HHS Public Access

Author manuscript

Drug Alcohol Depend. Author manuscript; available in PMC 2022 March 01.

Published in final edited form as:

Drug Alcohol Depend. 2021 March 01; 220: 108518. doi:10.1016/j.drugalcdep.2021.108518.

Hnrnph1 IS A NOVEL REGULATOR OF ALCOHOL REWARD

Elissa K. Fultz, B.S.[1], **Michal A. Coelho, B.S.**[1], **Dylan Lieberman, B.S.**[1], **C. Leonardo Jimenez-Chavez, M.S.**[1], **Camron D. Bryant, Ph.D.**[2], **Karen K. Szumlinski, Ph.D.**[1,3]

[1]Department of Psychological Brain Sciences, University of California, Santa Barbara

[2]Laboratory of Addiction Genetics, Department of Pharmacology and Experimental Therapeutics and Psychiatry, Boston University School of Medicine

[3]Department of Molecular, Developmental and Cellular Biology and the Neuroscience Research Institute, University of California, Santa Barbara

Abstract

Background: *Hnrnph1* is a validated quantitative trait gene for methamphetamine behavioral sensitivity that encodes for heterogeneous nuclear ribonucleoprotein H1 (hnRNP H1). This RNA-binding protein is involved in all stages of RNA metabolism that impacts mesocorticolimbic dopamine neurotransmission to influence addiction-related behavior.

Methods: We characterized the alcohol behavioral phenotypes of mice heterozygous for a deletion in the first coding exon of *Hnrnph1* (*Hnrnph1+/−*). We examined alcohol intake under both continuous- and limited-access procedures, as well as alcohol-induced place-conditioning. Follow-up studies examined genotypic differences in the psychomotor-activating and sedative-hypnotic effects of acute and repeated alcohol, and a behavioral test battery was employed to determine the effects of *Hnrnph1* deletion on the manifestation of negative affect during alcohol withdrawal.

Results: Relative to wild-type (WT) controls, *Hnrnph1+/−* males exhibited blunted intake of high alcohol concentrations under both drinking procedures. *Hnrnph1* deletion did not impact the conditioned rewarding properties of low-dose alcohol, but reversed the conditioned place-aversion elicited by higher alcohol doses (2 and 4 g/kg), with more robust effects in male versus female mice. No genotypic differences were observed for alcohol-induced locomotor activity. *Hnrnph1+/−* mice exhibited a modest increase in sensitivity to alcohol's sedative-hypnotic effects, but did not differ from WT mice with regard to tolerance to alcohol's sedative-hypnotic effects or alcohol

Correspondance: Karen K. Szumlinski, Ph.D. Department of Psychological and Brain Sciences MC 9660 University of California Santa Barbara Santa Barbara, CA 93106-9660, szumlinski@ucsb.edu.

Contributors

EFK, MAC, DL, CLJC and KKS conducted the experiments; CLJC and KKS analyzed the data; KKS supervised the research and graphically depicted the data; EFK composed the initial manuscript draft; KKS and CDB revised the manuscript; all authors contributed to the final editing of the manuscript prior to submission.

Conflict of Interest

Nothing declared

metabolism, Inconsistent effects of *Hnrnph1* deletion were observed in models for withdrawal-induced negative affect.

Conclusions: These data identify *Hnrnph1* as a novel, male-selective, driver of alcohol consumption and high-dose alcohol aversion that is potentially relevant to the neurobiology of alcohol abuse and alcoholism.

Keywords

hnRNP H1; binge-drinking; place-preference; intoxication; negative affect; ethanol; dysphoria

1. INTRODUCTION

hnRNP H1 (heterogenous nuclear ribonucleoprotein H1) is an RNA-binding protein (RBP) that is ubiquitously expressed in brain (Lein et al., 2007) and can regulate all aspects of RNA metabolism, including pre-mRNA splicing through binding at specific intron sites, mRNA stability, and translational regulation via 5'UTR and 3'UTR binding and poly-adenylation control (e.g., Han et al., 2010; Dreyfuss et al., 2002; Schaub et al., 2007). hnRNP H family proteins (including hnRNP H1) are considered critical regulators of neuron and oligodendrocyte differentiation (Aranburu et al., 2006; Tiruchiinapalli et al., 2008). Congenic mice harboring *Hnrnph1* polymorphisms associated with decreased methamphetamine sensitivity express a set of down-regulated genes involved in neurodevelopment, including a 1.5-fold decrease in the transcription factor *Nurr1/Nr4a2* (Yazdani et al., 2015). While RBPs, including hnRNP H1, are localized to the nucleus, exposure to extracellular stimuli (e.g., stressors, neuronal activity, drugs) can cause their translocation to cytoplasm where they can be positioned to regulate local translation underlying activity-dependent synaptic plasticity (Fukuda et al., 2009; Guil et al., 2006; Markmiller et al., 2018; Wall et al., 2020; Zhang et al., 2012).

As reviewed elsewhere (Bryant and Yazdani, 2016), there is a growing appreciation that RBPs play a pivotal role in addiction-related synaptic plasticity. Using an unbiased, forward genetic and fine mapping approach, we positionally cloned and validated *Hnrnph1* as a quantitative trait gene underlying sensitivity to the locomotor stimulant response to methamphetamine (Yazdani et al., 2015) that we subsequently showed is likely mediated by a set of four, 5' UTR variants that cause decreased 5' UTR usage and decreased hnRNP H protein expression (Ruan et al., 2020b). Homozygous deletion of *Hnrnph1* is lethal (Yazdani et al., 2015), however, we subsequently showed that a heterozygous mutation in the first coding exon of *Hnrnph1* also decreased the rewarding and reinforcing properties of methamphetamine (Ruan et al., 2020a). *Hnrnph1* contributes to post-transcriptional processing of *OPRM1,* including translational repression (Song et al., 2012) and splicing (Xu et al., 2014). *OPRM1* encodes the mu opioid receptor, which is the primary molecular target underlying the addictive and analgesic properties of opioid drugs (e.g., Matthes et al., 1996). Supporting a potential role for *Hnrnph1* in substance use disorders, an intronic variant in *HNRNPH1* was associated with the severity of heroin dependence and differential splicing of *OPRM1* in humans (Xu et al., 2014). Further, mice with a small frameshift deletion within the first coding exon of *Hnrnph1* (*Hnrnph1+/*) (Yazdani et al., 2015) self-administer less fentanyl than their wild-type (WT) counterparts, independent of any

observable effects of gene deletion on fentanyl-induced antinociception or physiological dependence (Bryant et al., 2020).

Decades of evidence from both human and laboratory animal studies implicate *OPRM1* polymorphisms in the etiology and treatment prognosis of alcoholism (see Berrentini 2016 for review). Moreover, a survey of 17 proteomic studies indicate an association between alcohol exposure and an increase in *Hnrnph* expression in the brains of laboratory rodents (Wang et al. 2011). To the best of our knowledge, the functional relevance of hnRNP H1 in alcohol drinking and dependence is unexplored. Thus, the present study characterized the effect of a heterozygous *Hnrnph1* deletion on AUD-related behaviors. Both female and male *Hnrnph1+/−* mice showed reduced sensitivity to the locomotor stimulant response to methamphetamine (Yazdani et al., 2015), while only *Hnrnph1+/−* females showed reduced fentanyl-induced locomotion (Bryant et al., 2020). Thus, we compared *Hnrnpnh1*+/− versus +/+ mice of both sexes for alcohol-induced locomotor activity and sedative-hypnotic effects. Prior co-administration studies indicated common neural adaptations contribute to both methamphetamine and alcohol intake and conditioned reward (Fultz et al., 2017; Fultz and Szumlinski, 2019; Sern et al., 2020). Thus, we also tested for genotypic differences in alcohol consumption and conditioned reward. Finally, as the severity of alcohol withdrawal correlates with the motivation to drink, we compared *Hnrnpnh1*+/− versus *+/+* mice in behavioral models for alcohol withdrawal-induced negative affect. These results identify select, sometimes sexually dimorphic, alcohol behavioral phenotypes that were modified by acute and repeated alcohol exposure in *Hnrnph1* mutant mice.

2. MATERIALS AND METHODS

2.1 Subjects.

Hnrnph1+/− and their wild-type (WT; +/+) littermates were originally generated on an isogenic C57BL/6J background using TALENs targeting the first coding exon which induced a small deletion and frameshift mutation resulting in a premature stop codon (Yazdani et al., 2015). *Hnrnph1*+/− mice were maintained at UCSB by mating *Hnrnph1+/−* males from a colony established at UC Santa Barbara with C57BL/6J females purchased from The Jackson Laboratory (Sacramento, CA). At weaning, offspring were housed with same-sex littermates (a minimum of 2 mice per cage) and genotyped as detailed below. Behavioral testing commenced no earlier than PND 50, and the mice ranged in age between PND50 and PND100, with a vast majority of mice aged PND56–70 at the start of testing. At least one week prior to commencement of experimental testing, mice involved in the place-conditioning or withdrawal-induced anxiety studies were relocated to a colony room maintained on a 12:12 h light–dark cycle (lights on at 0700 h), while those involved in the alcohol-drinking studies were relocated to a colony room maintained on a 12:12 h reverse light cycle (lights off: 1000 h). Food and water were available *ad libitum* in the home cage. All procedures were approved by the UC Santa Barbara Animal Care and Use Committee and were conducted in strict accordance with National Institute of Health guidelines for the care and use of laboratory animals.

2.2 Genotyping.

Genomic DNA was extracted from tail clips obtained upon weaning and used in a PCR reaction with primers amplifying approximately 100 base pairs upstream and downstream of the TALENs binding domains, as detailed in prior reports (Bryant et al., 2020; Ruan et al., 2018,2020; Yazdani et al., 2015). After PCR, samples were mixed with a restriction enzyme cocktail overnight (BstNI), run on a 2% ethidium bromide Tris-borate-EDTA gel for 1.2 hrs, and imaged with ultraviolet light. TALENs-edited mice were identified by bands that were uncut by the restriction enzyme due to the loss of the restriction enzyme binding site (Yazdani et al., 2015).

2.3 Alcohol Drinking Procedures:

Female and male (n=8/genotype) mice were single-housed under a 12-h reverse cycle (lights off: 1000 h) for at least 7 days prior to commencing alcohol drinking procedures, which began with an examination for genotypic differences in alcohol intake under continuous-access, followed by limited-access, conditions in the same mice. For the continuous-access procedure, mice were presented in the home cage with 4 sipper tubes containing 0, 5, 10 or 20% alcohol (v/v) for 14 consecutive days. This continuous-access procedure was employed by our group previously to generate a within-subjects dose-response function for alcohol intake and preferences (e.g., Lominac et al., 2006). Bottles were weighed daily at the same time each day and the volume consumed from each sipper tube was calculated to determine intake (expressed as a function of body weight, determined weekly). Then, following a 3-day respite, the same mice underwent testing for alcohol intake under limited-access conditions. For the limited-access procedure, we employed our 4-bottle-choice version of the Drinking-in-the-Dark (DID) paradigm (e.g., Cozzoli et al., 2014; Lee et al., 2015, 2016), in which mice were presented with 4 sipper tubes containing 5, 10, 20 and 40% alcohol (v/v) for 2 h/day, beginning at 3 h into the dark phase of the circadian cycle. Limited-access drinking procedures were conducted for 10 consecutive days. Alcohol intake was determined immediately at the end of each 2-h session as described for the continuous-access procedures.

