Intracranial self-stimulation and concomitant behaviors following systemic methamphetamine administration in *Hnrnph1* mutant mice

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ABSTRACT

Rationale. Addiction to methamphetamine (MA) is a public health issue in the United States. While psychostimulant use disorders are heritable, their genetic basis remains poorly understood. We previously identified heterogeneous nuclear ribonucleoprotein H1 (*Hnrnph1;* H1) as a quantitative trait gene underlying sensitivity to MA-induced locomotor activity. Mice heterozygous for a frameshift deletion in the first coding exon of H1 ($H1^{+/-}$) showed reduced MA-induced locomotor activity, dopamine release, and dose-dependent differences in MA conditioned place preference. However, the effects of $H1^{+/-}$ on innate and MA-modulated reward sensitivity are not known.

Objectives. We examined innate reward sensitivity and modulation by MA in $H1^{+/-}$ mice via intracranial self-stimulation (ICSS).

Methods. We used intracranial self-stimulation (ICSS) of the medial forebrain bundle to assess shifts in reward sensitivity following acute, ascending doses of MA (0.5-4.0 mg/kg, i.p.) at 10 min or 2 h post-MA. We also assessed video-recorded behaviors during ICSS testing sessions.

Results. Ten min post-MA, $H1^{+/-}$ mice displayed reduced maximum response rates, $H1^{+/-}$ females had lower M50 values than wild-type females, and $H1^{+/-}$ influenced ICSS responding relative to maximum control rates (MCR). Two h post-MA, higher response rates were observed in females, irrespective of genotype. There was a dose-dependent reduction in distance to the response wheel 10 min post-MA and reduced immobility time in the perimeter corners both 10 min and 2 h post-MA.

Conclusions. $H1^{+/-}$ mice displayed altered MA-induced reward modulation in a time-, sex-, and dose-dependent manner. This expands the set of MA-induced phenotypes observed in $H1^{+/-}$ mice.

Keywords: Intracranial Self-Stimulation (ICSS), methamphetamine, genetics, addiction, behavioral genetics, psychostimulants, mouse, forward genetics, QTL, sensitization

INTRODUCTION

Abuse of psychostimulant drugs, such as methamphetamine (**MA**), contributes to an ongoing public health crisis marked by unprecedented overdose rates in the United States. Deaths linked to psychostimulant misuse increased by 33.3 percent from 2016-2017 alone (Seth et al. 2018). Psychostimulant use disorder has a significant heritable component, but the genetic basis remains poorly understood (Bousman et al. 2009a; Jensen 2016; Goldman et al. 2005). Prior clinical studies designed to discover genes associated with MA dependence and/or abuse have been hindered by several limitations, including a lack of statistical power and environmental control (Uhl et al. 2008; Ikeda et al. 2013; Hancock et al. 2018). Rodent models help address some of these impediments and permit the study of both the genetic and neurobiological mechanisms underlying addiction-relevant quantitative traits. Forward genetic approaches, such as quantitative trait locus (**QTL**) mapping, can identify chromosomal loci and novel genes and variants linked to addiction-relevant behaviors (Flint et al. 2005; Spanagel 2013).

We recently used QTL mapping, positional cloning, and gene editing to validate *Hnrnph1* (heterogeneous nuclear ribonucleoprotein H1) as a quantitative trait gene underlying sensitivity to MA-induced locomotor stimulation. To validate the candidate gene within the identified 0.2 Mb locus, we used transcription activator-like effector nucleases (**TALENs**) to induce a frameshift deletion in the first coding exon of the H1 gene in B6 mice. H1 mutant mice heterozygous for the *H1* deletion (**H1$^{+/-}$**) recapitulated the decreased sensitivity to MA-induced locomotor activity observed in the congenic lines (Yazdani et al. 2015). Additionally, striatal transcriptomic analysis of H1$^{+/-}$ mice suggested a deficit in the development of dopaminergic (DA-ergic) innervation and transmission in the mesocorticolimbic reward pathway (Yazdani et al. 2015). More recently, we showed that a 0.1 Mb locus containing *Hnrnph1* was also sufficient to reduce MA behavior, including MA reinforcement, and can be linked to decreased 5' UTR usage of *Hnrnph1* and decreased hnRNP H protein (Ruan et al. 2020a).

H1 codes for an RNA-binding protein that is ubiquitously expressed throughout the mouse brain (Lein et al. 2007) and regulates multiple stages of RNA metabolism, including alternative splicing (Kim et al. 2002; Han et al. 2010). Accumulating evidence indicates a crucial role for RNA-binding proteins, such as H1, in neurobehavioral plasticity underlying **SUDs** (Bryant and Yazdani, 2016). Proteomic analysis of synaptosomal striatal tissue at 30 min following an acute dose of MA (2 mg/kg, i.p.) indicated that the molecular mechanisms underlying the MA-induced behavioral deficits in H1$^{+/-}$ mice could involve mitochondrial dysfunction as opposing effects of MA on several synaptosomally localized mitochondrial proteins were observed as a function of genotype and as a function of MA treatment (Ruan et al. 2020b).

Intracranial self-stimulation (**ICSS**) can assess innate reward sensitivity and the abuse potential and motivational effects of various addictive substances, including psychostimulants such as MA (Bauer et al. 2013; Carlezon and Chartoff 2007; Kesby et al. 2018; Negus and Miller 2014). One variant of the ICSS procedure involves indirect electrical stimulation of the mesolimbic dopaminergic reward pathway by targeting the medial forebrain bundle (**MFB**); a collection of axonal fibers of passage in the lateral hypothalamus (Bielajew and Shizgal 1986; Garris et al. 1999). Stimulation of several brain regions can support ICSS; within the MFB, stimulation activates mesolimbic dopaminergic projections from the ventral tegmental area (**VTA**) to the nucleus accumbens (**NAc**) to increase synaptic dopamine levels within the NAc (Fiorino et al. 1993; Miliaressis et al. 1991; Owesson-White et al. 2008), a brain region critical for endogenous reward signaling (Schultz 2000). MA also targets plasma membrane dopamine transporters and the vesicular monoamine transporters (Cruickshank and Dyer 2009) to increase synaptic dopamine in the NAc (Johnson et al. 2018; Sulzer et al. 2005) that tracks closely with an increase in locomotor activity (Koshikawa et al. 1989; Di Chiara and Imperato 1988; Zocchi et al. 1997). The rise in NAc DA levels elicited by amphetamine-type psychostimulants can increase ICSS response rates and sensitivity to brain stimulation reward (**BSR**) in rodent models. Conversely, drugs that deplete or inhibit DA transmission decrease sensitivity to BSR, an indicator of anhedonia (Negus and Miller 2014; Bauer et al. 2014; Bauer et al. 2013; Phillips et al. 1989; Riday et al. 2012).

In vivo microdialysis studies have shown that **H1$^{+/-}$** mice have significantly lower DA levels in the NAc compared to wild-type (**WT**) littermates following intraperitoneal (i.p.) injections of MA (0.5 and 2.0 mg/kg) (Ruan et al. 2020b). Following MA administration, DA deficits in the H1$^{+/-}$ mice emerged by 20 min after the 0.5 mg/kg dose but did not emerge until 100 min following the 2.0 mg/kg dose. The time-dependency of this neurochemical finding motivated the current study where we performed ICSS testing at 10 min versus 2 h post-MA administration. We established the effects of H1$^{+/-}$ on ICSS responding under baseline conditions and following "priming" of the reward circuitry with increasing doses of MA (0.5, 1.0, 2.0, 4.0 mg/kg). As ICSS and locomotor

activity both vary with DA release and because H1$^{+/-}$ mice showed a robust reduction in MA-induced locomotor activity (Yazdani et al. 2015; Ruan et al. 2020b), we hypothesized that the threshold-reducing effects of MA would be less robust in H1$^{+/-}$ mice. Additionally, we hypothesized that genotypic differences in ICSS responding would vary as a function of both MA dose and post-MA testing latency, consistent with the neurochemical and behavioral dynamics in H1$^{+/-}$mice (Ruan et al. 2020b). We also assessed concomitant behavioral activity and location during ICSS testing sessions to potentially identify unique, genotype-specific behaviors altered by MA treatment that coincide with MA-induced changes in ICSS operant responding.

METHODS

Mice

All procedures in mice were approved by the Boston University Institutional 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 (National Research Council 2011). Colony rooms were maintained on a 12:12 h light–dark cycle (lights on at 0630 h) and all experimental manipulations were performed during the light cycle (between 0900–1600 h). Mice were housed in same-sex groups of 2-5 mice per cage with standard laboratory chow and water available *ad libitum*. We analyzed data from a total of 34 mice, including 14 H1 WT [5 females (**F**); 9 males (**M**)] and 20 H1$^{+/-}$ (10 F, 10 M). Male mice heterozygous for the *Hnrnph1* deletion were generated on a C57Bl/6J background and were crossed at each generation with female wild-type C57BL/6J mice ordered from The Jackson Laboratory (Bar Harbor, ME USA) to prevent genetic drift of the isogenic C57BL/6J background. Offspring included approximately 50% WT mice and 50% H1$^{+/-}$ mice.

Genotyping

Genotyping was conducted as previously described (Yazdani et al. 2015). We designed forward (GTTTTCTCAGACGCGTTCCT) and reverse (ACTGACAACTCCCGCCTCA) primers upstream and downstream of the TALENs binding domain (used to generate the deletion) within exon 4 of *Hnrnph1*. DreamTaq Green PCR Mastermix (ThermoScientific) was used in PCR amplification a 204 bp region containing the deletion site. PCR products were incubated overnight at 60º with either BstNI restriction enzyme (New England Biolabs) or a control buffer solution without enzyme. Gel electrophoresis (2% agarose gel with 0.5 µg/mL ethidium bromide) was performed on enzyme-treated and control samples for band visualization under UV light. The 204bp PCR amplicon contained 2 BstNI restriction sites which flanked the cut sites of the TALENs *Fok*I nuclease. On the gel, mice carrying a heterozygous *Hnrnph1* deletion displayed 2 bands, while WT mice showed only a single band.

