Hnrnph1 is a quantitative trait gene for methamphetamine sensitivity

Neema Yazdani1,2, Clarissa C. Parker3,4, Ying Shen5, Michael A. Guido4, Loren A. Kole3, Stacey L. Kirkpatrick1, Jackie E. Lim2, Greta Sokoloff3,6, Riyan Cheng3,7, W. Evan Johnson5, Abraham A. Palmer4, Camron D. Bryant1

1 Laboratory of Addiction Genetics, Department of Pharmacology and Experimental Therapeutics and Department of Psychiatry, Boston University School of Medicine (BUSM),
2 NIGMS Ph.D. Program in Biomolecular Pharmacology, Department of Pharmacology and Experimental Therapeutics, BUSM, 3 Department of Human Genetics, The University of Chicago (UCH), 4 Department of Psychology, Middlebury College, 5 Department of Medicine, Division of Computational Biomimicry, BUSM, 6 Current address: Department of Pharmacology, University of Iowa, 7 Current address: Plant Sciences, Research School of Biology, Australian National University,
8 Department of Human Genetics and Department of Behavioral Neuroscience, UCH

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) F2 F0 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 F2 F0 cross and used phenotypic analysis of interval specific congenic lines containing various D2-derived segments of chromosome 11 on an inbred B6 background to uncover a 206 kb critical interval containing only two protein-coding genes, Ruly1 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 Ruly1 or Hnrnph1. Phenotypic analysis of replicate lines heterozygous for the Hnrnph1 deletion (ahntrhph1) recapitulated the congenic phenotype while those heterozygous for the Ruly1 deletion did not, thus identifying Hnrnph1 as the quantitative trait gene. With regard to the functional properties of Hnrnph1, ahntrhph1 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 revealed perturbations in ‘glutamate receptor signaling’ and ‘G protein signaling’, and identified ‘cellular development, nervous system development, integrative behavior’ as the top network. We hypothesize that Hnrnph1 regulates neurodevelopment of the mesoconctolimbic 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 dopaminergic neurotransmission, including addiction, schizophrenia, ADHD, OCD, and Parkinson’s disease.

BACKGROUND

Locomotor Activity Assessments

How do TALENs work?

Locomotor Activity Assessments

Hnrnph1 het qPCR Analysis

Localumotor Activity Protocol

Strain
cage

Locomotor Activity Protocol

How do TALENs work?

Conditioned Reward

TALENs Knockout Mice

DNA

DNA

Hnrnph1 het (Founder #20)

Hnrnph1 het (Founder #20)

WT Hnrnph1 transcript

Total Hnrnph1 transcript

RNA-Seq: SPLICE VARIANT ANALYSIS

RNA-Seq: SPLICE VARIANT ANALYSIS

RNA-Seq: PATHWAY ANALYSIS

RNA-Seq: PATHWAY ANALYSIS

FUNDING: R00 DA029635, R01 DA039168, T32 GM008541-18, Burroughs Wellcome Fund

CONCLUSIONS & FUTURE DIRECTIONS

CONCLUSIONS & FUTURE DIRECTIONS

Hnrnph1 hets present a B6.D2-like decrease in MA-induced locomotor activity, while Ruly1 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.

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.

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

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

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

List of alternatively spliced exons (line 4a B6.D2 congenic) from DESeq was run through RBMap to determine Hnrnph1 H1 target (76325 exons).

Top network from IPA analysis of the hnrnph1 h1 target list: ‘Call-to-cell signaling and interaction, nervous system development and function, gene expression.’

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

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.

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

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

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

List of alternatively spliced exons (line 4a B6.D2 congenic) from DESeq was run through RBMap to determine Hnrnph1 H1 target (76325 exons).

Top network from IPA analysis of the hnrnph1 h1 target list: ‘Call-to-cell signaling and interaction, nervous system development and function, gene expression.’

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

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.

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

List of alternatively spliced exons (line 4a B6.D2 congenic) from DESeq was run through RBMap to determine Hnrnph1 H1 target (76325 exons).

Top network from IPA analysis of the hnrnph1 h1 target list: ‘Call-to-cell signaling and interaction, nervous system development and function, gene expression.’

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

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.

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

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

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

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

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

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