2.4 Alcohol-induced place-conditioning:

A separate cohort of experimentally naïve female and male mice (n>8/genotype) underwent alcohol-induced place-conditioning procedures to determine how *Hnrnph1* deletion impacts the motivational valence of alcohol. The apparatus and procedures employed were similar to those described previously (e.g., Ary et al., 2012; Szumlinski et al., 2008). In brief, an unbiased place-conditioning procedure involving 8 pairings of alcohol (0.5, 1.0, 1.5, 2 or 4 g/kg) was conducted, with one compartment of a 2-compartment apparatus that differed in wall pattern (marbled vs. wood-paneled) and floor texture (rough vs. smooth). Random counterbalancing of the alcohol-paired side assignment was employed, irrespective of initial side preference. Conditioning commenced with a pre-conditioning test (PreTest) in which mice were allowed to explore both compartments for 15 min. This PreTest was conducted mid-day (around 1200 h) and mice were returned to the colony room. Then, in the morning (between 0900–1100 h), mice were injected IP with saline (vol=0.2 ml/10 g) and confined to one of the compartments for 15 min, with animals randomly assigned to the saline-

conditioned compartment. In the afternoon (between 1630–1830 h), mice were injected IP with their assigned dose of alcohol and confined to the opposite compartment. Following 8 conditioning days, mice were tested for preference for the alcohol-paired compartment in the absence of any injection and the total time spent in the alcohol- versus saline-paired side (CPP Score) served to index the motivational valence of alcohol (Post-Test). Similar to the PreTest, the Post-Test was conducted mid-day, approximately 18 h following the last alcohol- conditioning session. The locomotor activity of the mice was recorded during each of the alcohol-conditioning sessions to index drug-induced psychomotor activity and changes in psychomotor activity with repeated alcohol treatment. Both the time spent in the two compartments and the distance traveled during conditioning were tracked using AnyMaze™ tracking software (Stoelting Co., Wood Dale, IL, USA).

2.5 Alcohol withdrawal-induced negative affect:

To examine the possibility that genotypic differences in the direction of the alcohol-conditioned response under high-dose alcohol place-conditioning procedures might reflect differential sensitivity to withdrawal-induced anxiety, another separate cohort of experimentally naïve female and male mice were injected IP, once daily, with 4 g/kg alcohol for a total of 8 days of injections (to mimic the place-conditioning injection regimen). Alcohol was injected in this study to control for the precise amount of alcohol exposure and to avoid the interpretational confounds associated with genotypic differences in alcohol drinking (see Results). The day following the last injection, mice were then tested for alcohol withdrawal-induced anxiety using a behavioral test battery consisting of light-dark shuttle-box, marble-burying and forced swim tests. These paradigms were selected as they are pharmacologically-validated models for negative affect and are consistently sensitive to the negative affective state produced by withdrawal from alcohol drinking in C57BL/6J mice (e.g., Lee et al., 2016, 2017a,b; 2018a,b). Recently, we observed inconsistent effects of *Hnrnph1* deletion on indices of anxiety-like behavior expressed by male mice only (Bryant et al., 2020; but see Ruan et al., 2020). Thus, a subset of alcohol-naïve *Hnrnph1+/+* and *Hnrnph1−/−* mice were included to further examine the potential genotype by sex interaction in basal affective state.

The light/dark shuttle box test indexes anxiety-like behaviors (Bourin & Hascoet, 2003; Crawley, 1985) and involves placing mice into a polycarbonate box (46cm long×24cm high×22cm wide), which is equally subdivided into a white, uncovered compartment and a black, covered compartment, separated by a central divider with an opening. Testing began with the mice on the dark side and the latency to enter the light side, number of light-side entries, and total time spent in the light side of the shuttle box were recorded during the 15-min trial using Any-maze™ tracking software (Stoelting Co., Wood Dale, IL).

The marble-burying test provides an additional index for anxiety-like behavior (Nicolas et al., 2006). Here, we placed 10 square glass pieces (2.5 cm2 × 1.25 cm tall) in the animals' home cage, 5 at each end. The total number of marbles buried by at least 75% (i.e., at least ¾ of the marble was covered by bedding) at the end of the 20-min trial was recorded and video-recordings during the 20-min session were scored for the latency to begin burying and

the total time spent burying by experimenters, who were blinded to the treatment of the mice.

Behavioral testing ended in the Porsolt forced swim test, in which each mouse was placed into an 11-cm diameter cylindrical container and the latency to first exhibit immobility (defined as no horizontal or vertical displacement of the animal's center of gravity for ≥ 5 s), total time spent immobile, and the numbers of immobile episodes were monitored throughout the entire 6-min trial period using AnyMaze™ tracking software. All testing for negative affect was conducted during the animals' circadian light phase.

2.6 Alcohol-induced intoxication and sedation.

In another cohort of alcohol-naïve mice, genotypic differences in the intoxicating and sedative properties of alcohol were assayed, respectively, using rotarod and regain of righting reflex procedures. The rotarod procedures were similar to those employed previously to examine genotypic differences in basal motor coordination (Ruan et al., 2020a) and commenced with successive training of mice to walk on a fixed speed (10 rpm) rotarod for 2 min. The following day, mice were tested for baseline rotarod performance over a 3-min period and then were injected with either 2 or 3 g/kg alcohol (the doses were reported previously by our group to induce motor in-coordination in mice; see Quadir et al., 2016, 2017) and, 15 min later, the average time to fall from the rotarod was determined in 3 successive 3-min tests. To examine the development of tolerance to alcohol's intoxicating effects, mice were injected with their assigned dose of alcohol once daily (~1100 h) for 8 injections (i.e., the same number of injections as those employed in the place-conditioning study). Then, mice were assayed again for alcohol-induced changes in rotarod performance using procedures identical to the test for alcohol's acute intoxicating effects. For the righting reflex study, a distinct cohort of alcohol-naïve female and male mice (n=7–9/sex/genotype) were injected acutely (~1100 h) with 4 g/kg and placed in an empty home cage. Upon observing the loss of righting reflex (defined as the inability to turn over and place all 4 paws on the floor of the cage; occurred within 1–2 min post-injection), mice were placed in a supine position and the latency to right themselves was determined using a stop-watch by an observer who was blinded to the genotype of the mice. The mice tested for righting reflex were then injected once daily (~1100 h), with 4 g/kg alcohol, followed by testing for withdrawal-induced anxiety as described earlier.

2.7 Alcohol pharmacokinetics:

To test for the potential relationship between genotypic differences in behavior and alcohol metabolism, mice were injected IP with 1.5 g/kg and blood was sampled from the submandibular vein at 5, 15, 30 and 60 min post-injection. Samples were analyzed by gas chromatography due to its effectiveness and accuracy in determining ethanol levels in various substances, including blood (Tiscione et al., 2011). Blood alcohol concentrations (BACs) were determined using a Shimadzu GC-2014 gas chromatography system (Shimadzu, Columbia, MD) and GC Solutions 2.10.00 software. Samples were diluted at 1:9 with non-bacteriostatic saline (50 μl of sample). Acetone and dichloromethane were used as the pre-solvents due to their lower boiling point versus ethanol. Each sample was tested within 1-week of blood collection to reduce the potential for alcohol evaporation during

storage. The determination of ethanol concentration from each sample was derived using the standard curve equation determined prior to analyses of the samples. A new standard curve was formulated for each cohort of blood samples to ensure maximal accuracy. After the ethanol peak area was determined, the peak area was used to determine the ethanol concentration and subsequently the percent of ethanol in the blood (Campbell et al., 2019; Jimenez-Chavez et al., 2020).

2.8 Statistical Analyses:

The data were analyzing using multi-factorial ANOVAs, with sex and genotype included as between-subjects factors for all initial analyses. Failure to detect sex effects or interactions prompted removal of the factor and data re-analysis. Significant interactions were deconstructed along the relevant factor(s), followed by t-tests (when fewer than 3 comparisons were conducted), tests for simple main effects or LSD post-hoc tests, when appropriate. Alpha = 0.05 for all analyses.

3. RESULTS

3.1 Alcohol intake under continuous-access.

The effect of *Hnrnph1* deletion on consumption of 5, 10 and 20% alcohol (v/v) under free-access conditions was sex-dependent (Fig.1) [Sex X Genotype X Concentration X Day: $F(26,728)=1.81$, $p=0.008$]. Interestingly, *Hnrnph1* deletion did not produce any detectable effect on intake of alcohol at any concentration in female mice (Fig.1, left) [Dose effect: $F(2,364)=29.36$, $p<0.0001$; Dose X Day: $p=0.08$; Genotype effect and interactions: all p's>0.20]. In contrast, the effect of gene deletion in males varied as a function of alcohol concentration (Fig.1, right) [Genotype X Dose X Day: $F(26,364)=1.87$, $p=0.007$]. Specifically, gene deletion did not influence the intake of 5 or 10% alcohol in male mice (Fig.1B,D) [for 5% alcohol, Genotype X Day ANOVA: all p's>0.06; for 10% alcohol, Day effect: $F(13,182)=5.92$, $p<0.0001$; Genotype effect and interaction, p's>0.25]. However, *Hnrnph1* +/− males exhibited lower intake of the 20% solution, particularly during the 2nd week of testing (Fig.1F) [Genotype X Day: $F(13,182)=2.45$, $p=0.004$; *post-hoc* tests for simple main effects]. In contrast to alcohol intake, water intake declined in all groups over the course of testing [Day effect: $F(13,364)=1.91$, $p=0.03$], but we failed to observe any overt sex or genotypic difference in this regard (Fig.1G,H; Genotype or Sex effects/interactions: all p's>0.07)

The sex difference in the effect of *Hnrnph1* deletion on alcohol drinking was also apparent with respect to the total alcohol intake exhibited by the mice drinking under continuous-access conditions (Fig.2A) [Sex X Genotype interaction: $F(1,31)=8.11$, $p=0.008$]. Male *Hnrnph1+/−* mice exhibited lower alcohol intake than their male *Hnrnph1+/+* counterparts [$t(14)=2.99$, $p=0.01$], while no genotypic difference was apparent in females (t-test: $p=0.25$). Although the average water intake exhibited by male *Hnrnph1+/−* mice was also lower than their respective male controls (Fig.2B), this difference was not statistically reliable (Sex X Genotype ANOVA: all p's>0.10). Taken together, these data implicate *Hnrnph1* in regulating alcohol intake under continuous-access procedures, with the *Hnrnph1* mutation reducing alcohol consumption only in males.