Surgery and Histology

Age-matched mice were 50-70 days old at the time of surgery. Mice were anesthetized with 1-4% isoflurane and underwent stereotaxic surgery to implant a bipolar stimulating electrode (Plastics1, Roanoke, VA; MS308) aimed at the medial forebrain bundle (**MFB**) at the level of the lateral hypothalamus (-1.7, 1.0, -5.0 mm; relative to Bregma) (Paxinos and Franklin 2004). The analgesic meloxicam was administered subcutaneously at 5.0 mg/kg on the day of surgery and on the following 3 days. The mice recovered for 1 week prior to beginning ICSS training.

Following testing, mice were anesthetized with isoflurane and were transcardially perfused with 0.9% saline followed by 4.0% paraformaldehyde in 0.1 M PBS to fix brain tissue. Brains were later sectioned with a cryostat (40 µm), stained with 1% cresyl violet (Nissl stain), and imaged under a light microscope (4x) to confirm accurate electrode placement in the MFB.

ICSS Training

FR1 training

Mice were trained on a fixed-ratio 1 (**FR1**) schedule to respond for stimulation via a wheel manipulandum (ENV-113AM; Med Associates, St. Albans VT USA) within an operant chamber (15.24 x 13.34 x 12.7 cm; ENV-307A-CT; Med Associates) as previously described (Fish et al. 2012, Carlezon and Chartoff 2007). Operant chambers were placed inside closed sound-attenuating cubicles (ENV-022V; Med Associates). For every ¼ turn of the response wheel, a 500 ms train of square-wave, cathodal current (100-ms pulses) was delivered at a constant frequency of 142 Hz through a stimulator (PHM-150B/2; Med Associates). Each stimulation was followed by a

500 ms time-out period during which responses were counted but not reinforced by stimulation. The current intensity was adjusted for each subject to the lowest value that produced >500 responses during a 45-min period across at least 3 consecutive training sessions (-50 to -140 uA). The minimum effective current (**MEC**) for each subject was held constant throughout the rest of the study. All behavioral procedures were performed using Med-PC V software (Med Associates).

Rate-frequency training (Carlezon and Chartoff, 2007).

Mice were introduced to stimulation delivered at a constant current (MEC) over a series of 15 descending frequencies ($\log_{0.05}$ steps, 142-28 Hz). Each frequency trial consisted of 5 s of non-contingent priming stimulation, a 50-s response period during which responses were rewarded with 500 ms stimulation, and a 5-s time-out period. Each response-contingent stimulation was followed by a 500 ms timeout. This sequence was repeated for all 15 frequencies (1 pass = 15 min), and the procedure was repeated 2 more times in the same session (3 total passes = 45 min). The number of responses (including those emitted during timeout periods) and stimulations delivered were recorded for each trial. Training was repeated until subjects consistently responded at maximum rates during the highest frequency trials and did not respond during the lowest frequency trials. Subjects were required to demonstrate less than 15% between-day variability in mean response threshold for at least 3 consecutive training days prior to testing with MA.

ICSS Testing

Mice were weighed and assessed for baseline (BL), drug-naïve ICSS responding via a rate-frequency procedure identical to that used during training (see above). Upon completion of the 45-min BL procedure, mice were removed from the operant chambers, injected with MA (0.5, 1.0, 2.0, 4.0 mg/kg; i.p.), and placed individually into new, clean cages with fresh bedding. Ten min post-MA injection, mice were placed back into the operant chambers and underwent the same protocol used during BL assessment (3 passes of 15 min, 45 min total). Testing was performed every other day and all mice received escalating doses of MA using a within-subjects design. Three weeks after the completion of these tests, one cohort of mice (n=23; 9 H1 WT (5M/4F), 14 $H1^{+/-}$ (8M/6F) additionally underwent an altered ICSS testing protocol with testing at BL and at 2 h post-MA administration. On testing days, videos of both BL and post-MA sessions were recorded for later behavioral tracking and analysis using ANY-maze V software (Stoelting Co., Wood Dale, IL).

ANALYSIS

Minimum effective current

MECs were analyzed with a 2-way analysis of variance (**ANOVA**) test with Genotype and Sex as between-subject factors.

M50

M50 values reflect the frequency at which an animal responds at half its maximum response rate for a given pass; this measure is analogous to an effective concentration (**EC**) 50 value of a pharmacological dose-response curve. Detailed descriptions of this approach are published (Miliaressis et al. 1986; Carlezon and Chartoff 2007). M50 values for each pass were determined using a custom-built analysis program, and these values were averaged across all 3 passes in a BL or MA session. % BL M50 values represent the average post-MA M50 value divided by the average BL M50 value x 100. For each dose, post-MA M50 values were compared to same-day BL M50 values. %BL M50 values were analyzed via three-way repeated measures (**RM**-) ANOVAs with Genotype and Sex as between-subjects factors and Dose (0.5, 1.0, 2.0, 4.0 mg/kg) as the repeated measure. In the presence of significant interactions between Genotype and Sex, the model was deconstructed along the Sex factor, and two-way RM-ANOVAs were used with Genotype as the between-subjects factors to determine sex-specific effects of H1 genotype. Post-hoc pairwise differences were determined with 2-tailed Student's t-tests that were either unpaired (for between-group differences) or paired (for within-group differences) with significance set at α=0.05 and Bonferroni correction for multiple comparisons. All within-group comparisons were made to the 0.5 mg/kg MA %BL M50 values because these values preceded any MA exposure, including the 0.5 mg/kg dose.

Operant Responding

Maximum response rates (**RRs**) during any given frequency trial were determined for each pass during BL and MA sessions. The average maximum response rate was calculated for each BL and post-MA session. Data are presented as %BL to reflect how maximum RRs are affected by each MA dose. Similarly, the average sum of total responses was determined for each BL and MA session. Data are presented as % BL total responses to reflect the effect of MA on overall responding at a specific dose. Both average maximum RRs and total response % BL values were analyzed using 3-way RM-ANOVAs with Genotype and Sex as the between-subjects factors and Dose as the within-subjects factor. RM-ANOVAs were deconstructed along the Sex factor in the case of significant interactions between Genotype and Sex, and additional 2-way RM-ANOVAs were run for each sex individually with Genotype as the between-subjects factor. Post-hoc pairwise differences were determined with 2-tailed Student's t-tests that were either unpaired (for between-group differences) or paired (for within-group differences) with significance set at α=0.05 and Bonferroni correction for multiple comparisons. All within-group comparisons were made to 0.5 mg/kg MA % BL M50 values.

Maximum Control Rate (MCR)

This approach has been previously described (Altarifi and Negus 2011). Changes in post-MA responding at individual stimulation frequencies were assessed relative to the average maximum RRs at BL. Here, the average maximum RRs calculated for BL sessions are used as Maximum Control Rates (MCRs) to normalize post-MA response rates as %MCR for each frequency. Post-MA responses were averaged across all three passes for each frequency. %MCR values were then calculated for each frequency as: average post-MA response rate ÷ MCR x 100. For each dose, %MCR values were analyzed using 3-way RM-ANOVAs with Genotype and Sex as the between-subjects factors and Dose as the within-subjects factor. RM-ANOVAs were deconstructed along the Sex factor in the case of significant interactions between Genotype and Sex, and additional 2-way RM-ANOVAs were run for each sex individually with Genotype as the between-subjects factor. Post-hoc differences between groups across stimulation frequencies were determined with 2-tailed unpaired Student's t-tests with significance set at α=0.05 and Bonferroni correction for multiple comparisons.

Concomitant behaviors

All behavioral measures were collected using custom tracking protocols in ANY-maze V (Stoelting Inc. City, State, USA). The average distance to the response wheel was determined by setting a point of interest at the central-most position of the response wheel using ANY-maze. Additionally, time immobile in the corners of the testing chambers was determined by demarcating the far corners of the testing chamber (opposite the response wheel) as a zone of interest. Post-MA averages for distance from the response wheel and corner time are presented as % BL. For each dose, % BL values were analyzed using 3-way RM-ANOVAs with Genotype and Sex as the between-subjects factors and Dose as the within-subjects factor. Post-hoc pairwise differences relative to 0.5 mg/kg MA % BL values were determined for subsequent doses with 2-tailed paired Student's t-tests with significance set at α=0.05 and Bonferroni correction for multiple comparisons.

RESULTS

Electrode placement

Histological analysis confirmed accurate electrode placement in the MFB or in a proximity to these fibers sufficient to produce activation (**Fig. 1**) in all mice that completed MA testing (n=34). Average electrode placements were -1.89 ± 0.04 (mean ± SEM) AP, 1.00 ± 0.03 ML, and -5.05 ± 0.03 DV (mm relative to Bregma). A total of 6 mice were omitted from the study because they failed to meet training criteria or detachment of the electrode prior to study completion, and thus, these mice were not included in histological analyses nor were their data used in the behavioral analyses.

Minimum Effective Current (MEC)

The average MEC was -95.0 ± -3.4 (SEM) µA. A 2-way ANOVA revealed no significant effect of Genotype ($F_{1,30}$= 0.27, p=0.61) or Sex ($F_{1,30}$=1.22, p= 0.28) and no interaction ($F_{1,30}$=0.005, p= 0.94; **Fig. 2**).

