-end adaptor library generation → RNA-seq pipeline: Illumina HiSeq2500 → FASTQC & FASTX Trimmer → TopHat for read alignments with *Mus musculus* genome (UCSC, mm10 or NCBIM37) → HTSeq & LIMMA for differential gene expression & splice variant analysis

- 179 downregulated and 189 upregulated genes (FDR<0.05; FC>1.2)
- Top network from Ingenuity Pathway analysis (IPA): "Cellular Development, Cellular Growth & Proliferation, Nervous System Development & Function" (Score=44)
- Top canonical pathways include: EIF2, Neuropathic Pain, Calcium, and Corticotropin Releasing Hormone signaling (P<4xE^06)

- Top Diseases & Bio Functions:
  - **Neurological & Psychiatric Disorders**: movement disorders, Schizophrenia, Parkinson's Disease, Anxiety, Depression, Drug Dependence (Opioid & Alcohol), etc.
  - **Cell Morphology**: neuron growth & morphology
  - **Nervous Development & Function**: synaptic transmission, quantity of neurons, excitatory postsynaptic potential, etc.



RNA-SEQ: SPLICE VARIANT ANALYSIS



- *Pppc3a* (calcineurin catalytic subunit) is one of five alternatively spliced genes shared between *Hnrnph1+/-* mice and CI congenics
- SNPs affecting splicing of human *PPP3CA* is associated with addiction vulnerability as well as Alzheimer's disease (Chiocco et al., *Subst Use Misuse*. 2010)

CONCLUSIONS & FUTURE DIRECTIONS

- In addition to reduced sensitivity to the stimulant effects of MA, *Hnrnph1+/-* also display reduced sensitivity to the rewarding effects of MA in CPP.
- With respect to WT B6 littermates, *Hnrnph1+/-* mice do not display a significant reduction in FENT sensitivity at 0.2 mg/kg.
- hnRNP H appears to be expressed within the nuclei of most if not all neurons throughout the brain. Thionin counterstain reveals exclusion of glia.
- Through IHC and Western Blot we detected a significant increase in TH staining in the striatum of *Hnrnph1+/-* mice.
- Transcriptome and pathway analysis of *Hnrnph1+/-* striatal tissue suggests deficits in neuronal development/function, and groups differentially expressed genes in neurobiological and psychiatric categories.
- Future directions, in collaboration with **Dr. Karen Szumlinski** at UC Santa Barbara:
  - Striatal microdialysis of dopamine, GABA, norepinephrine, and glutamate
  - MA oral self-administration (almost complete!)

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