3.2 Binge Alcohol intake under limited-access.

As reported previously (e.g., Lee et al., 2016), alcohol intake under limited-access procedures was stable across the 14 days of testing [Day effect and interactions, $F(13,364)<1.2$, p's>0.26] and mice of both genotypes consumed amounts of alcohol that are predicted to result in BAC's in excess of the 0.08 g/dL criterion for binge-drinking (e.g., Rhodes et al. 2005; Lee et al., 2016). Although we did not detect a significant Genotype X Sex X Dose interaction (p=0.53) for alcohol intake under DID procedures, *Hnrnph1* deletion reduced alcohol intake in this paradigm [Genotype effect: $F(1,28)=7.24$, p=0.01; Genotype X Dose interaction [$F(3,84)=4.18$, p=0.008], with a non-significant trend for a Sex X Dose interaction [$F(3,84)=2.38$, p=0.07]. Given the sex-specific effect of *Hnrnph1* deletion on alcohol intake under continuous-access procedures (Fig.1; Fig.2A), we deconstructed the data for binge-intake along the sex factor for re-analysis of potential sex-specific effects and confirmed no effect of *Hnrnph1* deletion on the dose-intake function for female mice drinking under DID procedures (Fig.2C) [Dose effect: $F(3,42)=68.34$, $p<0.0001$; Genotype effect: p=0.10; interaction: p=0.48]. In contrast, male *Hnrnph1+/−* mice tended to binge-drink less 20% and 40% alcohol than their WT counterparts, but the genotypic differences were statistically unreliable (Fig.2D) [Genotype X Dose: $F(3,42)=3.67$, p=0.02; *post-hoc* tests for simple main effects, p's>0.05]. Thus, while not as robust as the results observed for alcohol intake under continuous-access procedures, these data nonetheless are consistent with a sex-specific effect of *Hnrnph1* deletion also on binge alcohol-drinking.

3.3 Alcohol-induced locomotor activity.

No genotypic difference was apparent with respect to distance traveled during the Pre-Test, when mice had access to both compartments of the place-conditioning apparatus, although females tended to locomote more than males (Fig.3) [Sex effect: $F(1,185)=3.54$, p=0.06; Genotype effect: $F(1,185)=2.75$, p=0.09; interaction, p=0.61]. However, no sex or genotype differences were apparent with respect to the locomotor response to an acute saline injection (Genotype X Sex ANOVA, p's>0.10; data not shown).

Analysis of the dose-response function for acute alcohol-induced locomotion (0.5, 1, 1.5, 2 and 4 g/kg) indicated a shift upwards in females versus males (Fig.4A,B) [Dose effect: $F(4,185)=12.16$, $p<0.0001$; Sex effect: $F(1,185)=14.16$, $p<0.0001$], but no genotypic difference (Genotype X Sex X Dose ANOVA, all other p's>0.15). As no genotypic difference was noted for the acute locomotor response to alcohol, we next examined the effect of the *Hnrnph1* mutation on the change in alcohol-induced locomotion during the course of place-conditioning by subtracting the distance traveled on Injection 1 from that on Injection 8. While the analysis of this dose-response function revealed a significant Genotype X Sex X Dose interaction [$F(4,185)=2.89$, p=0.02], deconstruction of the interaction along the sex factor failed to detect any dose or genotype effect in females (data not shown; Genotype X Dose ANOVA, p's>0.10), and only a statistical trend for lower responding in male *Hnrnph1* mutants [data not shown; Dose effect: $F(1,96)=3.10$, p=0.02; Genotype effect: p=0.08; interaction, p>0.20]. Thus, in contrast to both methamphetamine (Yazdani et al., 2015; Ruan et al., 2020) and fentanyl (Bryant et al. 2020), *Hnrnph1* deletion does not significantly affect the acute or sensitized locomotor response to alcohol.

3.4 Alcohol-induced place-conditioning.

In contrast to sex-dependent genotypic differences in drinking, a robust genotypic difference was detected with respect to the dose-response function for alcohol-induced place-conditioning, irrespective of Sex [Genotype X Side X Dose: F(4,166)=4.45, p=0.002; 4-way interaction, p=0.24], with *Hnrnph1+/+* mice exhibiting a strong alcohol-conditioned place-aversion at the two highest doses tested, whereas *Hnrnph1+/−* mice did not show any significant aversion at either dose of alcohol (Fig.4C) [for 2 g/kg, Side X Genotype: F(1,37)=11.63, p=0.002; for 4 g/kg, Side X Genotype: F(1,28)=11.60, p=0.002; for other doses, Side X Genotype ANOVAs, all p's>0.17]. Further, direct comparisons of the time spent in the alcohol- versus saline-paired side during the conditioning test confirmed a place-aversion in *Hnrnph1+/+* controls at both the 2 g/kg [F(1,18)=11.21, p=0.004] and 4 g/kg doses [F(1,15)=6.77, p=0.02]. In contrast, *Hnrnph1*+/−mutants were place-ambivalent at the 2 g/kg dose (p=0.31) and instead, exhibited a significant place-preference at the 4 g/kg dose [F(1,13)=4.96, p=0.04]. While the results of the ANOVA failed to indicate any sex effect or interactions, the prior Sex by Genotype interactions that we observed for alcohol-drinking prompted a comparison of the dose-response functions for alcohol-induced place-conditioning between female versus male mice. As illustrated in Fig.4D, no signs of high-dose alcohol-conditioned place-aversion were apparent in either *Hnrnph1*+/− male or female mice. However, the large genotypic difference in CPP scores observed when the data are collapsed across sex (Fig.4C) is driven primarily by the larger, less variable genotypic differences in conditioning of the male mice, including a more robust preference in +/− males and a more robust aversion in +/+ males (Fig.4D). These data indicate that *Hnrnrph1* deletion reduces sensitivity to the aversive effects of high-dose alcohol, without impacting the rewarding properties of lower alcohol doses, which is a finding in line with our previous study indicating greater high-dose methamphetamine CPP compared to wild-types (Ruan et al., 2020a). However, in contrast to our previous methamphetamine study, the effect of *Hnrnph1* deletion on alcohol's motivational valence is more pronounced in males.

3.5 Alcohol Intoxication and Sedation.

No genotype or sex differences were noted in the number of trials required for alcohol-naïve mice to remain on the fixed speed rotarod for 2 min during training (*Hnrnph1+/+*: 3.0 ± 0.0 trials, n=11; *Hnrnph1+/−*: 3.1 ± 0.1 trials, n=11; Genotype X Sex ANOVA, all p's>0.30) and both alcohol-naïve *Hnrnph1*+/+ and +/− mice remained on the rotarod for the entire 3-min period prior to alcohol injection. Overall, the latency to fall from the rotarod appeared to be longer in mice injected repeatedly with 3 g/kg alcohol, with *Hnrnrph+/+* mice exhibiting better rotarod performance than *Hnrnph1+/−* mice (Fig.5A). However, an analysis of these data failed to support the development of tolerance to alcohol's intoxicating effects (no Injection effect or interactions, p's>0.20), nor did it indicate any overall effects of, or interaction between, the Genotype and Sex factors (all p's>0.20). Likewise, *Hnrnph1+/+* controls tended to right themselves in a shorter period of time than *Hnrnph1+/−* mice following an acute injection with 4 g/kg alcohol (Fig.5B), but no significant genotype or sex differences were detected for this variable (Genotype X Sex ANOVA, all p's>0.20). Thus, *Hnrnph1* deletion does not reliably alter the intoxicating or sedative effects of higher alcohol doses.

3.6 Blood Alcohol Levels.

We next tested for the relationship between genotypic differences in alcohol intake and alcohol aversion to alcohol metabolism. As expected, BACs declined over time following injection with 1.5 g/kg alcohol [Time effect: $F(2,32)=50.23$, $p<0.0001$], but there were no genotype or sex differences in this regard (Fig.5C; Genotype X Sex X Time ANOVA, other p's>0.40). These BAC data are consistent with no overt effect of gene deletion on the locomotor, intoxicating, and sedative properties of alcohol.

3.7 Alcohol Withdrawal-Induced Anxiety.

3.7.1 Light-Dark Box.—No group differences were observed in the latency to first enter the light side (data not shown; Genotype X Sex X Treatment ANOVA, all p's>0.16). Overall, alcohol withdrawal reduced the number of light-side entries (Fig.6A) [Treatment effect: $F(1,65)=16.14$, $p<0.0001$], indicative of anxiety-like behavior. However, this alcohol withdrawal effect was more pronounced in *Hnrnph1−/−* mice as indicated by a significant Genotype X Treatment interaction [$F(1,65)=3.96$, $p=0.05$] and the results of within-genotype comparisons between alcohol- and saline-experienced mice [for +/+: $t(28)=1.61$, $p=0.12$; for +/−: $t(34)=4.01$, $p<0.0001$]. Alcohol withdrawal also reduced the time spent in the light side [Treatment effect: $F(1,65)=44.46$, $p<0.0001$]; however, the magnitude of this effect did not vary significantly with Genotype (Fig.6B; Genotype effects and interactions, p's>0.50) or with Sex (Sex effects and interactions, p's>0.08).

3.7.2 Marble-burying.—Compared to alcohol-naïve controls, mice in alcohol withdrawal exhibited a significantly shorter latency to begin marble-burying (Fig.6C) [Treatment effect: $F(1,65)=10.22$, $p=0.002$], and buried more marbles than alcohol-naïve controls (Fig.6D) [Treatment effect: $F(1,65)=191.56$, $p<0.0001$]. While, *Hnrnph1−/−* mice tended to exhibit a shorter latency to bury overall (Fig.6C; Genotype effect, $p=0.07$), neither Genotype nor Sex significantly interacted with the Treatment factor for this variable (Genotype effect, $p=0.07$; other p's>0.20) or the number of marbles buried (Genotype X Sex X Treatment ANOVA, other p's>0.16]. In contrast, no group differences were observed regarding the time spent burying (data not shown; Genotype X Sex X Treatment ANOVA, all p's>0.30). Thus, while alcohol withdrawal-induced anxiety was also observed in the marble-burying test, the intensity of this state was not affected by *Hnrnph1* deletion.

3.7.3 Forced Swim.—Analysis of the latency to first float in the forced swim test revealed a modest Genotype X Sex X Treatment interaction [$F(1,65)=3.94$, $p=0.05$]. Deconstruction of this interaction along the Sex factor indicated a significant Genotype X Treatment interaction only in female mice (Fig.7A) [$F(1,30)=4.83$, $p=0.037$], but not for males (Fig.7B) [p's>0.65]. As illustrated in Fig. 7A, the interaction in females reflected a shorter latency to float in alcohol-withdrawn *Hnrnph1+/+* mice versus their alcohol-naïve controls [$t(17)=1.81$, $p=0.08$], while no alcohol withdrawal effect was apparent in the mutant females (t-test, $p=0.15$). While it appeared that alcohol-naïve female +/− mice also exhibited a shorter latency to float than their +/+ counterparts, follow-up analyses failed to indicate any significant genotypic differences in either alcohol-naïve or -experienced females (t-tests, all p's>0.10).