Operant Responding

Maximum Response Rate

10 min post-MA: There was a main effect of Genotype (n=34: 14 H1 WT (5F/9M) and 20 $H1^{+/-}$ (10F/10M); $F_{1,30}$=6.29; p=0.02; **Fig. 3a** and no other significant main effects or interactions (all p>0.27). There were no significant differences between genotypes at individual MA doses (all adj. p > 0.30), however, $H1^{+/-}$ mice had significantly lower %BL values than WT mice when averaged across all doses (95% confidence interval for mean difference: -71.30, -7.817).

2 h post-MA: There was a significant Genotype x Dose x Sex interaction (n=23: 9 H1 WT (5M/4F) and 10 $H1^{+/-}$ (8M/6F); $F_{1,19}$=3.40; p=0.02; **Fig. 3b**). After breaking down the data along the Sex factor, we performed additional RM-ANOVAs for each sex individually. There were no significant main effects (Dose, Genotype) or interactions for these analyses (males: n=13: 5 H1 WT and 8 $H1^{+/-}$; all p's>0.16; females: n=10: 4 H1 WT and 6 $H1^{+/-}$; all p's>0.15).

Total Responses

10 min post-MA: There was a significant main effect of Dose ($F_{3,90}$=3.85; p=0.01; **Fig. 3c**) and no other significant main effects or interactions (all p>0.12). Post-hoc analysis revealed significant increases in %BL responses at 2.0 (t_{33}=2.95, adj. p=0.02) and 4.0 (t_{33}=2.97, adj. p=0.02) mg/kg MA relative to 0.5 mg/kg %BL values (2-tailed paired t-tests with Bonferroni correction; significant increases indicated by arrows in panel **Fig. 3c**).

2 h post-MA: There were no significant main effects or interactions (all p>0.16; **Fig. 3d**).

M50

10 min post-MA: There was a significant Genotype x Sex interaction ($F_{3,90}$=4.18, p=0.04; **Fig. 4a**). There were no other significant interactions and no significant main effects (all p>0.10). We deconstructed the RM-ANOVAs along the Sex factor and analyzed the sexes separately. In the males (n=19), there was no significant main effect of Genotype or Dose and no interaction (all p>0.52; **Fig. 4c**). In the females (n=14), there was a main effect of Genotype ($F_{1,14}$=6.734, p=0.02; **Fig. 4d**), a main effect of Dose ($F_{3,42}$=3.85, p=0.02), and no interaction ($F_{3,42}$=1.28, p=0.30). Post-hoc 2-tailed paired t-tests with Bonferroni correction revealed a significant decrease in % BL M50 values following 2.0 mg/kg compared to those after 0.5 mg/kg across both genotypes (t_{16}=2.89, p=0.01). Pairwise comparisons revealed no significant differences at any individual dose (all p>0.05) between genotypes. When %BL M50 values were assessed in a dose-independent manner, we found $H1^{+/-}$ females showed significantly lower values compared to H1 WT females (95% CI for mean difference: -28.87, -15.38; p=0.03).

2 h post-MA: There were no significant main effects or interactions (all p's>0.18; **Fig. 4b**).

%MCR by Frequency

10 Min Post-MA

For all RM-ANOVAs there was a significant main effect of Frequency (all p<0.0001).

0.5 mg/kg: There were significant Genotype x Frequency ($F_{14,420}$=3.75; p<0.0001; **Fig. 5a**:) and Genotype x Sex x Frequency ($F_{14,420}$=1.78; p=0.04) interactions, but no main effects of Genotype or Sex (both p>0.10). Data were deconstructed along the Sex factor prior to further analysis. For the males, there was a significant Genotype x Frequency interaction ($F_{14,238}$=3.93; p<0.0001) and no main effect of Genotype ($F_{1,17}$=2.05; p=0.17). Post-hoc analysis revealed no Frequency-specific differences between Genotypes (all adj. p>0.54; 2-tailed unpaired t-tests with Bonferroni correction; **Fig. 5e**). Analysis of females revealed no main effect of Genotype nor a significant Genotype x Frequency interaction (both p>0.19; data not shown).

1.0 mg/kg: We found a significant Genotype x Sex x Frequency interaction ($F_{14,420}$=2.03; p=0.01; **Fig. 5b**) and no main effect of Genotype or Sex (both p>0.75). Data were again broken down along the Sex factor for further analysis. For the males, there was a significant Genotype x Frequency interaction ($F_{14,238}$=2.80; p<0.001) and no main effect of Genotype ($F_{1,17}$=0.29; p=0.60; **Fig. 5f**). Post-hoc analysis revealed no Frequency-specific differences between Genotypes (all adj. p>0.54; 2-tailed unpaired t-tests with Bonferroni correction). Analysis of female data revealed no main effect of Genotype nor a significant Genotype x Frequency interaction (both p>0.49; data not shown).

2.0 mg/kg: There was a near significant Genotype x Sex x Frequency interaction ($F_{14,420}$=1.64; p=0.06; **Fig. 5c**) and no main effects of Genotype or Sex (both p>0.18).

4.0 mg/kg: There was a significant Genotype x Frequency interaction ($F_{14,420}$=3.65; p<0.0001; **Fig. 5d**) and no main effects of Genotype or Sex (both p>0.45). Post-hoc analysis revealed no Frequency-specific differences between Genotypes (all adj. p>0.88; 2-tailed unpaired t-tests with Bonferroni correction).

2h Post-MA

For all RM-ANOVAs there was a significant main effect of Frequency (all $p<0.02$).

0.5 mg/kg: There were no significant interactions and no main effects of Genotype or Sex (both $p>0.13$; **Fig. 6a**).

1.0 mg/kg: We found a significant Sex x Frequency interaction ($F_{14,266}=3.02$; $p<0.001$; **Fig. 6b**), a main effect of Sex ($F_{1,19}=8.82$; $p=0.008$), and no effect of Genotype ($F_{1,19}=1.14$; $p=0.30$). Post-hoc analysis revealed females had significantly higher %MCR values at 112 Hz relative to males ($T_{21}=3.45$; adj. $p=0.03$; 2-tailed unpaired t-test with Bonferroni correction; **Fig. 7a)**.

2.0 mg/kg: We found significant Genotype x Sex x Frequency ($F_{14,266}=2.04$; $p=0.02$; **Fig. 6c**) and Sex x Frequency ($F_{14,266}=2.99$; $p<0.001$) interactions, a main effect of Sex ($F_{1,19}=6.76$; $p=0.02$), and no main effect of Genotype ($F_{1,19}=0.02$; $p=0.89$). For further analysis, we deconstructed the data along the Sex factor and performed additional RM-ANOVAs. For both males and females, there was no significant Genotype x Frequency interaction (both p's>0.10) and no main effect of Genotype (both p's>0.57; data not shown). Post-hoc analysis revealed females had significantly higher %MCR values at 89 Hz relative to males ($t_{21}=3.41$; adj. $p=0.04$; 2-tailed unpaired t-test with Bonferroni correction; **Fig. 7b**).

4.0 mg/kg: There was a significant Sex x Frequency interaction ($F_{14,266}=2.31$; $p=0.005$; **Fig. 6d**), a main effect of Sex ($F_{1,19}=11.56$; $p=0.003$), and no main effect of Genotype ($F_{1,19}=0.59$; $p=0.45$). Post-hoc analysis revealed females had significantly higher %MCR values at 126 ($t_{21}=3.36$; adj. $p=0.04$), 79 ($t_{21}=3.43$; adj. $p=0.04$), 71 ($t_{21}=3.82$; adj. $p=0.01$), and 63 ($t_{21}=4.10$; adj. $p=0.01$) Hz compared to males (2-tailed unpaired t-tests with Bonferroni correction; **Fig. 7c**).

Concomitant Behaviors

Distance to the Response Wheel

10 m post-MA: There was a main effect of Genotype ($F_{1,30}=6.29$; $p=0.02$; **Fig. 8a**) but no other significant main effects or interactions (all $p>0.27$).

2 h post-MA: There was a significant Genotype x Dose x Sex interaction ($F_{1,19}=3.40$; $p=0.02$; **Fig. 8b**). After deconstructing the data along the Sex factor, we performed additional RM-ANOVAs for each sex individually. There were no significant main effects (Dose, Genotype) or interactions for these analyses (males: all p's>0.16; females: all p's>0.15).

Time Spent Immobile in Corners

10 min post-MA: There was a significant main effect of Dose ($F_{3,90}=3.85$; $p=0.01$; **Fig. 8c**) and no other significant main effects or interactions (all $p>0.12$). Post-hoc analysis revealed significant increases in %BL responses at 2.0 ($T_{33}=2.95$, adj. $p=0.02$) and 4.0 ($T_{33}=2.97$, adj. $p=0.02$) mg/kg MA relative to 0.5 mg/kg %BL values (2-tailed paired t-tests with Bonferroni correction; significant increases indicated by arrows in panel **Fig. 8c**).

2 h post-MA: There were no significant main effects or interactions (all $p>0.16$; **Fig. 8d**).

DISCUSSION

$H1^{+/-}$ mice showed lower RRs than WT mice relative to BL levels at 10 min post-MA injection (**Fig. 3a**). This effect was present in the absence of significant differences in total number of responses relative to BL, indicating that the reduction in MA-induced potentiation of ICSS responding in $H1^{+/-}$ mice could not be explained by MA-induced performance deficits in operant responding. Irrespective of genotype, there was an increase in total operant responses with increasing doses of MA (post-2.0 and 4.0 mg/kg relative to 0.5 mg/kg %BL levels; **Fig. 3C**). There was no significant effect of $H1^{+/-}$ genotype on maximum response rates or total responses when assessed at 2 h post-MA (**Fig. 3b&d**), suggesting a time-dependence of H1 genotype-linked ICSS response phenotypes.

