Hnrnph1 is a quantitative trait gene for methamphetamine sensitivity

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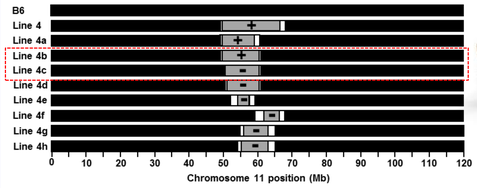
ABSTRACT

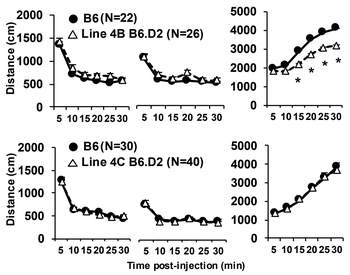
Sensitivity to the locomotor stimulant effects of amphetamines is a heritable trait in mice that may aid in our understanding of the genetic and neurobiological basis of neuropsychiatric disorders involving perturbations in dopaminergic transmission. We previously used short-term selected mouse lines derived from a C57BL/6J (B6) x DBA/2J (D2)-F_2 cross to identify a quantitative trait locus on chromosome 11 that was causally associated with reduced methamphetamine-induced locomotor activity (D2 < B6). We replicated this QTL in a standard B6 x D2-F_2 cross and used phenotypic analysis of interval specific congenic lines containing various D2-derived segments of chromosome 11 on an isogenic B6 background to uncover a 206 Kb critical interval containing only two protein-coding genes, *Rufy1* and *Hnrnph1*, that was necessary for reduced MA sensitivity. Here, we used transcription activator-like effector nucleases (TALENs) to induce small deletions in the first coding exon of *Rufy1* or *Hnrnph1*. Phenotypic analysis of replicate lines heterozygous for the *Hnrnph1* deletion (*Hnrnph1* hets) recapitulated the congenic phenotype while those heterozygous for the *Rufy1* deletion did not, thus identifying *Hnrnph1* as the quantitative trait gene. With regard to addiction-like phenotypes, *Hnrnph1* hets displayed increased MA-induced conditioned place preference (MA-CPP) relative to WT B6 littermates at the 2 mg/kg dose. Transcriptome analysis via mRNA sequencing of B6.D2 congenic (chr.11: 50-60 Mb) striatal tissue followed by pathway analysis revealed perturbations in "glutamate receptor signaling" and "GalphaQ signaling", and identified "Cellular development, nervous system development and function, behavior" as the top network. We hypothesize that *Hnrnph1* regulates neurodevelopment of the mesocorticolimbic circuitry, thereby affecting both dopaminergic neuron development and glutamate signaling, and hence the stimulant response to amphetamines. These results will likely have widespread implications for understanding the genetic and neurobiological bases of disorders comprising perturbations in dopamine neurotransmission, including addiction, schizophrenia, ADHD, OCD, and Parkinson's disease.

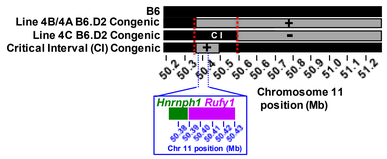
BACKGROUND



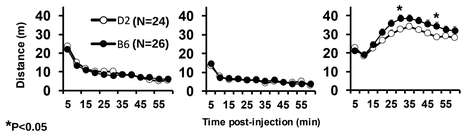
LOCOMOTOR ACTIVITY PROTOCOL

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





CI Congenics



*P<0.05

➢***Congenic backcrossing (Line 4a-4h) and behavioral assessments were conducted in Dr. Abraham Palmer's lab by Dr. Camron Bryant at the University of Chicago.***

➢From back-crossing to the C57BL/6J (**B6**) background strain, a 206 Kb critical interval was deduced.

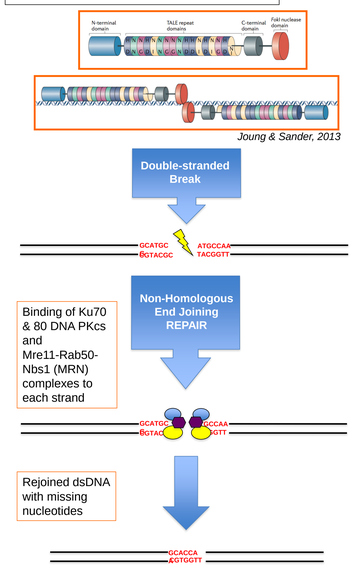
➢**(+)** = captures QTL for decreased MA-induced locomotor activity

➢**(-)**= fails to capture QTL

➢Critical interval (**CI**) congenic possesses a mere 112kb DBA/2J (**D2**) interval that includes only *Hnrnph1* and *Rufy1*. CI congenics homozygous for this D2 interval display decreased MA sensitivity compared to WT B6 controls.