A sex difference was detected for the withdrawal-induced increase in the number of immobile episodes [Sex X Treatment: $F(1,65)=5.35$, $p=0.02$], but there was no effect of Genotype (all p's>0.07). Alcohol withdrawal doubled the number of immobile episodes exhibited by female mice [$t(29)=4.79$, $p<0.0001$], but had no effect on immobile episodes in males (Fig.7C; t-test, $p=0.13$). Alcohol-withdrawal also increased the time spent immobile (Fig.7D) [Treatment effect: $F(1,65)=36.31$, $p<0.0001$], but this effect did not vary with sex or genotype (all other p's>0.13).

Taken altogether, these data for withdrawal-induced negative affect provide little evidence that hnRNP H1 plays a key role in regulating the basal affective state or alcohol withdrawal-induced changes therein.

4. Discussion

The present study sought to characterize the alcohol-related behavioral phenotype of mice with a heterozygous deletion of *Hnrnph1*. When allowed 24-h concurrent access to water and alcohol (10, 20 and 40%, v/v), male *Hnrnph1+/−* mice consumed less alcohol than WT controls, while no effect of gene deletion on drinking was apparent in female mice, with a similar pattern of results being observed under limited-access drinking procedures. The larger genotypic difference observed in male drinking in the DID versus continuous access procedure likely reflects the timing of alcohol presentation in DID, which coincides with the time of peak fluid intake during the circadian cycle (Gill et al., 1996; Rhodes et al. 2005). However, the fact that *Hnrnph1* heterozygous males exhibited lower alcohol intake under two distinct drinking paradigms is consistent with the results of meta-analysis indicating a correlation between continuous-access alcohol drinking (when water is freely available) and DID drinking, both in WT and mutant mice (see Blednov et al., 2012) and suggests that *Hnrnph1* deletion impacts a common underlying psychobiological mechanism to curb alcohol intake in males.

Interestingly, while no overt Sex by Genotype interaction was observed with respect to the effects of *Hnrnph1* deletion on oral methamphetamine intake (Ruan et al., 2020b), for intake with the mu opioid receptor agonist fentanyl, only male *Hnrnph1+/−* mice exhibited lower operant self-administration (Bryant et al., 2020). Further, as reported previously for fentanyl intake (Bryant et al., 2020), the genotypic difference in alcohol intake observed herein was only observed at the higher alcohol concentrations tested. This result suggests that heterozygous *hnrnpn1* deletion induces a sex-specific shift in both alcohol and opioid sensitivity. Although the mechanism by which male *Hnrnph1+/−* mice exhibit reduced alcohol or fentanyl consumption is unclear, a gene homolog, *Hnrnph2*, is located on the X chromosome in both rodents and humans. Mutations in both *Hnrnph1* and *Hnrpnh2* are linked to a rare, x-linked neurodevelopmental disorder in females (Bain *et al.* 2016; Pilch *et al.* 2018; Harmsen *et al.* 2019). If *Hnrnph2* undergoes variable X-inactivation, heterozygous deletion of *Hnrnph1* could induce sex-dependent changes in *hnrnph2* expression to influence the self-administration of certain drugs of abuse by males. While cocaine (Reynolds et al., 2011) and opioids (Suder et al., 2009) are reported to alter hnRNP H2 expression in rodent brain, it remains to be determined if alcohol can also regulate hnRNPH 1/2 mRNA or protein expression. Alternatively, sex hormones are well-characterized to influence alcohol

intake in both humans and laboratory rodents (for recent reviews, Finn, 2020; Verplaetse et al., 2020) and may contribute to sex differences in the effect of *Hnrnph1* deletion on alcohol drinking. While the molecular mechanisms by which *Hnrnph1* deletion exerts sex-specific effects on alcohol (and fentanyl) intake are unknown, the present findings provide novel evidence that *Hnrnph1* function is necessary for alcohol drinking behavior in males.

We employed alcohol-induced place-conditioning procedures to relate genotypic differences in alcohol intake to the affective/motivational valence of alcohol, with the hypothesis that reduced alcohol intake by *Hnrnph1*+/− males would reflect either less sensitivity to the conditioned rewarding properties of alcohol (as reported for both low-dose methamphetamine- and low-dose fentanyl-induced place-conditioning; Bryant et al., 2020; Ruan et al., 2020a) and/or greater sensitivity to the conditioned aversive properties of the drug. While the results of the statistical analyses failed to indicate a significant sex difference in the effect of *Hnrnph1* deletion on the dose-response function for alcohol-induced place-conditioning, a comparison across sexes suggests that the marked genotypic difference in the direction of the conditioned response to high-dose alcohol was driven, in large part, by male subjects. While the direction of the observed *Hnrnph1*+/− effect on place-conditioning is opposite our original hypothesis, these data nevertheless provide additional support for a male-selective effect of *Hrnrnph1* deletion on measures of alcohol reward and argue instead that the low alcohol intake exhibited by male *Hnrnph1*+/− mice might reflect a compensation for their increased sensitivity to alcohol's positive interoceptive effects.

It is interesting to note that, akin to the present findings for alcohol, *Hnrnph1*+/− mice also exhibited blunted sensitivity to the conditioned aversive properties of 2 mg/kg methamphetamine, as indicated by a greater conditioned place-preference in mutant mice, relative to WT controls at this dose (Ruan et al., 2020a). A similar trend was also observed with fentanyl (Bryant et al., 2020) which together, raises the intriguing possibility that *Hnrnph1*+/− blocks the negative affective/motivational valence of a variety of drugs of abuse. At least in the case of methamphetamine, the attenuated aversion exhibited by *Hnrnph1*+/− mice cannot be readily explained by an effect of gene deletion on nucleus accumbens dopamine, as no genotypic difference is observed for basal dopamine content (Ruan et al., 2020a). Moreover, *Hnrnph1*+/− blunted the capacity of acute methamphetamine to elevate nucleus accumbens extracellular dopamine levels (Ruan et al., 2020a) and blunted dopamine release within the nucleus accumbens is reported to promote, rather than prevent, a methamphetamine-conditioned place-aversion (Lominac et al., 2014). Likewise, we know from prior our work that methamphetamine-induced place-conditioning is bidirectionally regulated by nucleus accumbens glutamate levels (Szumlinski et al., 2016); however, *Hnrnph1*+/− did not alter either basal, or acute methamphetamine-induced changes in, extracellular glutamate within the nucleus accumbens (Ruan et al., 2020a). Of relevance to the manifestation of place-preference/aversion, we have yet to determine the effects of *Hnrnph1*+/− on drug-induced neurotransmitter levels within the nucleus accumbens of mice following repeated drug exposure, nor do we know how gene deletion alters neurotransmitter levels following acute or repeated alcohol. Alcohol-induced place-aversion is linked to anomalies in glutamate plasticity within both the nucleus accumbens (Szumlinski et al., 2005) and bed nucleus of the stria terminalis (Campbell et al., 2019),

implicating the extended amygdala as at least one potential neurocircuit affected by *Hnrnph1* deletion.

Under taste-conditioning procedures, binge alcohol-drinking inversely correlated with magnitude of a conditioned taste aversion in WT mice across various genetic backgrounds (Blednov et al., 2012; Rhodes et al., 2007) and this relationship was disrupted in a number of different transgenic mutations (see Blednov et al., 2012). While we did not assay *Hnrnph1+/−* mice for alcohol-conditioned taste-aversion, our place-conditioning data indicate that heterozygous *Hnrnph1* deletion not only blocked, but reversed the negative affective/motivational valence of high-dose alcohol. As C57BL/6J mice are reported to exhibit weak alcohol-induced place-preference (Cunningham, 2014; Cunningham et al., 1992), the failure of alcohol to elicit a place-preference in our WT mice likely reflects their genetic background rather than the alcohol doses selected and it remains to be determined whether or not the *Hnrnph1+/−* mutation would exert a similar effect on alcohol place-conditioning or any of our other measures in mice of a different genetic background more prone to exhibit place-preference or less likely to consume alcohol (e.g., DBA/2J). Nevertheless, the incongruency in results between our drinking and place-conditioning studies indicates that heterozygous *Hnrnph1* deletion blurs the inverse relationship between the conditioned aversive properties of alcohol and alcohol intake. This “blurring” is consistent with the results of alcohol-conditioned taste aversion studies of other mutant mouse lines (Blednov et al., 2012). and highlights the importance of conducting multiple assays of drug reward when phenotyping mice of both sexes.

In humans, the perception of alcohol’s interoceptive effects as aversive or appetitive typically relates to individual variation in sensitivity to alcohol-induced intoxication (e.g., Krystal et al., 2003; Schuckit and Smith, 2000) or the severity of alcohol withdrawal (e.g., Anton and Becker, 1995; Schuckit et al., 1998), as well as individual differences in alcohol metabolism (c.f., Cederbaum, 2012). However, a number of results from the present study argue against these psychopharmacological factors as contributing to the alcohol reward phenotype of *Hnrnph1+/−* mice. For one, we did not detect any consistent effect of *Hnrnph1* deletion on acute alcohol-induced locomotor activity, locomotor sensitization, intoxication or sedation, nor did we detect differences in alcohol pharmacokinetcs. Thus, the alcohol reward phenotype of *Hnrnph1* mutants is unrelated to changes in sensitivity to any of alcohol’s effects on motor behavior or alcohol metabolism. Our findings for alcohol-induced locomotor activity contrast sharply with our prior results for both methamphetamine-(Yazdani et al., 2015; Ruan et al., 2020a, 2020b) and fentanyl-induced locomotion (Bryant et al. 2020), suggesting that hnRNP H1 does not play a universal role in regulating all drug-induced psychomotor activity. Alternatively, our lack of genotypic differences in alcohol-induced locomotion may reflect procedural differences related to the duration of locomotor testing as genotypic differences in methamphetamine-induced locomotion were most robust when saline and drug trials were conducted over a 1-h period compared to a 30-min period (Yazdani et al., 2015). This being said, we have successfully detected large genotypic differences in alcohol-induced locomotion and/or sensitization using our place-conditioning procedures (e.g., Ary et al., 2012; Campbell et al., 2019; Szumlinski et al., 2005; 2008). Unfortunately, the limited number of alcohol-naïve mice available at the time of study precluded further investigation of this procedural issue.

Notably, we also failed to detect consistent effects of *Hnrnph1* deletion on negative affect-like measures in alcohol-naïve mice – a finding replicating our results indicating that *Hnrnph1*+/− does not affect baseline emotionality in mice (Bryant et al., 2020; Ruan et al., 2020a). We have shown repeatedly that early withdrawal from a history of binge-drinking induces a negative affective state in mice (e.g., Jimenez Chavez et al., 2020; Lee et al., 2015, 2016, 2017a,b, 2018a,b; Szumlinski et al., 2019), raising the possibility that the marked genotypic differences in alcohol-induced place-conditioning could reflect reduced sensitivity to a withdrawal-induced negative affective state. Given group differences in place-conditioning, we opted to inject mice repeatedly with high-dose alcohol (4 g/kg) in a manner consistent with the regimen employed during place-conditioning procedures and showed that this injection regimen was sufficient to increase anxiety- and depression-like behaviors when assessed during early alcohol withdrawal. However, as reported for fentanyl withdrawal (Bryant et al., 2020), we did not detect a consistent Genotype effect or Sex by Genotype interactions in the alcohol withdrawal-induced negative affective state. Thus, there does not appear to be a relationship between either reduced alcohol intake or an absence of alcohol-conditioned place-aversion and the severity of alcohol withdrawal in *Hnrnph1*+/− mice.