A leftward shift in the M50 value (or decrease relative to the baseline M50 value) is interpreted as potentiation of reward sensitivity, such that the rewarding properties of MFB stimulation (as indicated by an increase in the number of operant responses) are enhanced at lower stimulation frequencies relative to BL levels. In females (10 min post-MA), there was a significant decrease in %BL M50 values following 2.0 mg/kg MA, relative to 0.5 mg/kg level, suggesting greater reward potentiation at this dose (**Fig. 4d**). We also found that $H1^{+/-}$ females, independent of MA dose, had lower %BL M50 values than WT females, indicating heightened sensitivity to the reward-potentiating effects of ICSS. It is not clear why increased reward potentiation was only observed in $H1^{+/-}$ females. Percent BL M50 values did not differ by genotype, dose, or sex at 2 h post-MA

administration (**Fig. 4b**), which could indicate time-dependence in the effect of $H1^{+/-}$, or alternatively, this negative result could be explained by prior MA exposure 2 weeks earlier when the 10 min dataset was collected.

We observed male-specific effects of H1 genotype on %MCR responding when measured 10 min post-MA. Specifically, following 0.5 and 1.0 mg/kg, $H1^{+/-}$ males showed a decrease and increase in %MCR responding, respectively, in a manner that depended on frequency (**Fig. 5e&f**). Additionally, following 4.0 mg/kg MA, we observed a significant decrease in %MCR values in $H1^{+/-}$ mice, irrespective of Sex (**Fig. 5d**). The combined results indicate that $H1^{+/-}$ genotype modulates %MCR responding in a Sex- and Dose- dependent manner, suggesting nuanced mechanisms of reward modulation mediated by the interaction of $H1^{+/-}$, MA treatment, and underlying Sex-dependent molecular mechanisms.

The lack of genotypic differences in %BL M50 values in males indicates that genotypic differences in %MCR following 0.5 mg/kg and 1.0 mg/kg MA are mediated by differences in the *magnitude* of reward potentiation post-MA rather than differences in *sensitivity* to the stimulation. In other words, a selective change in %MCR values indicates a modulation of reward efficacy, and the lack of shift in M50 values reflects no significant change in potency of stimulation to produce BSR. $H1^{+/-}$ mice (females and males combined) also showed a decrease in reward magnitude following 4.0 mg/kg MA, indicating potential modulation of reward magnitude induced by the H1 mutation at this dose.

When mice were tested 2 h post-MA, there were no overall genotypic differences in %MCR responding (**Fig. 6a-d**), suggesting that H1+/- only modulates reward magnitude early post-MA, but not after its peak pharmacological and behavioral effects have subsided. It is interesting to note that males (irrespective of genotype) showed significantly lower %MCR response values compared to females following the 1.0, 2.0, and 4.0 mg/kg doses MA (**Fig. 7a-c**). Our results indicate that male mice have robust deficits in reward magnitude relative to female mice following the peak onset of MA that could potentially be attributable to differing pharmacokinetic and/or metabolic profiles between the sexes (Lominac et al. 2014) or to differences in pharmacodynamics of MA-induced regulation of DAT or VMAT that can affect the time course of changes in DA neurotransmission (Dluzen et al. 2008; Dluzen & McDermott 2008; Milesi-Hallé et al. 2005).

A caveat for the dataset collected at 2 h post-MA is that these mice were previously exposed to MA 3 weeks prior for the dataset collected at 10 min post-MA. Prior exposure to MA can potentiate the effects of MA re-exposure on behavior (Jing et al. 2014) and impact DA concentration in the striatum (McFadden et al. 2015; Lominac et al. 2014). $H1^{+/-}$ mice could have disrupted transcriptional regulation of key genes involved in psychostimulant sensitization and physical dependence (Cates et al. 2018; Zhu et al. 2016; Piechota et al. 2012), resulting in effects on reward sensitivity and magnitude at the time of re-exposure that depend on prior exposure. The effects of prior MA exposure on reinforcement depends on several factors, including dose, length of exposure, and latency to re-exposure. For example, while acute MA treatment typically *reduces* ICSS thresholds, chronic MA treatment can *increase* ICSS thresholds (Jang et al. 2013). Future studies could parse out whether the differences in BSR magnitude and sensitivity in $H1^{+/-}$ mice can be explained by the shorter time interval between MA administration and ICSS assessment or by a lack of prior MA exposure. Despite this limitation, our study highlights the utility of assessing reward function at different time points following drug exposure (e.g., peak onset versus post-peak onset), which can reveal time-dependent genotypic effects. The hedonic valence associated with the acute “come-down” phase following acute/limited administration is not typically assessed in ICSS (as opposed to more delayed assessments of withdrawal-induced anhedonia following extensive high-dose chronic administration (Paterson et al. 2000)) and likely impacts later responding to rewarding stimuli and drug seeking.

The effects of $H1^{+/-}$ on Post-MA ICSS responding could be mediated by differences in MA pharmacokinetics (i.e., transport or metabolism) or differences in the pharmacodynamics of DA release and signaling in the NAc (Otani et al. 2008; P Chan et al. 1994; Albers and Sonsalla 1995; Bousman et al. 2009b). Testing mice at 2 h post-MA administration partly addresses the pharmacokinetic explanation as it encompasses a time-frame that avoids peak MA concentrations in the brain based on the fact that the half-life for MA elimination from the brain of mice following i.p. injection at the doses used herein is approximately 1 h (Brien et al. 1978). Furthermore, we previously found no difference in brain concentration of MA or its metabolite at 30 min post-MA (Ruan et al. 2020b). Nevertheless, a full time-course of brain and plasma concentrations at different doses would more fully address the question of potential pharmacokinetic differences and MA bioavailability.

A novel component of this study was measuring concomitant behavioral differences during ICSS testing. While we did not identify genotypic differences in these behavioral phenotypes, we observed dose-dependent changes in behavior with increasing MA doses. The average distance to the response wheel significantly

decreased following the 2.0 and 4.0 mg/kg MA doses when assessed at 10 min post-MA (**Fig. 8a**). This behavioral trend intuitively aligns with the significant increase in %BL total responses that was observed following these same doses (**Fig. 8c**). Furthermore, decreased distance to the response wheel potentially reflects reinforcement-induced place preference within the testing chamber, as it could reflect the association of MA-modulated reward potentiation with the ICSS response apparatus. Additionally, time spent immobile in the corners of the testing chamber was significantly reduced following 4.0 mg/kg MA when assessed at both 10 min and 2 h post-MA. Thigmotaxis (spending more time in the periphery of a behavioral apparatus) is considered an anxiety-like behavioral pattern typical of rodents ((Walz et al. 2016, Simon et al. 1994). Here, we interpret the time spent immobile in the corners of the ICSS testing chambers to be a proxy for anxiety-like behavior, akin to spending more time in the corners or time away from the center in the open field assay (Prut and Belzung 2003; Harro 2018). This behavior could also be attributable to reduced reward sensitivity/magnitude and therefore reduced tendency to leave the corners and respond for BSR. The tendency for mice to spend less time in the corners of the apparatus could indicate increased motivation to leave the corners and res pond for BSR and/or that they are experiencing fewer anxiety-related effects of MA, which have been observed following acute MA exposure (Miladi-Gorji et al. 2015). While the ICSS chamber is notably smaller than a typical open field arena, these behavioral differences offers novel insight and complement the trends observed in the ICSS responding.

Despite the significant decrease in MA-induced locomotor activity we have repeatedly observed in $H1^{+/-}$ mice (Yazdani et al. 2015; Ruan et al. 2020b), we did not detect any genotypic differences in MA-induced locomotor activity during ICSS testing sessions. This discrepancy is likely attributable to the small size of the operant chambers used during testing (relative to the larger open field used to map this quantitative phenotype), as well as restricted movement due to attachment to the stimulation apparatus. In support, the reduction in MA-induced locomotor activity in $H1^{+/-}$ mice was more robust in an undivided open field arena (Yazdani et al. 2015) compared to the enclosed MA side of the CPP chamber (Ruan et al. 2020b).

We present evidence of altered reward modulation in $H1^{+/-}$ mice that was only observed after MA administration. The data suggest the reward-related effects of H1 mutation depend on MA exposure, as there was no effect of Genotype on key measurements in the MA-naïve state including MECs determined during training (**Fig. 2**) and drug-naïve M50 values (data not shown). All significant effects of Genotype were exclusively observed following MA exposure. These observations suggest that $H1^{+/-}$ mice do not exhibit basal neurobehavioral dysfunction in response to MFB stimulation.

In conclusion, $H1^{+/-}$ mice showed time-, sex-, and dose-dependent differences in reward sensitivity (% BL M50), maximum responses (% BL maximum RRs), and reward magnitude (%MCR). We also identified concomitant behavioral differences in activity and location within the ICSS chambers which, at times, aligned with the operant data and could offer novel insight into changes in reward sensitivity and salience. As an extension of our previous findings indicating that $H1^{+/-}$ mice show reduced MA-induced locomotor activity, reduced low-dose MA MA-CPP and reduced MA oral self-administration (Yazdani et al. 2015; Ruan et al. 2020b), the present results provide further evidence that $H1^{+/-}$ mice show changes in MA-induced reward processing that could behaviorally reflect the altered dynamics and magnitude of MA-induced extracellular dopamine levels (Ruan et al. 2020b). Ongoing studies are deciphering the molecular mechanisms driving deficits in MA-induced release and behavior in $H1^{+/-}$ mice. These findings provide support that normal *Hnrnph1* function contributes to MA-induced changes in reward processing with different effects on reward sensitivity depending on the drug dose, incubation period following drug administration, and sex.