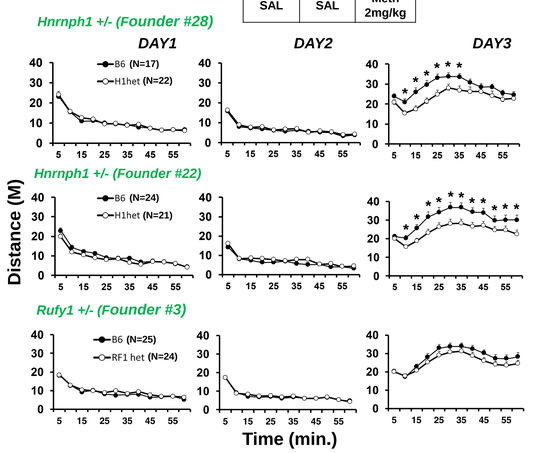
TALENs KNOCK-OUT MICE

How do TALENs work?

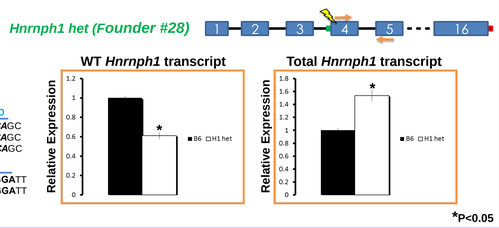


Locomotor Activity Assessments

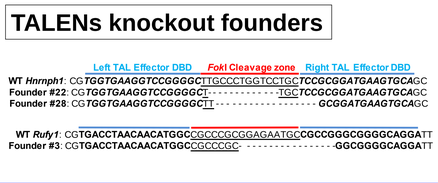
DAY1	DAY2	DAY3
SAL	SAL	Meth 2mg/kg



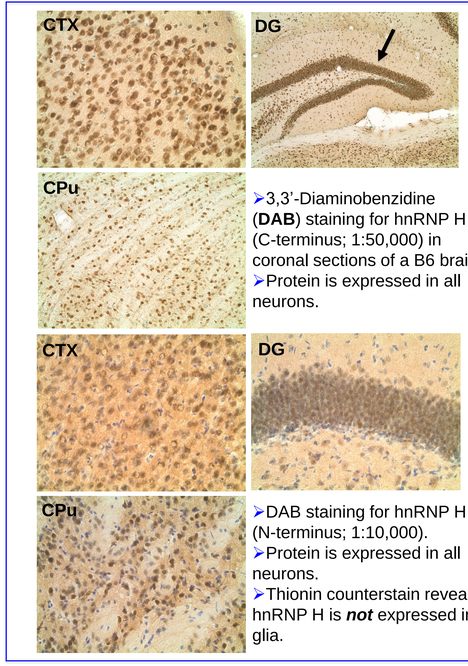
Hnrnph1 het qPCR Analysis



TALENs knockout founders



hnRNP H IN THE BRAIN



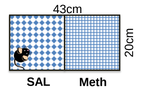
➢3,3'-Diaminobenzidine (**DAB**) staining for hnRNP H (C-terminus; 1:50,000) in coronal sections of a B6 brain.
➢Protein is expressed in all neurons.

➢DAB staining for hnRNP H (N-terminus; 1:10,000).
➢Protein is expressed in all neurons.
➢Thionin counterstain reveals hnRNP H is ***not*** expressed in glia.

CONDITIONED REWARD

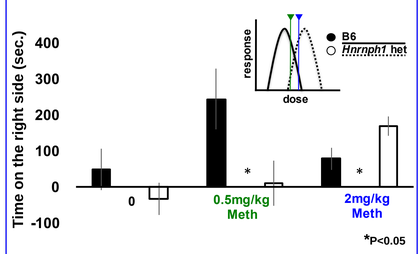
CONDITIONED PLACE PREFERENCE PROTOCOL

DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7	DAY8	DAY9
SAL	SAL	Meth	SAL	Meth	Home cage	Home cage	SAL	Meth
OPEN	CLOSED	CLOSED	CLOSED	CLOSED			OPEN	OPEN



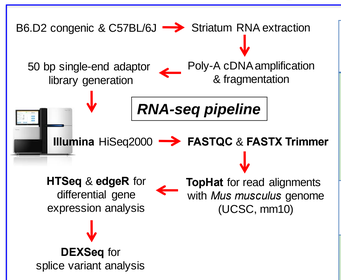
Hnrnph1 het (Founder #28)

SAL (0)	0.5mg/kg Meth	2mg/kg Meth
B6 (N=15)	B6 (N=14)	B6 (N=19)
H1het (N=16)	H1het (N=16)	H1het (N=15)



➢*Hnrnph1* hets are less sensitive to the rewarding properties of MA, shifting their MA-CPP dose-response curve to the right.