In conclusion, heterozygous deletion of *Hnrnph1* reduced high-concentration alcohol intake under two distinct drinking paradigms in male mice only. *Hnrnph1*+/− profoundly reversed the negative affective/motivational valence of high-dose alcohol – an effect that was more pronounced and less variable in males. The effects of *Hnrnph1* deletion on these measures of alcohol reward were unrelated to changes in alcohol pharmacokinetics, sensitivity to the psychomotor-activating, intoxicating or sedative properties of the drug, or the severity of alcohol withdrawal. These findings further support a general and surprisingly selective role for *Hnrnph1* function specifically following exposure to multiple addictive substances, although the underlying mechanisms regarding the effect of *Hnrnph1*+/− on behavior are likely to differ among drug classes and sex.

Acknowledgements

This project was funded by NIH grants DA039168 (CDB), U01DA050243 (CDB), and AA024044 (KKS).

Role of Funding Source

Nothing declared

References

Anton RF, Becker HC, 1995 Pharmacotherapy and pathophysiology of alcohol withdrawal In: Kranzler HR, (Ed.). The Pharmacology of Alcohol Abuse. Berlin: Springer-Verlag; 1995 pp. 315–367.

Aranburu A, Liberg D, Honorø B, Leanderson T, 2006 CArG box-binding factor—a interacts with multiple motifs in immunoglobulin promoters and has a regulated subcellular distribution, Eur. J. Immunol. 36, 2192–2202. [PubMed: 16874739]

Ary AW, Cozzoli DK, Finn DA, Crabbe JC, Dehoff MH, Worley PF, Szumlinski KK, 2012 Ethanol up-regulates nucleus accumbens neuronal activity-dependent pentraxin (Narp): implications for alcohol-induced behavioral plasticity. Alcohol 46, 377–387. [PubMed: 22444953]

Berrettini W, 2016 Alcohol addiction and the mu-opioid receptor. Prog. Neuropsychopharmacol. Biol. Psychiatry. 65, 228–233. [PubMed: 26226591]

Bain JM, Cho MT, Telegrafi A, Wilson A, Brooks S, Botti C, Gowans G, Autullo LA, Krishnamurthy V, Willing MC, Toler TL, Ben-Zev B, Elpeleg O, Shen Y, Retterer K, Monaghan KG & Chung WK, 2016 Variants in HNRNPH2 on the X Chromosome Are Associated with a Neurodevelopmental Disorder in Females. Am. J. Hum. Genet. 99, 728–734. [PubMed: 27545675]

Bourin M, and Hascoet M, 2003 The mouse light/dark box test. European Journal of Pharmacology, 463, 55–65. [PubMed: 12600702]

Brabant C, Guarnieri DJ, Quertemont E, 2014 Stimulant and motivational effects of alcohol: lessons from rodent and primate models. Pharmacol. Biochem. Behav, 122, 37–52. [PubMed: 24632178]

Bryant CD, Healy AF, Ruan QT, Coehlo MA, Lustig E, Yazdani N, Luttik KP, Tran T, Swancy I, Brewin LW, Chen MM, Szumlinski KK, 2020. Sex-dependent effects of an Hnrnph1 mutation on fentanyl addiction-relevant behaviors but not antinociception in mice. Genes Brain Behav, 3:e12711. doi: 10.1111/gbb.12711. Epub ahead of print.

Bryant CD and Yazdani N, 2016 RNA-binding proteins, neural development and the addictions. Genes Brain Behav, 15, 169–186. [PubMed: 26643147]

Campbell RR, Domingo RD, Williams AR, Wroten MG, McGregor HA, Waltermire RS, Greentree DI, Goulding SP, Thompson AB, Lee KM, Quadir SG, Jimenez Chavez CL, Coelho MA, Gould AT, von Joquieres G, Klugmann M, Worley PF, Kippin TE, Szumlinski KK, 2019 Increased alcohol-drinking induced by manipulations of mGlu5 phosphorylation within the bed nucleus of the stria terminalis. J. Neuroscience, 39, 2745–2761.

Cederbaum AI, 2012 Alcohol metabolism. Clin Liver Dis, 16, 667–685. [PubMed: 23101976]

Collins D, Reed B, Zhang Y, Kreek MJ, 2016 Sex differences in responsiveness to the prescription opioid oxycodone in mice. Pharmacol. Biochem. Behav, 148, 99–105. [PubMed: 27316549]

Cozzoli DK, Courson J, Wroten MG, Greentree DI, Lum EN, Campbell RR, Thompson AB, Worley PF, Jonquieres G, Klugmann M, Finn DA, Szumlinski KK, 2014 Binge alcohol drinking by mice requires intact Group1 metabotropic glutamate receptor signaling within the central nucleus of the amygdala. Neuropsychopharmacology, 39, 435–444. [PubMed: 23966068]

Crabbe JC, Phillips TJ, Harris RA, Arends MA, Koob GF, 2006 Alcohol-related genes: contributions from studies with genetically engineered mice. Addict Biol, 11, 195–269. [PubMed: 16961758]

Crawley JN (1985). Exploratory behavior models of anxiety in mice. Neurosci. Biobehavi. Reviews, 9, 37–44.

Cunningham CL, 2014 Genetic relationship between ethanol-induced conditioned place preference and other ethanol phenotypes in 15 inbred mouse strains. Behav. Neurosci, 128, 430–445. [PubMed: 24841742]

Cunningham CL, Niehus DR, Malott DH, Prather LK, 1992 Genetic differences in the rewarding and activating effects of morphine and ethanol. Psychopharmacology (Berl), 107, 385–393. [PubMed: 1352057]

Dreyfuss G, Kim VN, Kataoka N, 2002 Messenger-RNA-binding proteins and the messages they carry. Nat. Rev. Mol. Cell. Biol, 3, 195–205. [PubMed: 11994740]

Finn DA, 2020 The Endocrine System and Alcohol Drinking in Females. Alcohol Res, 40: 02. doi: 10.35946/arcr.v40.2.02.

Fukuda A, Nakadai T, Shimada M, Hisatake K, 2009 Heterogeneous nuclear ribonucleoprotein R enhances transcription from the naturally configured c-fos promoter in vitro. J. Biol. Chem, 284, 23472–23480. [PubMed: 19581295]

Fultz EK, Martin DL, Hudson CN, Kippin TE, Szumlinski KK, 2017 Methamphetamine-alcohol interactions in murine models of oral drug-taking. Drug Alcohol Dep, 177, 178–186.

Fultz EK, Szumlinski KK, 2018 Prior binge-drinking history promotes methamphetamine-preference in mice. Drug Alcohol Dep, 183, 150–154.

Gill K, France C, Amit Z, 1986 Voluntary ethanol consumption in rats: an examination of blood/brain ethanol levels and behavior. Alcohol Clin. Exp. Res, 10, 457–462. [PubMed: 3530027]

Guil S, Long JC, Cáceres JF, 2006 hnRNP A1 relocalization to the stress granules reflects a role in the stress response. Mol. Cell. Biol, 26, 5744–5758. [PubMed: 16847328]

Han SP, Tang YH, Smith R, 2010 Functional diversity of the hnRNPs: past, present and perspectives. Biochem. J, 430, 379–392. [PubMed: 20795951]

Hansson AC, Gründer G, Hirth N, Noori HR, Spanagel R, Sommer WH, 2019 Dopamine and opioid systems adaptation in alcoholism revisited: Convergent evidence from positron emission tomography and postmortem studies. Neurosci. Biobehav. Rev, 106, 141–164. [PubMed: 30243576]

Harmsen S, Buchert R, Mayatepek E, Haack TB, Distelmaier F, 2019 Bain type of X-linked syndromic mental retardation in boys. Clin. Genet, 95, 734–735. [PubMed: 30887513]

Heinz A, Reimold M, Wrase J, Hermann D, Croissant B, Mundle G, Dohmen BM, Braus DF, Schumann G, Machulla HJ, Bares R, Mann K, 2005 Correlation of stable elevations in striatal mu-opioid receptor availability in detoxified alcoholic patients with alcohol craving: a positron emission tomography study using carbon 11-labeled carfentanil. Arch. Gen. Psychiatry, 62, 57–64. [PubMed: 15630073]

Jimenez Chavez CL, Coelho MA, Brewin LW, Swancy I, Tran T, Albanese T, Laguna A, Garbriela I, Szumlinski KK, 2020 Incubation of negative affect during protracted alcohol withdrawal is age-, but not sex-selective. Brain Sciences, 10, 405. doi:10.3390/brainsci10060405

Kim J, Park A, 2018 A systematic review: Candidate gene and environment interaction on alcohol use and misuse among adolescents and young adults. Am. J. Addict, 10:10.1111/ajad.12755.. doi: 10.1111/ajad.12755 Epub ahead of print.

Krystal JH, Petrakis IL, Mason G, Trevisan D'Souza DC, 2003 N-methyl-D-aspartate glutamate receptors and alcoholism: reward, dependence, treatment and vulnerability. Pharmacol. Ther, 99, 79–94. [PubMed: 12804700]

Lee KM, Coelho MA, Class MA, Szumlinski KK, 2018a mGlu5-dependent modulation of anxiety during withdrawal from binge-drinking in adult and adolescent male mice. Drug Alcohol Dep, 68, 71–79.

Lee KM, Coelho MA, McGregor HA, Solton NR, Cohen M, Szumlinski KK, 2016 Adolescent Mice Are Resilient to Alcohol Withdrawal-Induced Anxiety and Changes in Indices of Glutamate Function within the Nucleus Accumbens. Frontiers Cell. Neurosci. 10: 265

Lee KM, Coelho M, McGregor HA, Waltermire RS, Szumlinski KK, 2015 Binge Alcohol Drinking Elicits a Persistent Negative Affective State in Mice. Behav. Brain Res, 291, 385–398. [PubMed: 26048424]

Lee KM, Coelho MA, Sern KR, Class MA, Bocz MD, Szumlinski KK, 2017a Anxiolytic effects of buspirone and MTEP in the Porsolt Forced Swim Test. Chronic Stress 1:2470547017712985. doi: 10.1177/2470547017712985.

Lee KM, Coelho MA, Sern KR, Szumlinski KK, 2018b Homer2 within the central nucleus of the amygdala gates withdrawal-induced anxiety in a mouse model of binge-drinking. Neuropharmacology, 128, 448–459. [PubMed: 29109058]

Lee KM, Coehlo MA, Solton NR, Szumlinski KK, 2017b Negative affect and excessive alcohol intake incubate during protracted withdrawal from binge-drinking in adolescent, but not, adult mice. Front Psychology, 8, 1128.

Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, et al., 2007 Genome-wide atlas of gene expression in the adult mouse brain. Nature, 445, 168–176. [PubMed: 17151600]

Lominac KD, Kapasova Z, Hannun RA, Patterson C, Middaugh LD, Szumlinski KK (2006) Behavioral and neurochemical interactions between Group 1 mGluR antagonists and ethanol: potential insight into their anti-addictive properties. Drug Alcohol Dep, 85, 142–156.

Lominac KD, McKenna CL, Schwartz LM, Ruiz PN, Wroten MG, Miller BW, Holloway JJ, Travis KO, Rajasekar G, Maliniak D, Thompson AB, Urman LE, Phillips TJ, Szumlinski KK, 2014 Mesocorticolimbic monoamine correlates of methamphetamine sensitization and motivation. Front. Systems Neurosci, 8, 70

Markmiller S, Soltanieh S, Server KL, Mak R, Jin WJ, Fang MY, Luo E-C, Krach F, Yang D, Sen A, et al., 2018 Context-Dependent and Disease-Specific Diversity in Protein Interactions within Stress Granules. Cell, 172, 590–604. [PubMed: 29373831]

Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, Befort K, Dierich A, Le Meur M, Dolle P, Tzavara E, Hanoune J, Roques BP & Kieffer BL, 1996 Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature, 383, 819–823. [PubMed: 8893006]

Nicolas LB, Kolb Y, Prinssen EP, 2006 A combined marble burying locomotor activity test in mice: a practical screening test with sensitivity to different classes of anxiolytics and antidepressants. Eur. J. Pharmacol, 547, 106–115. [PubMed: 16934246]

Njung'e K, Handley SL, 1991 Evaluation of marble-burying behavior as a model of anxiety. Pharmacol. Biochem. Behav, 38, 63–67. [PubMed: 2017455]

Phillips TJ, Shen EH (1996) Neurochemical bases of locomotion and ethanol stimulant effects. Int Rev Neurobiol. 1996;39:243–82. [PubMed: 8894850]

Pilch J, Koppolu AA, Walczak A, Murcia Pienkowski VA, Biernacka A, Skiba P, Machnik-Broncel J, Gasperowicz P, Kosińska J, Rydzanicz M, Emich-Widera E & Płoski R (2018) Evidence for HNRNPH1 being another gene for Bain type syndromic mental retardation. Clin Genet 94, 381–385. [PubMed: 29938792]

Quadir SG, Guzelian E, Palmer MA, Martin DL, Kim J, Szumlinski KK, 2019 Complex interactions between the subject factors of biological sex and prior histories of binge-drinking and unpredictable stress influence behavioral sensitivity to alcohol and alcohol intake. Physiol. Behav, 203, 100–112 [PubMed: 28803118]

Quadir SG, Santos JR, Campbell RR, Wroten MG, Singh N, Holloway JJ, Bal SK, Camarini R, Szumlinski KK, 2016 Homer2 regulates alcohol and stress cross-sensitization. Addiction Biol, 21, 613–633.

Ray LA, Barr CS, Blendy JA, Oslin D, Goldman D, Anton RF, 2012 The role of the Asn40Asp polymorphism of the mu opioid receptor gene (OPRM1) on alcoholism etiology and treatment: a critical review. Alcohol Clin. Exp. Res, 36, 385–394. [PubMed: 21895723]

Reynolds JL, Mahajan SD, Bindukumar B, Sykes D, Schwartz SA, Nair MP, 2006 Proteomic analysis of the effects of cocaine 284 Mol Neurobiol (2011) 44:269–286 on the enhancement of HIV-1 replication in normal human astrocytes (NHA). Brain Res, 1123, 226–236 [PubMed: 17034766]

Rhodes JS, Best K, Belknap JK, Finn DA, and Crabbe JC, 2005 Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiol. Behav. 84, 53–63. [PubMed: 15642607]

Rhodes JS, Ford MM, Yu CH, Brown LL, Finn DA, Garland T Jr, Crabbe JC, 2007 Mouse inbred strain differences in ethanol drinking to intoxication. Genes Brain Behav, 6, 1–18. [PubMed: 17233637]

Ruan QT, Yazdani N, Beierle JA, Hixson KM, Hokenson KE, Apicco DJ, Luttik KP, Zheng K, Maziuk BF, Ash PEA, Szumlinski KK, Russek SJ, Wolozin B, Bryant CD, 2018 Changes in neuronal immunofluorescence in the C- versus N-terminal domains of hnRNP H following D1 dopamine receptor activation. Neurosci. Lett, 684, 109–114. [PubMed: 30003938]

Ruan QT, Yazdani N, Blum BC, Beierle JA, Lin W, Coelho MA, Fultz EK, Healy AF, Shahin JR, Kandola AK, Luttik KP, Zheng K, Smith NJ, Cheung J, Mortazavi F, Apicco DJ, Ragu Varman D, Ramamoorthy S, Ash PEA, Rosene DL, Emili A, Wolozin B, Szumlinski KK, Bryant CD, 2020 A Mutation in *Hnrnph1* That Decreases Methamphetamine-Induced Reinforcement, Reward, and Dopamine Release and Increases Synaptosomal hnRNP H and Mitochondrial Proteins. J. Neurosci, 40, 107–130. [PubMed: 31704785]

Schaub MC, Lopez SR, Caputi M 2007 Members of the heterogeneous nuclear ribonucleoprotein H family activate splicing of an HIV-1 splicing substrate by promoting formation of ATP-dependent spliceosomal complexes, J. Biol. Chem, 282, 13617–13626. [PubMed: 17337441]

Schuckit MA, Smith TL, 2000 The relationships of a family history of alcohol dependence, a low level of response to alcohol and six domains of life functioning to the development of alcohol use disorders. J. Stud. Alcohol, 61 827–835. [PubMed: 11188488]

Schuckit MA, Smith TL, Daeppen JB, Eng M, Li TK, Hesselbrock VM, Nurnberger JI Jr, Bucholz KK, 1998 Clinical relevance of the distinction between alcohol dependence with and without a physiological component. Am. J. Psychiatry, 155, 733–740. [PubMed: 9619144]

Sern KR, Fultz EK, Coelho MA, Bryant CD, Szumlinski KK, 2020 A prior history of binge-drinking increases sensitivity to the motivational valence of methamphetamine in female C57BL/6J mice. Subst Abuse, 20, 14:1178221819897073.

Song KY, Choi HS, Law PY, Wei LN, Loh HH, 2012 Post-transcriptional regulation of mu-opioid receptor: role of the RNA-binding proteins heterogeneous nuclear ribonucleoprotein H1 and F. Cell. Mol. Life Sci, 69, 599–610. [PubMed: 21739230]

Suder P, Bodzon-Kulakowska A, Mak P, Bierczynska-Krzysik A, Daszykowski M, Walczak B, Lubec G, Kotlinska JH, Silberring J, 2009 The proteomic analysis of primary cortical astrocyte cell culture after morphine administration. J. Proteome Res, 8, 4633–4640 [PubMed: 19642706]

Szumlinski KK, Ary AW, Lominac KD, Klugmann M, Kippin TE, 2008 Accumbens Homer2 over-expression facilitates alcohol-induced neuroplasticity in C57BL/6J mice. Neuropsychopharmacology, 33, 1365–1378. [PubMed: 17568396]

Szumlinski KK, Lominac KD, Campbell RR, Cohen M, Fultz EK, Brown CN, Miller BW, Quadir SG, Martin D, Thompson AB, von Jonquieres G, Klugamann M, Phillips TH, Kippin TE, 2017 Methamphetamine addiction vulnerability: The glutamate, the bad and the ugly. Biol. Psychiat, 81, 959–970 [PubMed: 27890469]

Szumlinski KK, Lominac KD, Oleson EB, Walker JK, Mason A, Dehoff MH, Klugmann M, Cagle S, Welt K, During MT, Worley PF, Middaugh LD, Kalivas PW, 2005 Homer2 is necessary for ethanol-induced neuroplasticity. J. Neurosci., 25, 7054–7061. [PubMed: 16049182]

Tiruchinapalli DM, Ehlers MD, Keene JD, 2008 Activity-dependent expression of RNA binding protein HuD and its association with mRNAs in neurons. RNA Biol, 5, 157–168. [PubMed: 18769135]

Verplaetse TL, Cosgrove KP, Tanabe J, McKee SA, 2020 Sex/gender differences in brain function and structure in alcohol use: A narrative review of neuroimaging findings over the last 10 years. J. Neurosci. Res, 24:10.1002/jnr.24625. [PubMed: 32103538]

Wall ML, Bera A, Wong FK, Lewis SM, 2020 Cellular stress orchestrates the localization of hnRNP H to stress granules. Exp. Cell Res, 394, 112111. [PubMed: 32473225]

Xu J, Lu Z, Xu M, Pan L, Deng Y, Xie X, Liu H, Ding S, Hurd YL, Pasternak GW, Klein RJ, Cartegni L, Zhou W, Pan YX, 2014 A heroin addiction severity-associated intronic single nucleotide polymorphism modulates alternative pre-mRNA splicing of the mu opioid receptor gene OPRM1 via hnRNPH interactions. J. Neurosci, 34, 11048–11066. [PubMed: 25122903]

Yazdani N, Parker CC, Shen Y, Reed ER, Guido MA, Kole LA, Kirkpatrick SL, Lim JE, Sokoloff G, Cheng R, Johnson WE, Palmer AA & Bryant CD (2015) Hnrnph1 Is A Quantitative Trait Gene for Methamphetamine Sensitivity. PLoS Genet 11, e1005713. [PubMed: 26658939]

Zaso MJ, Maisto SA, Glatt SJ, Belote JM, Park A, 2017 Interaction Between the μ-Opioid Receptor Gene and the Number of Heavy-Drinking Peers on Alcohol Use. Alcohol Clin. Exp. Res. 41, 2041–2050. [PubMed: 28992386]

Zhang G, Neubert TA, Jordan BA, 2012 RNA binding proteins accumulate at the postsynaptic density with synaptic activity. J. Neurosci, 32, 599–609. [PubMed: 22238095]

Highlights

- Heterozygous deletion of *Hnrnph1* (+/−) reduces alcohol intake by mice under continuous- and limited-access procedures.
- *Hnrnph1*+/− mice are resistant to the conditioned aversive properties of high-dose alcohol.
- The effects of *hnrnph1* deletion on alcohol reward are male-selective.
- *Hnrnph1* deletion does not alter alcohol metabolism, withdrawal-induced anxiety, or its sedative-hypnotic effects.
- *Hnrnph1* is a novel regulator of alcohol reward.