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FIGURES

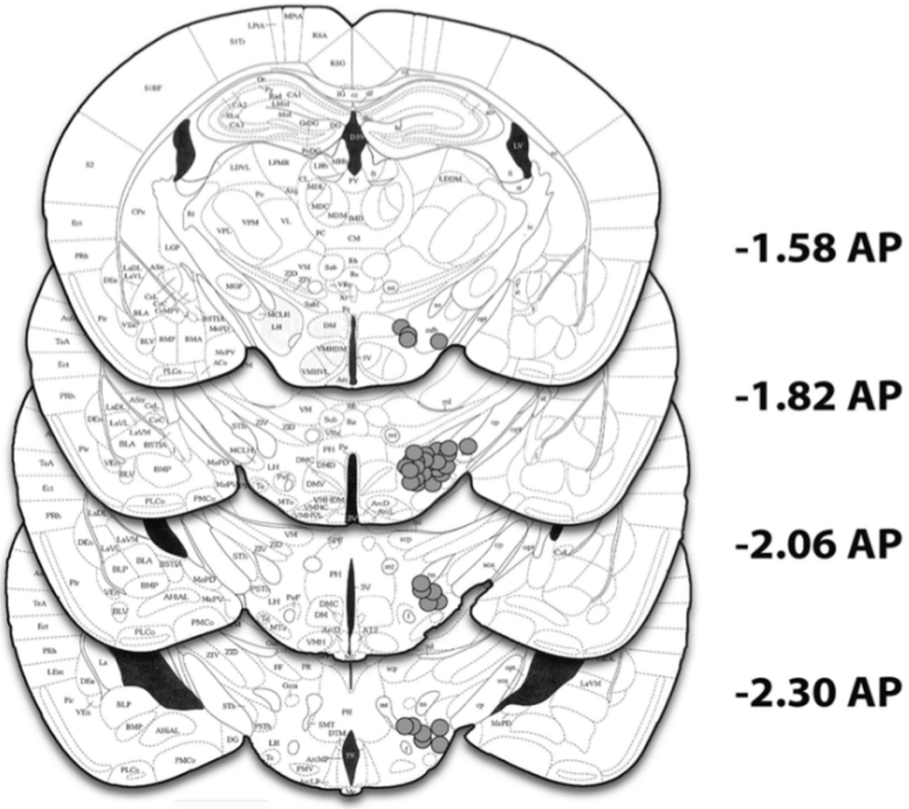

Fig. 1 Schematic of electrode placements in the MFB determined via histological sectioning and staining. Anterior/posterior (AP) coordinates (relative to Bregma) corresponding to each coronal section (Paxinos and Franklin 2004) are listed on the right

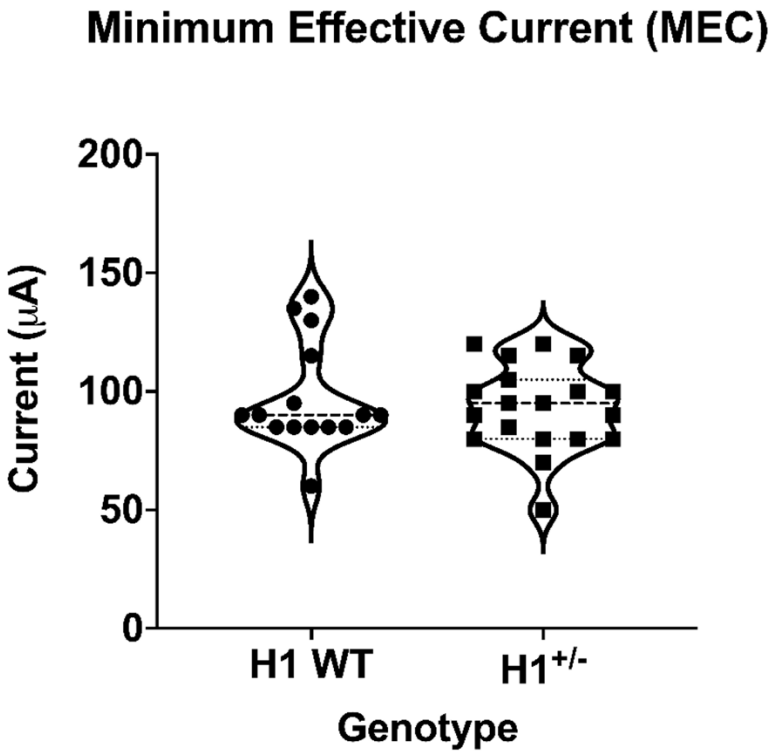

Fig. 2 Minimum Effective Current (MEC) in H1$^{+/-}$ mice. Violin plot of MEC distribution. A 2-way ANOVA revealed no significant effect of Genotype or Sex on MECs and no interaction (all p's>0.28)

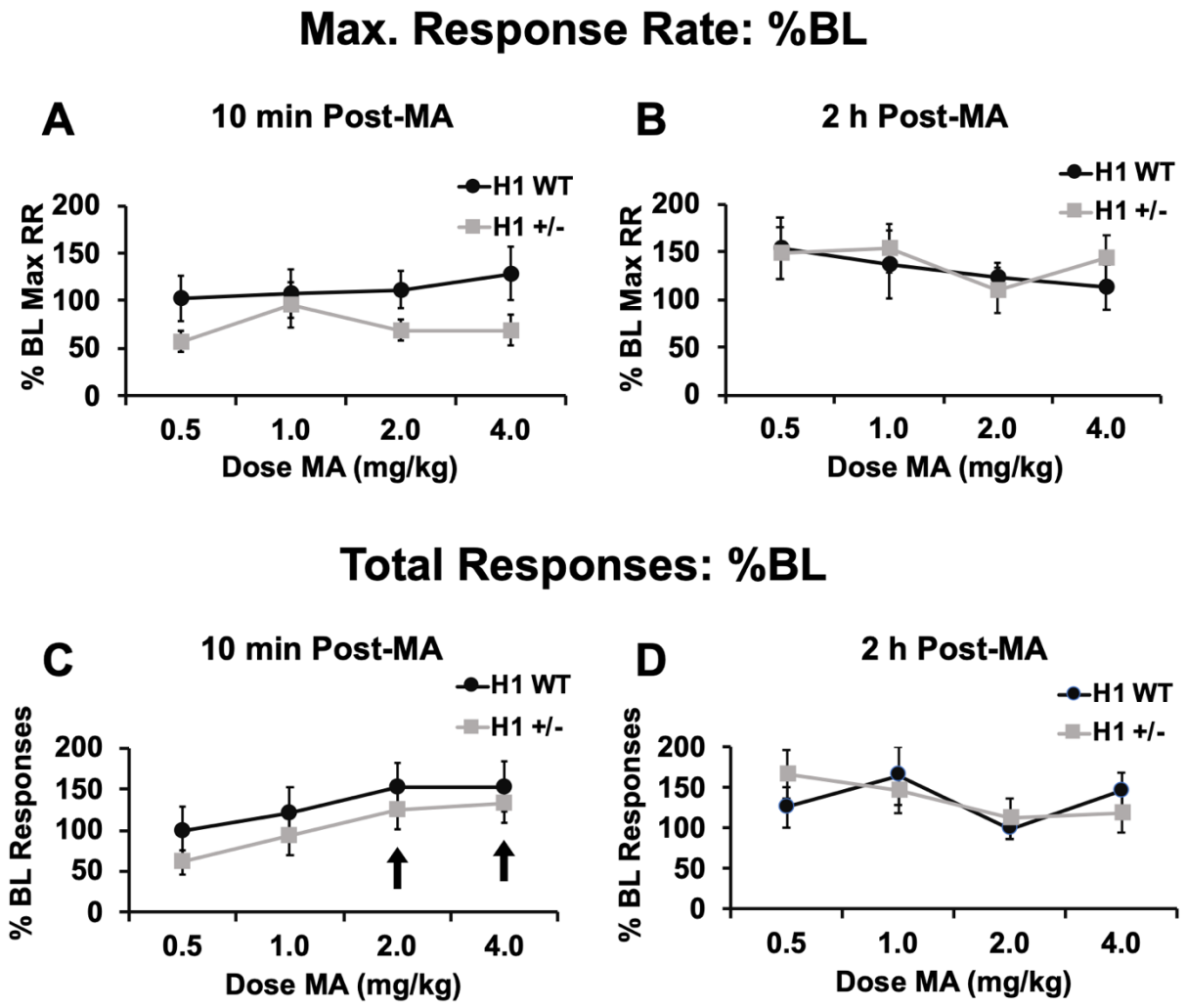

Fig. 3 ICSS response rates in H1$^{+/-}$ mice at 10 min and 2 h post-MA administration. Post-MA response rates during ICSS testing sessions are presented relative to same-day baseline rates for each genotype and dose MA (% BL ± S.E.M.). **a-b**: Average maximum response rates post-MA are presented relative to baseline values (%BL) for the 10 min (**a**) and 2 h (**b**) post-MA experiments. **a**: 10 min post-MA: There was a main effect of Genotype ($F_{1,30}$=6.29; p=0.02) and no other significant main effects or interactions (all p>0.27)., However, H1$^{+/-}$ mice had significantly lower %BL values than WT mice when averaged across all doses (95% CI for mean difference: -71.30, -7.817; p=0.02). **b**: 2 h post-MA: There was a significant Genotype x Dose x Sex interaction ($F_{1,19}$=3.40; p=0.02). After deconstructing the data along the Sex factor, additional RM-ANOVAs for each sex indicated no significant main effects (Dose, Genotype) or interactions for these analyses (males: all p's>0.16; females: all p's>0.15). **c-d**: The percent increase in responses relative to BL responses (%BL) is presented for 10 min post-MA (**c**) and 2 h post-MA (**d**). **c** 10 min post-MA: There was a significant main effect of Dose ($F_{3,90}$=3.85; p=0.01) and no other significant main effects or interactions (all p>0.12). Post-hoc analysis revealed significant increases in % BL responses at 2.0 (t_{33}=2.95, adj. p=0.02) and 4.0 (t_{33}=2.97, adj. p=0.02) mg/kg MA relative to 0.5 mg/kg % BL values (2-tailed paired t-tests with Bonferroni correction; significant increases indicated by arrows in panel **c**). **d**: 2 h post-MA: There were no significant main effects or interactions (all p>0.16)