RNA-SEQ: PATHWAY ANALYSIS

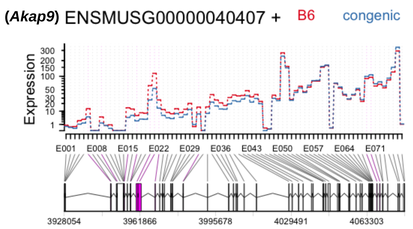


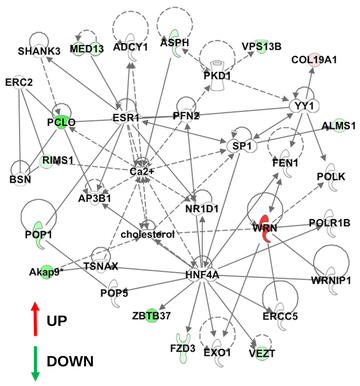
➢Top network from Ingenuity Pathway analysis (IPA): "Cellular development, nervous system development and function, behavior."
➢Disease & functions analysis suggests deficits in neural development (i.e. dopaminergic neurons), morphology, and electrophysiological properties.
➢Predicted perturbations in the dopaminergic & glutamatergic systems.
➢Down-regulated genes in the dopaminergic system: ***Nr4a2, Bdnf, Adra1b, Adra1d***
➢Down-regulated genes in the glutamatergic system: ***Slc17a6, Slc17a7, Gng2, Gria4***

Diseases or Functions Annotation	P-Value	Predicted Activation State	Activation z-score	Genes	#
Movement Disorders	***4.46E-09***	***Increased***	***2.199***	***ADRA1B,ADRA1D,BAIAP2,BDNF,CBLN1,CHRM1,CPNE5,CRYM,ELAVL2,GABRA3,GJC2,KCNC2,NR4A2,OXR1,PDE1B,PPARGC1A,PTPN3,RGS14,SERPINI1,SEZ6,SLC14A1,SLC17A7,SLC26A4,TBR1***	***24***
coordination	***6.76E-09***	***Decreased***	***-2.375***	***ADRA1D,BDNF,CADPS2,CBLN1,ELAVL4,GJC2,NR4A2,NXPH3,SEZ6,SLC17A7,SLC26A4***	***11***
excitatory postsynaptic potential	6.52E-08			BDNF,CADPS2,CHRM1,CPLX3,GRIA4,NRN1,SEZ6,SLC17A7	8
morphology of neurites	1.07E-04			BDNF,CADPS2,GFRA2,NRN1,SEZ6,SLC17A7	6
concentration of dopamine	***8.05E-05***	***Decreased***	***-2.041***	***ADRA1B,ADRA1D,BDNF,CHRM1,NR4A2***	***5***
abnormal morphology of nervous system	5.72E-05			BDNF,CADPS2,CBLN1,CHRM1,GFRA2,NEUROD6,NR4A2,NRN1,PPARGC1A,SEZ6,SLC17A6,SLC17A7,SLC26A4,TBR1	14
differentiation of dopaminergic neurons	4.11E-03			BDNF,NR4A2	2
Neurodegeneration of dopaminergic neurons	4.11E-03			BDNF,NR4A2	2
action potential of dopaminergic neurons	4.37E-03			BDNF	1

RNA-SEQ: SPLICE VARIANT ANALYSIS

(***Akap9***) ENSMUSG00000040407 + B6 congenic





➢List of alternatively spliced exons (line 4a B6.D2 congenic) from DEXSeq was run through RBPmap to determine hnRNP H1 targets (79/230 exons)
➢Top network from IPA analysis of the hnRNP H1 target list: "Cell-to-cell signaling and interaction, nervous system development and function, gene expression."

CONCLUSIONS & FUTURE DIRECTIONS

➢*Hnrnph1* hets present a B6.D2-like decrease in MA-induced locomotor activity, while *Rufy1* hets do not. Findings, suggest *Hnrnph1* is the QTG responsible for differential MA sensitivity in mice.

➢*Hnrnph1* hets present reduced sensitivity to the rewarding properties of MA in the conditioned place preference assessment.

➢hnRNP H appears to be expressed in neurons throughout the brain. Thionin counterstain reveals exclusion of glia.

➢In analyzing the top differentially expressed genes in the striatum of line 4a B6.D2 congenics, down-regulated genes in the dopaminergic system are of particular interest, since differential expression is predicted to result in deficits in dopaminergic neuron development and function.

➢DEXSeq splice variant analysis and hnRNP H1 target analysis reveal a list of genes involved in nervous system development and function, and gene expression.

➢Future directions:
- ✧TH staining of midbrain dopaminergic neurons projecting to striatum
- ✧Striatal microdialysis of dopamine, GABA, norepinephrine, and glutamate
- ✧MA oral self-administration
- ✧Behavioral testing with other drugs of abuse such as opiates

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