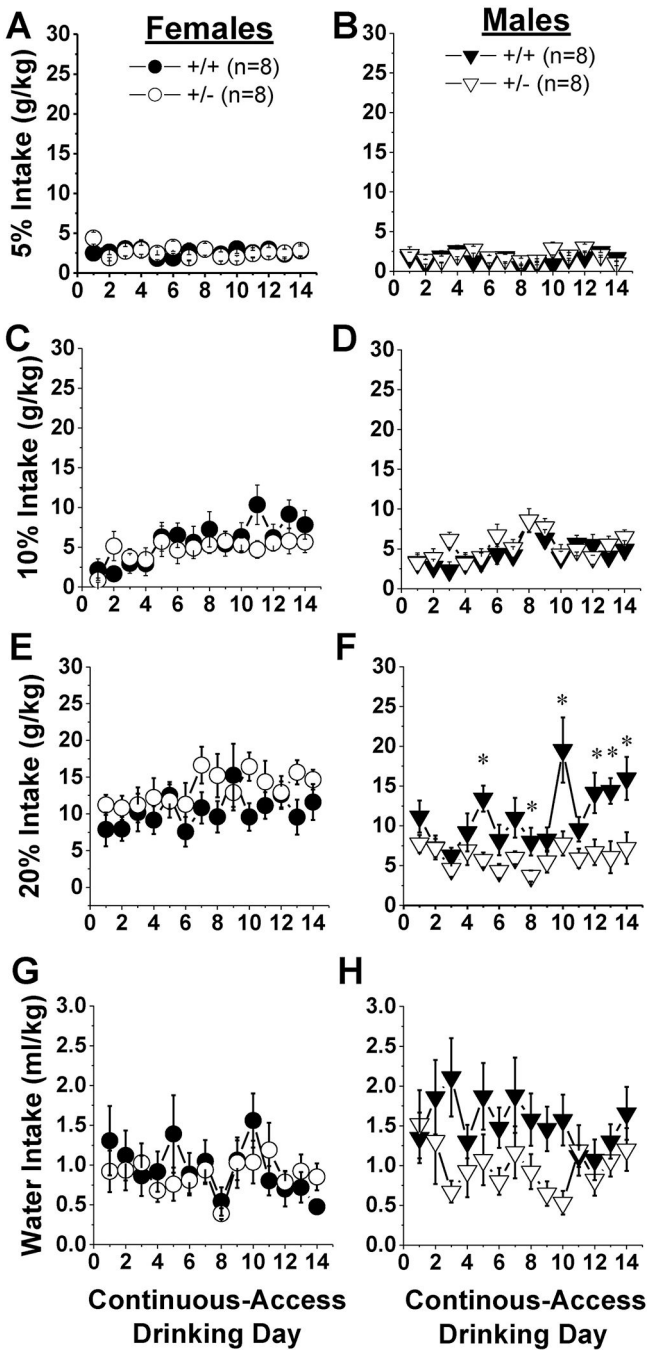

Figure 1: Male Hnrnph1+/− mice consume less high-concentration alcohol under continuous-access procedures.

Wild-type mice (+/+) and their littermates with a heterozygous deletion of *Hnrnph1* (+/−) were offered 24-h, concurrent, access to 0, 5, 10 and 20% alcohol (v/v) in the home-cage over the course of a 14-day period. No genotypic difference was detected for water intake in female ***(A)*** or male ***(B)*** mice. Likewise, no genotypic difference was detected for intake of 5% alcohol in either sex (**C,D**). Furthermore, for females, there was no genotypic difference in the intake of 10% ***(E)*** or 20% ***(G)*** alcohol. In contrast, male +/− mice exhibited blunted

intake of both 10% ***(F)*** and 20% alcohol ***(H)***. Data represent the means ± SEMs of the number of mice for each genotype (n) indicated in Panels A and B. *p<0.05 vs. +/+.

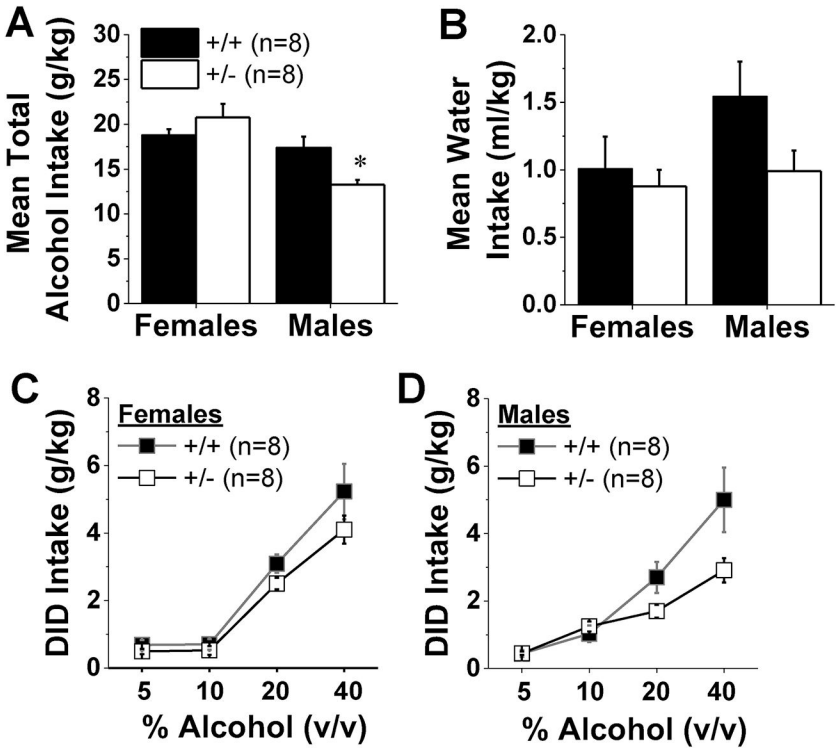

Figure 2: Male Hnrnph1+/− mice consume less high-concentration alcohol under limited-access procedures.

(A) The data from Fig. 1 are expressed as the average total alcohol intake over the 14-day course of drinking under continuous-access procedures and highlight the male-selective effect of gene deletion on alcohol-drinking. ***(B)*** No significant genotypic difference was observed for the average water intake during continuous-access procedures in either female or male mice. In assessing alcohol intake under Drinking-in-the-Dark (DID) procedures (concurrent access to 10, 20 and 40% alcohol v/v for 2 h/day), no genotypic difference was detected in female mice ***(C)***, while male *Hnrnph1+/−* mice consumed less high-concentration alcohol than their +/+ counterparts ***(D)***. The data represent the means ± SEMs of the number of mice indicated in Panel A. *$p<0.05$ vs. +/+.

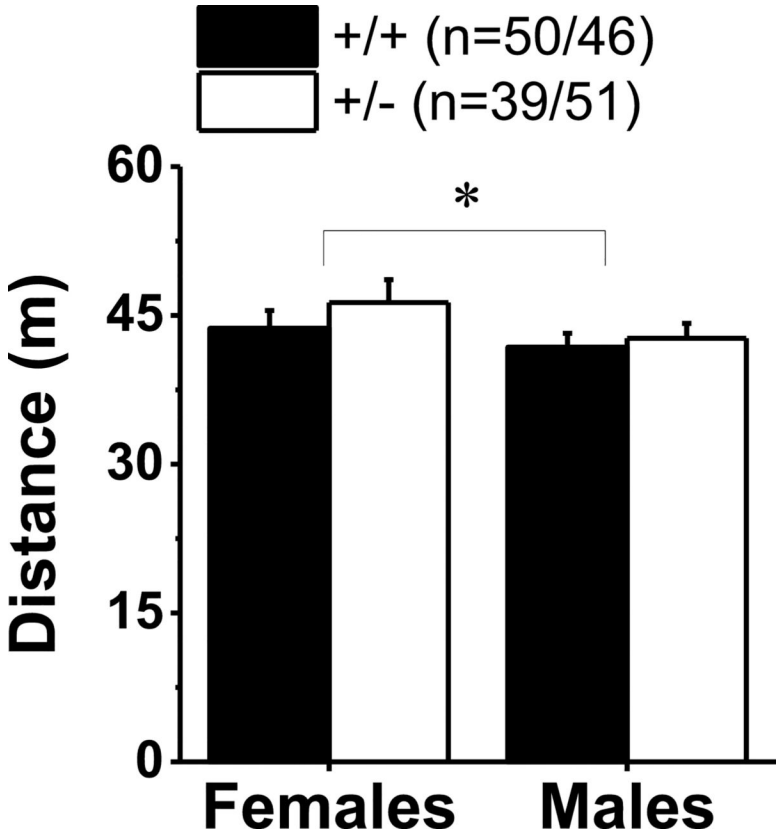

Figure 3:

Hnrnph1 deletion does not alter spontaneous locomotion. Wild-type mice (+/+) and their littermates with a heterozygous deletion of Hnrnph1 (+/−) were allowed to habituate to the place-conditioning apparatus for 15 min. Females locomoted more than males, irrespective of genotype with no effect of gene deletion detected. The data represent the means ± SEMs of the number of mice indicated. *$p < 0.05$ vs. males.

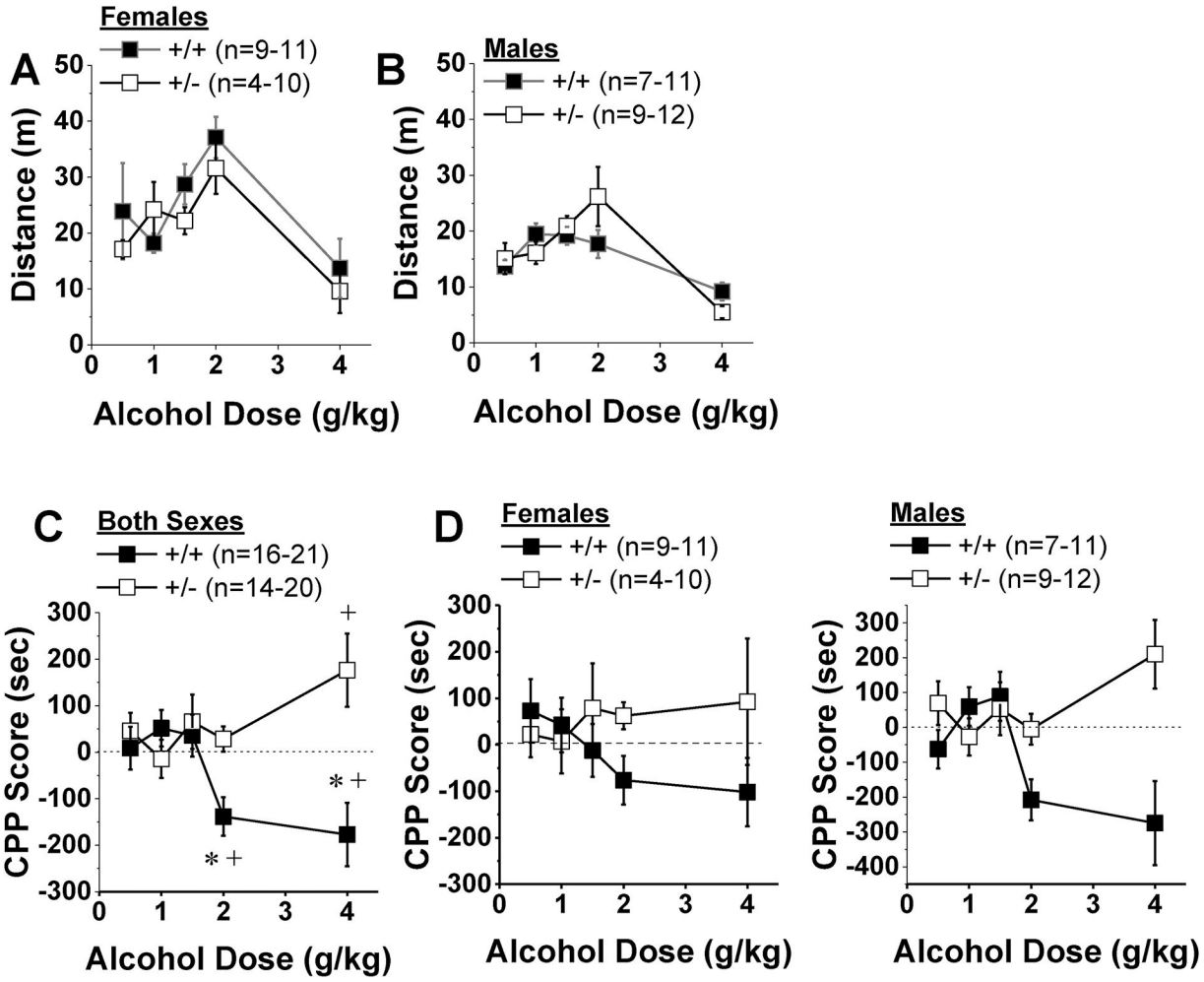

Figure 4:

Hnrnph1+/− mice do not exhibit alcohol-induced place-aversion. Wild-type mice (+/+) and their littermates with a heterozygous deletion of Hnrnph1 (+/−) underwent an alcohol-induced place-conditioning procedure involving 8 pairings of alcohol (0.5–4 g/kg) with a distinct compartment of a 2-compartment apparatus. While female mice (A) locomoted more than males (B) in response to alcohol injection during the first conditioning session, no genotypic differences were detected in the shape of the dose-response function for acute alcohol-induced locomotion. (C) When allowed free-access to both compartments following conditioning, we detected no sex difference in alcohol-induced place-conditioning. Thus, the data were collapsed across sexes to illustrate the large genotypic difference in the direction of the conditioned response between +/+ (aversion) and +/− mice (preference). The data represent the means ± SEMs of the number of mice indicated in Panels A and B. *$p < 0.05$ vs. +/+; +$p < 0.05$ vs. unpaired side (place-conditioning)

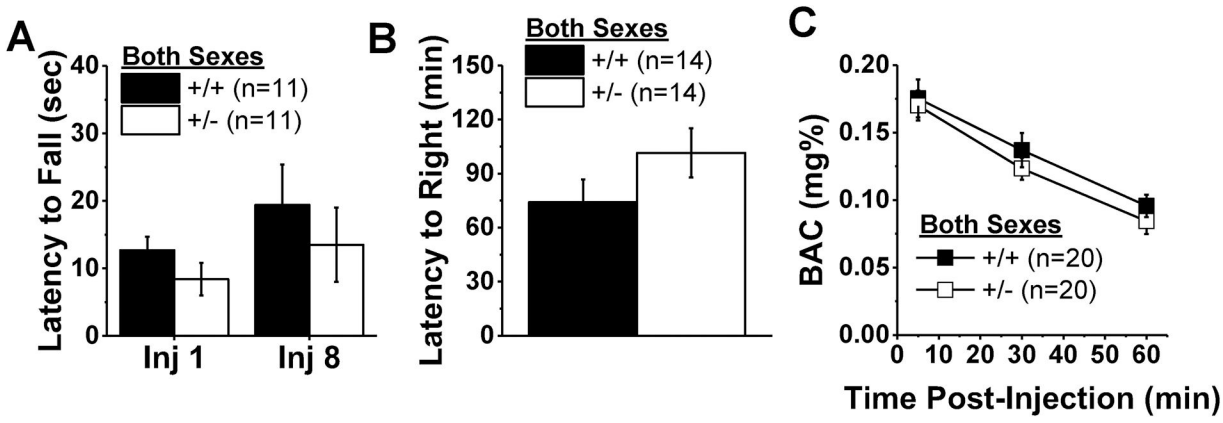

Figure 5:

Hnrnph1 deletion does not alter alcohol intoxication, sedation or metabolism. (A) Wild-type mice (+/+) and their littermates with a heterozygous deletion of Hnrnph1 (+/−) did not differ with regard to time spent on a fixed speed rotarod following their first and eighth injection of 3 g/kg alcohol. (B) Similarly, no genotypic difference was detected for the time taken to right themselves following an acute injection of 4 g/kg alcohol. (C) No genotypic difference in plasma alcohol levels were detect over the course of a 1-h period following injection with 3 g/kg alcohol. The data represent the means ± SEMs of the number of mice indicated in each panel. *$p < 0.05$ vs. +/+.

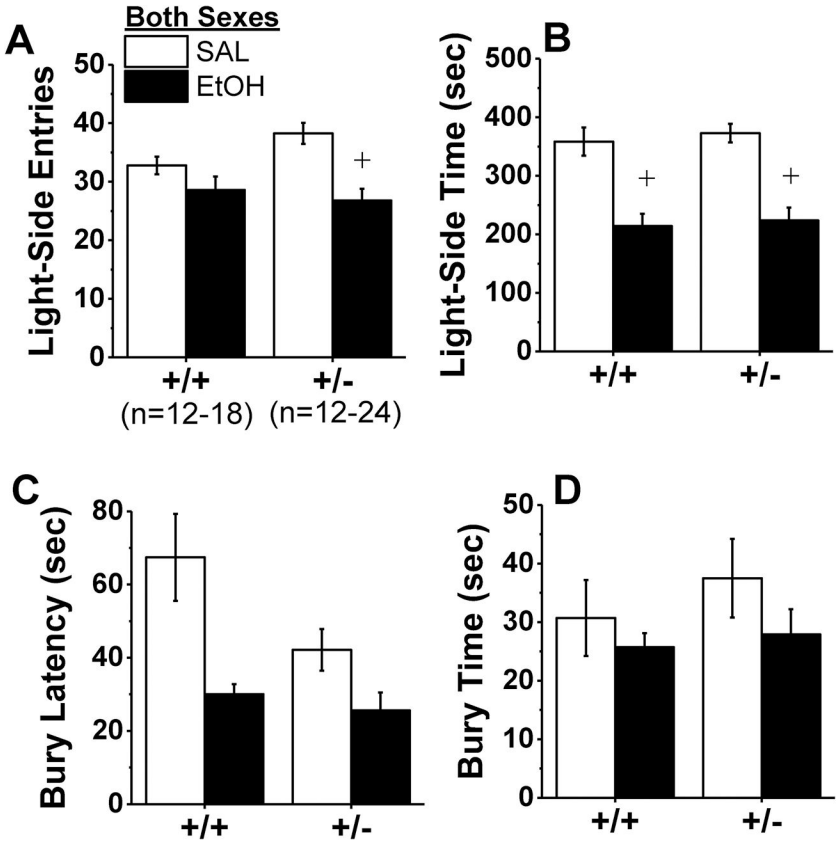

Figure 6:

Hnrnph1+/− deletion does not consistently alter baseline, or alcohol withdrawal-induced increases in anxiety-like behavior. Wild-type mice (+/+) and their littermates with a heterozygous deletion of Hnrnph1 (+/−) were injected repeatedly with 4 g/kg alcohol (EtOH) or saline (SAL) and then assayed for negative affect using a test battery including the light-dark shuttle-box and marble-burying tests. (A) +/− mice but not +/+ mice showed a significant withdrawal-induced decrease in the latency to enter the light side of a light-dark shuttle-box compared to their +/− control counterparts while (B) no genotypic difference was detected for the withdrawalinduced reduction in the time spent in the light side. In the marble-burying assay, alcohol withdrawal produced a nonsignificant reduction in the latency to begin burying marbles (C) and the time spent burying (D), but no genotypic differences were detected for either variable. The data represent the means ± SEMs of the number of mice indicated in Panel A. +$p < 0.05$ vs. SAL (alcohol withdrawal effect).

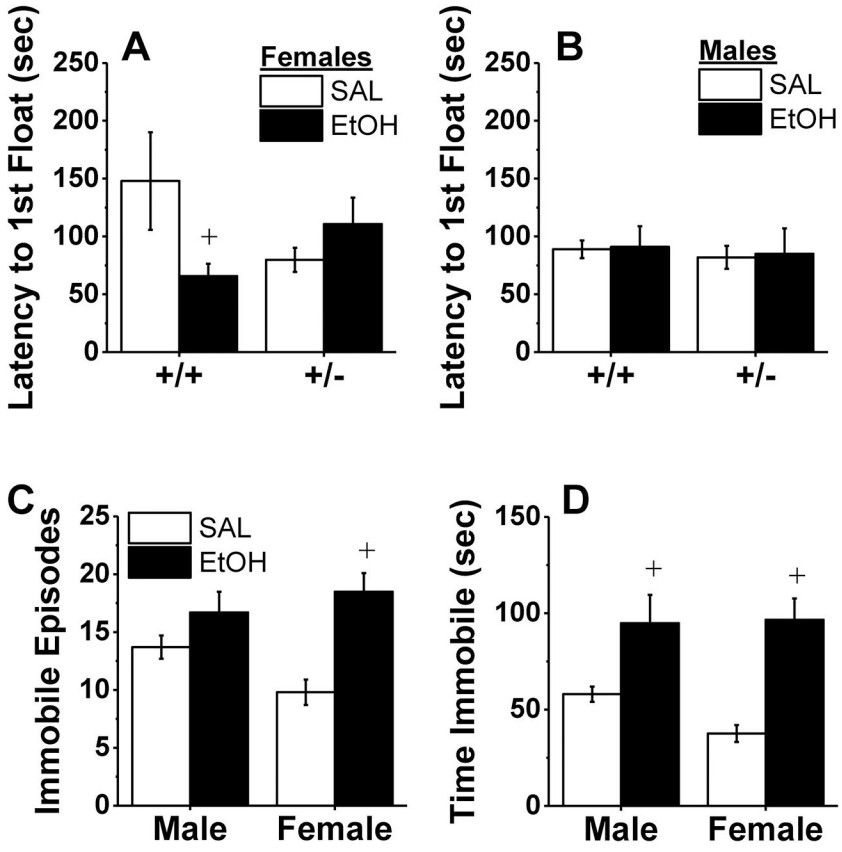

Figure 7.

Hnrnph1+/− deletion does not consistently alter baseline, or alcohol withdrawal-induced increases in depressivelike behavior. Wild-type mice (+/+) and their littermates with a heterozygous deletion of Hnrnph1 (+/−) were also assayed for genotypic differences in behavior in the forced swim test during early alcohol withdrawal. (A) A withdrawalinduced decrease in the latency to first float was detected in female +/+, but not female +/− mice, while no genotypic difference in float latency was observed in males (B). Alcohol withdrawal increased (C) the incidences of floating moreso in female versus male mice, while a withdrawal-induced increase in the time spent floating was comparable in male and female mice. No genotypic differences were detected for the number of floats or time spent floating, thus the data in Panels C and D are collapsed across genotype. The data represent the means ± SEMs of the number of mice indicated in Fig. 5, Panel A. +$p < 0.05$ vs. SAL (alcohol withdrawal effect).