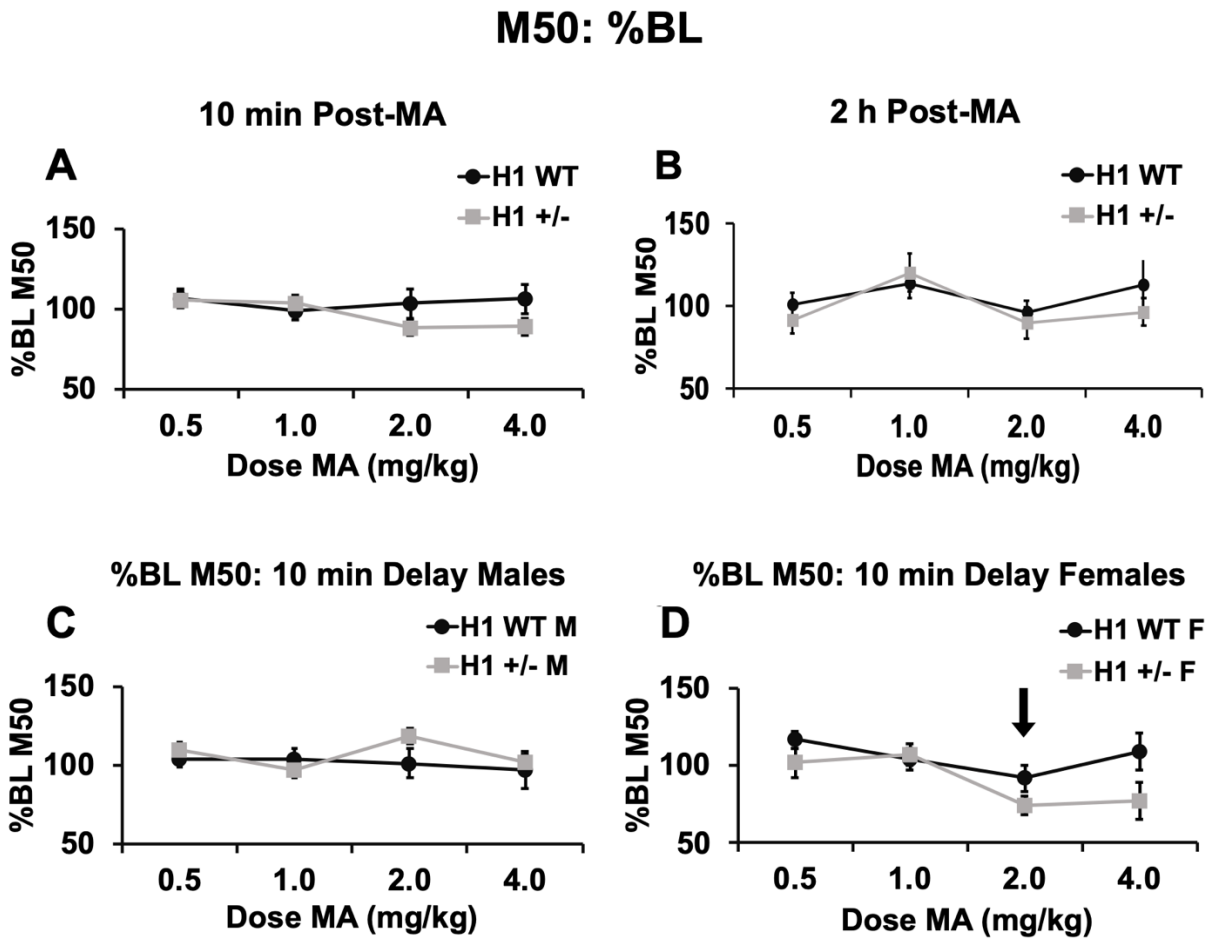

Fig. 4 M50 values presented as percentage of same-day baseline M50 values following intermittent, increasing doses of MA in H1$^{+/-}$ versus H1 WT mice. Data reflect % BL M50 values ± S.E.M. % BL M50 values are shown for each Genotype and Dose 10 min (**a**) and 2 h (**b**) post-MA. **a:** 10 min delay: There was a significant Genotype x Sex interaction ($F_{3,90}$=4.18, p=0.04). There were no other significant interactions and no significant main effects (all p>0.10). The data are shown broken down by Sex in panels **c** and **d**. **b**: 2 h delay: There were no significant main effects or interactions (all p>0.18). **c:** 10 min post-MA males: We found no significant main effects nor a significant interaction (all p>0.52). **d**: 10 min post-MA females: There was a main effect of Genotype ($F_{1,14}$=6.734, p=0.02), a main effect of Dose ($F_{3,42}$=3.85, p=0.02), and no significant interaction ($F_{3,42}$=1.28, p=0.30). Post-hoc 2-tailed paired t-tests with Bonferroni correction revealed a significant decrease in % BL M50 values following 2.0 mg/kg MA compared to those after 0.5 mg/kg MA across both genotypes (t_{16}=2.89, p=0.01; decrease indicated by arrow in panel **d**). Pairwise comparisons revealed no significant differences at any individual dose (all p>0.05) between genotypes. When % BL M50 values were assessed in a dose-independent manner, H1$^{+/-}$ females had significantly lower values compared to WT females (95% CI for mean difference: -28.87, -15.38; p=0.03)

10 Min Post-MA: %MCR

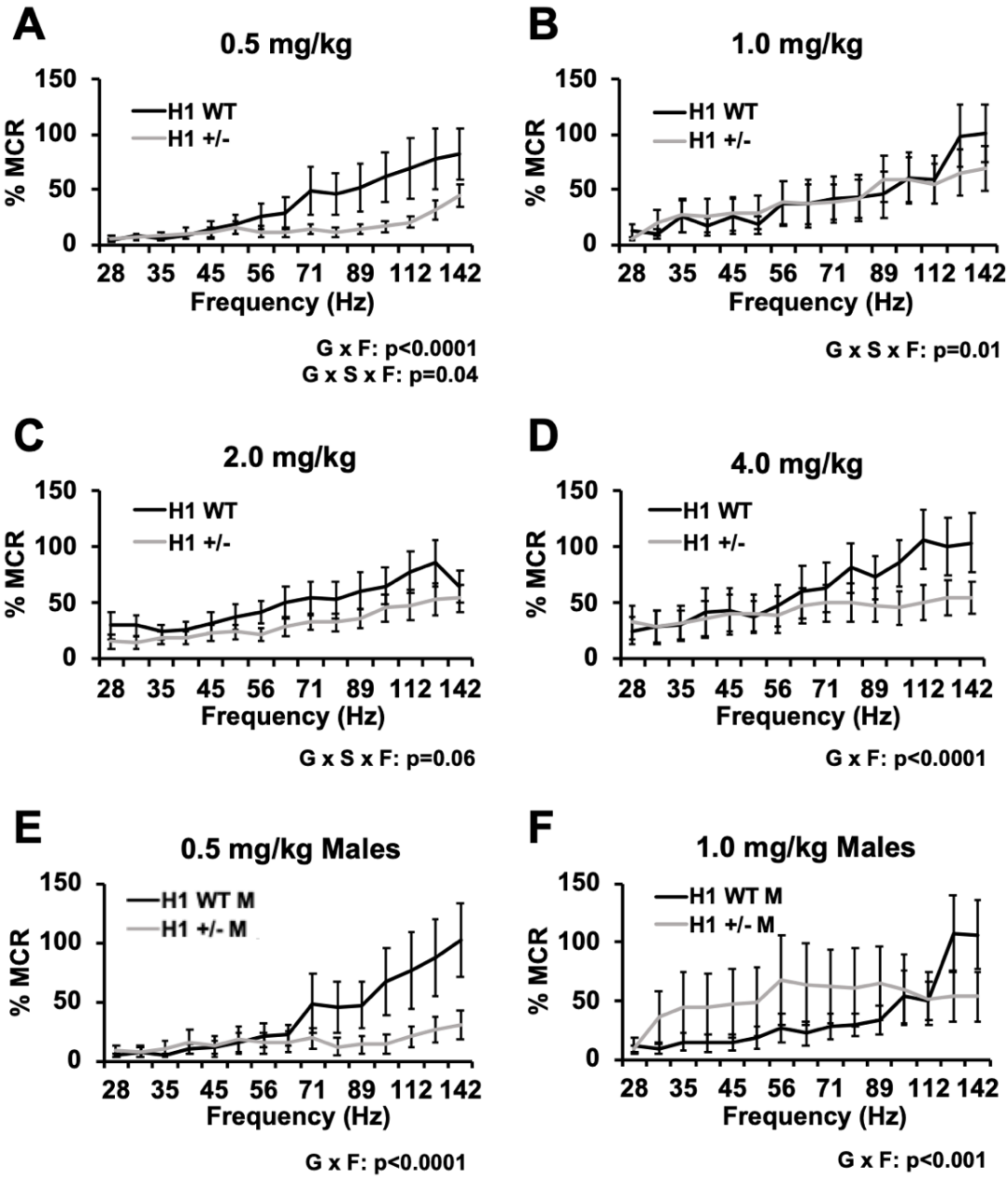

Fig. 5 ICSS in H1$^{+/-}$ mice: 10 min post-MA % Maximum Control Rate (MCR) across frequencies. Data reflect % MCR values ± S.E.M. **a-d:** Sex-combined % MCR values for 0.5, 1.0, 2.0, and 4.0 mg/kg MA, respectively, are shown for each genotype across increasing stimulation frequencies. **e-f**: % MCR values for the 0.5 mg/kg MA dose are shown for males (**e**) and 1.0 mg/kg MA (**f**) doses across increasing stimulation frequencies. For all RM-ANOVAs there was a significant main effect of Frequency (all $p<0.0001$). **a**: 0.5 mg/kg MA: There were significant Genotype x Frequency ($F_{14,420}=3.75$; $p<0.0001$) and Genotype x Sex x Frequency ($F_{14,420}=1.78$; $p=0.04$) interactions and no main effects of Genotype or Sex (both $p>0.10$). Data were deconstructed along the Sex factor prior to further analysis. Results for analysis of males is described in **e.** Analysis of females revealed no main effect of Genotype nor a significant Genotype x Frequency interaction (both $p>0.19$; data not shown). **b**: 1.0 mg/kg MA: There was a significant Genotype x Sex x Frequency interaction ($F_{14,420}=2.03$; $p=0.01$) and no main effect of Genotype or Sex (both $p>0.75$). Data were again deconstructed along the Sex factor for further analysis. Results for males are shown in **f.** Analysis of females revealed no main effect of Genotype nor a significant Genotype x Frequency interaction (both $p>0.49$; data not shown). **c**: 2.0 mg/kg MA: The Genotype x Sex x Frequency interaction did not quite reach statistical significance ($F_{14,420}=1.64$; $p=0.06$) and there were no main effects of Genotype or Sex (both $p>0.18$). **d**: 4.0 mg/kg MA: There was a significant Genotype x Frequency interaction ($F_{14,420}=3.65$; $p<0.0001$) and no main effects of Genotype or Sex (both $p>0.45$). Post-hoc analysis revealed no frequency-specific differences between Genotypes (all adj. $p>0.88$; 2-tailed unpaired t-tests with Bonferroni correction). **e**: 0.5 mg/kg MA in males: There was a significant Genotype x Frequency interaction ($F_{14,238}=3.93$; $p<0.0001$) and no main effect of Genotype ($F_{1,17}=2.05$; $p=0.17$). Post-hoc analysis revealed no frequency-specific differences between Genotypes (all adj. $p>0.54$; 2-tailed unpaired t-tests with Bonferroni correction). **f**: 1.0 mg/kg MA in males: There was a significant Genotype x Frequency interaction ($F_{14,238}=2.80$; $p<0.001$) and no main effect of Genotype ($F_{1,17}=0.29$; $p=0.60$). Post-hoc analysis revealed no frequency-specific differences between Genotypes (all adj. $p>0.54$; 2-tailed unpaired t-tests with Bonferroni correction). Significant main effects (other than frequency) and interactions indicated below Fig. panels with their respective p values. G=Genotype, S=Sex, F=Frequency

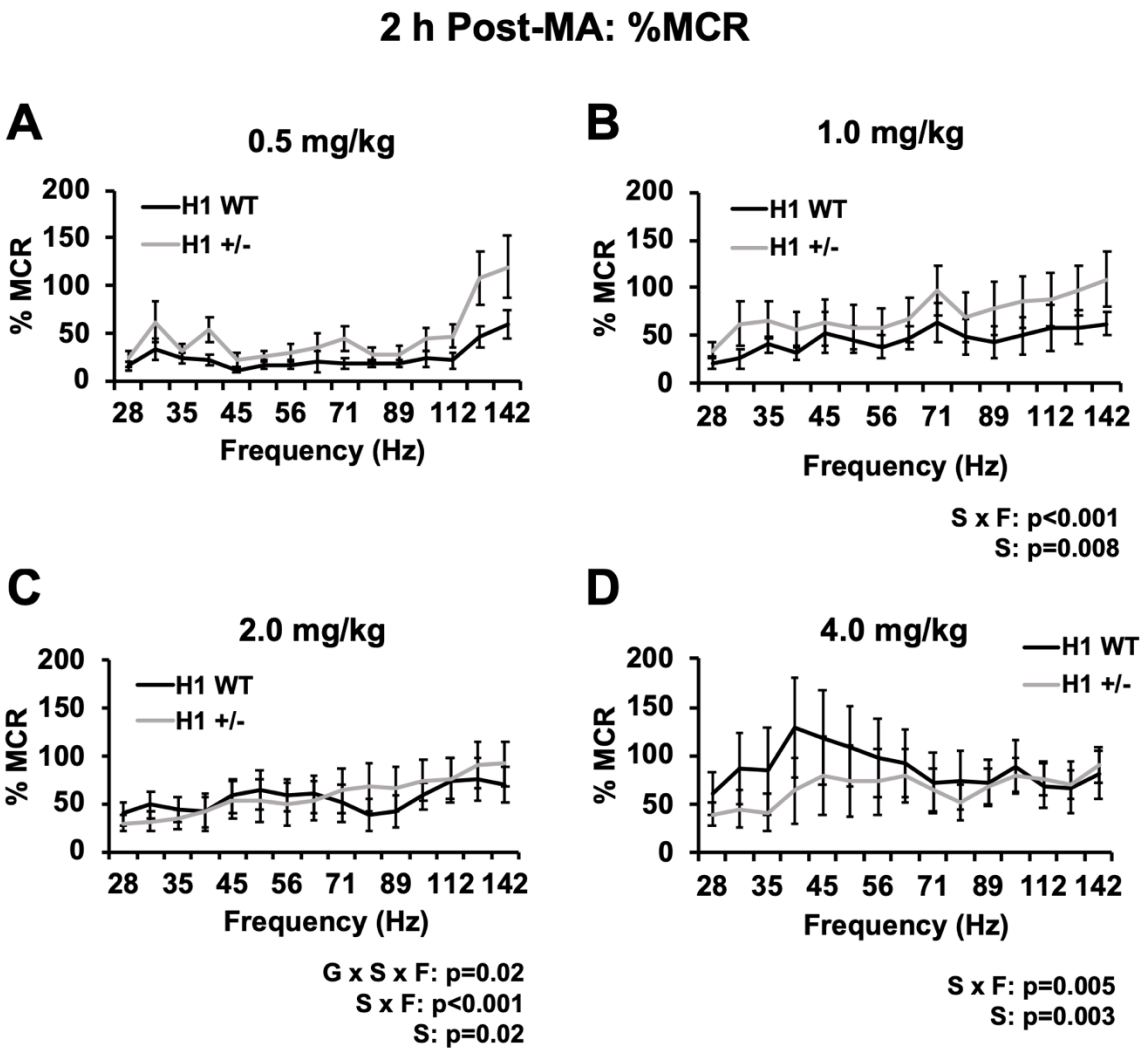

Fig. 6 ICSS in H1$^{+/-}$ mice: 2 h post-MA % Maximum Control Rate (MCR) by Frequency. Data reflect %MCR values ± SEM **a-d:** %MCR values for each dose (**a**: 0.5 , **b**: 1.0, **c**: 2.0, and **d**: 4.0; mg/kg) MA shown for each genotype across increasing stimulation frequencies. For all RM-ANOVAs there was a significant main effect of Frequency (all p<0.02). **a**: 0.5 mg/kg: There were no significant interactions and no main effects of Genotype or Sex (both p>0.13). **b**: 1.0 mg/kg: We found a significant Sex x Frequency interaction ($F_{14,266}$=3.02; p<0.001), a main effect of Sex ($F_{1,19}$=8.82; p=0.008), and no effect of Genotype ($F_{1,19}$=1.14; p=0.30). Results showing Frequency-specific differences by Sex are shown in **Fig. 7a**. **c**: 2.0 mg/kg MA: We found significant Genotype x Sex x Frequency ($F_{14,266}$=2.04; p=0.02) and Sex x Frequency ($F_{14,266}$=2.99; p<0.001) interactions, a main effect of Sex ($F_{1,19}$=6.76; p=0.02), and no main effect of Genotype ($F_{1,19}$=0.02; p=0.89). For further analysis, we deconstructed the data along the Sex factor and performed additional RM-ANOVAs. For both females and males, there was no significant Genotype x Frequency interaction (both p's>0.10) and no main effect of Genotype (both p's>0.57; data not shown). Results showing frequency-specific differences by Sex are shown in **Fig. 7b**. **d**: 4.0 mg/kg MA: There was a significant Sex x Frequency interaction ($F_{14,266}$=2.31; p=0.005), a main effect of Sex ($F_{1,19}$=11.56; p=0.003), and no main effect of Genotype ($F_{1,19}$=0.59; p=0.45). Results showing Frequency-specific differences by Sex are shown in **Fig. 7c**. Significant main effects (other than frequency) and interactions indicated below Fig. panels with their respective p values. G=Genotype, S=Sex, F=Frequency

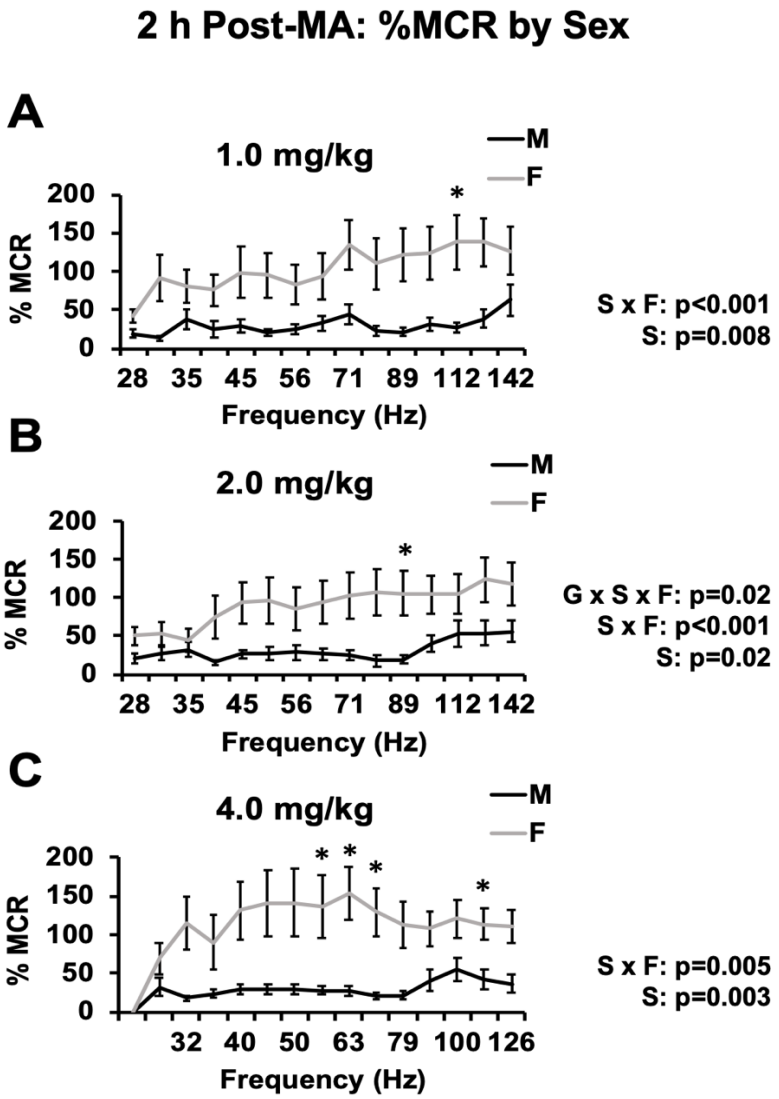

Fig. 7 Females show a lower % Maximum Control Rate (MCR) compared to males at 2 h post-MA administration. Data reflect % MCR values +/- S.E.M. **a-c**: % MCR values shown for each dose (**a**: 1.0 , **b**: 2.0, and **c**: 4.0; mg/kg) MA shown for each sex across increasing stimulation frequencies. **a**: 1.0 mg/kg MA: There was a significant Sex x Frequency interaction ($F_{14,266}$=3.02; p<0.001), a main effect of Sex ($F_{1,19}$=8.82; p=0.008), and no effect of Genotype ($F_{1,19}$=1.14; p=0.30). Post-hoc analysis revealed females had significantly higher %MCR values at 112 Hz relative to males (t_{21}=3.45; adj. p=0.03; 2-tailed unpaired t-test with Bonferroni correction). **b**: 2.0 mg/kg: We found significant Genotype x Sex x Frequency ($F_{14,266}$=2.04; p=0.02) and Sex x Frequency ($F_{14,266}$=2.99; p<0.001) interactions, a main effect of Sex ($F_{1,19}$=6.76; p=0.02), and no main effect of Genotype ($F_{1,19}$=0.02; p=0.89). For further analysis, we deconstructed the data along the Sex factor and performed additional RM-ANOVAs. For both females and males, there was no significant Genotype x Frequency interaction (both p's>0.10) and no main effect of Genotype (both p's>0.57; data not shown). We then analyzed frequency-specific differences in % MCR between the sexes. Post-hoc analysis revealed females had significantly higher % MCR values at 89 Hz relative to males (t_{21}=3.41; adj. p=0.04; 2-tailed unpaired t-test with Bonferroni correction). **c**: 4.0 mg/kg: There was a significant Sex x Frequency interaction ($F_{14,266}$=2.31; p=0.005), a main effect of Sex ($F_{1,19}$=11.56; p=0.003), and no main effect of Genotype ($F_{1,19}$=0.59; p=0.45). Post-hoc analysis revealed females had significantly higher % MCR values at 126 (t_{21}=3.36; adj. p=0.04), 79 (t_{21}=3.43; adj. p=0.04), 71 Hz (T_{21}=3.82; adj. p=0.01), and 63 Hz (t_{21}=4.10; adj. p=0.01) compared to males (2-tailed unpaired t-tests with Bonferroni correction). * = frequencies at which there are significant differences (p<0.05) in % MCR values between the sexes. Significant main effects (other than frequency) and interactions indicated to the right of Fig. panels with their respective p values. G=Genotype, S=Sex, F=Frequency

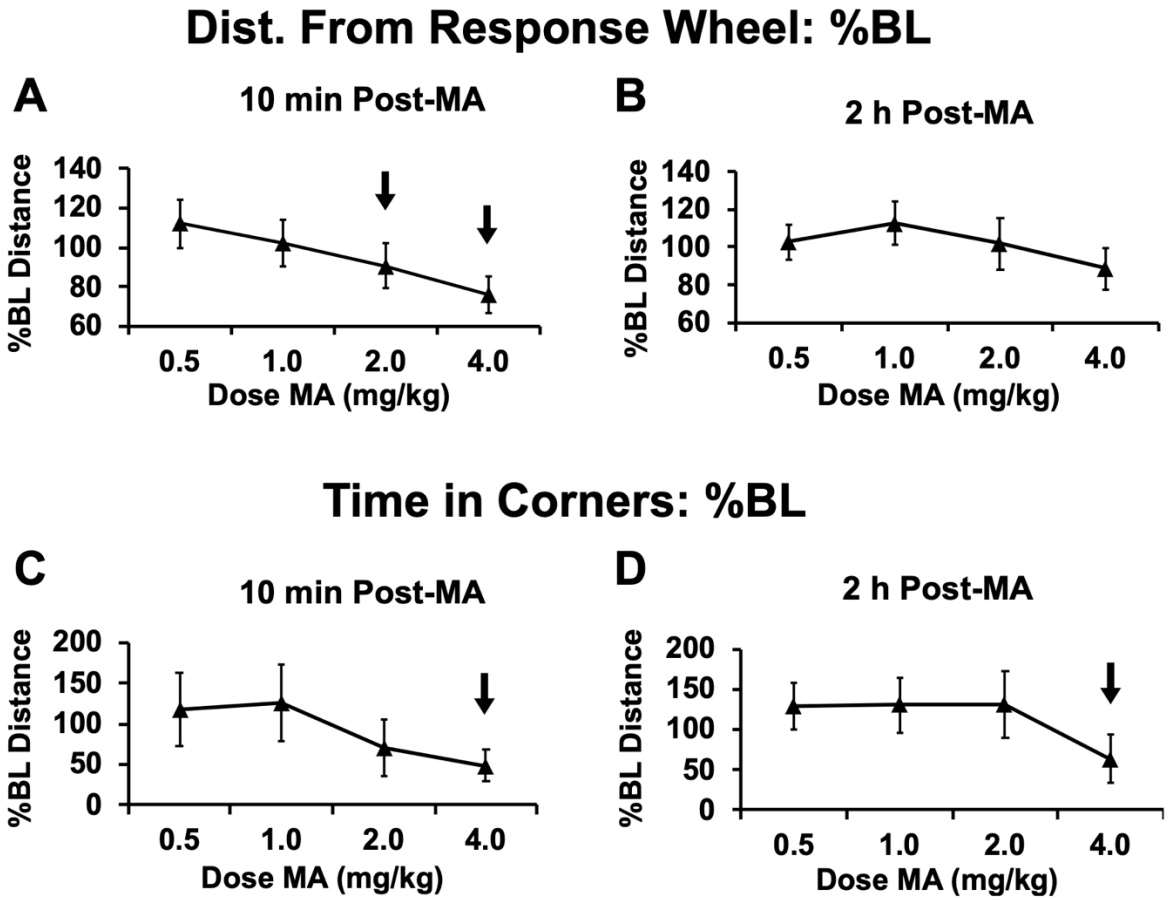

Fig. 8 Dose-dependent changes in distance to the response wheel and time spent immobile in corners in response to MA. Data are presented as % BL ± S.E.M. for average distance to the response wheel (**a-b**) and average time spent in the corners of the operant chambers (**c-d**) during ICSS testing sessions. **a**: 10 min post-MA: There was a main effect of Dose ($F_{3,66}$=8.08, p=0.0001) and no other significant main effects or interactions (all p>0.15). Post-hoc analysis revealed % BL values at 2.0 mg/kg MA (t_{25}=2.56, adj. p=0.04) and at 4.0 mg/kg MA (t_{25}=4.49, adj. p<0.001) mg/kg MA were significantly lower than 0.5% BL values, indicating that mice were located closer to the response wheel with increasing MA doses (2-tailed paired t-tests with Bonferroni correction; significant increases indicated by arrows in panel **a**). **b**: 2 h post-MA: Again, there was a significant main effect of Dose on wheel distance ($F_{3,57}$=2.84, p=0.04) and no other significant main effects or interactions (all p>0.11). Post-hoc analysis did not reveal any significant pairwise differences at 1.0, 2.0, or 4.0 mg/kg relative to 0.5 mg/kg % BL values (all p>0.27). **c**: 10 min post-MA: There was a main effect of Dose ($F_{3,57}$=4.13, p=0.01) and no other significant main effects or interactions (all p>0.09). Post-hoc analysis revealed a significant decrease following 4.0 mg/kg MA (t_{22}=2.80, p=0.03; decrease indicated by arrow in panel **c**) relative to 0.5 mg/kg MA %BL values indicating immobile corner time decreased after the highest MA dose (4.0 mg/kg) relative to corner time after the lowest MA dose (0.5 mg/kg). **d**: 2 h post-MA: Again, there was a significant main effect of Dose on corner time ($F_{3,57}$=3.77, p=0.01) and no other main effects or interactions (all p>0.17). Post-hoc analysis again revealed a significant decrease following 4.0 mg/kg MA (t_{22}=3.02, p=0.02; decrease indicated by arrow in panel **d**) relative to 0.5 mg/kg MA % BL values. Similar to the results for the 10 min post-MA experiment, mice spent less time immobile in the corners of the operant chamber following the 4.0 mg/kg MA dose relative to the 0.5 mg/kg MA dose