Reciprocal regulation of the H_3 histamine receptor in Rett syndrome and *MECP2* Duplication syndrome: implications for therapeutic development

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Abstract

Rett syndrome (RTT) and *MECP2* Duplication syndrome (MDS) are disorders caused by reciprocal decreases and increases, respectively, in expression of the methyl reader protein, Methyl CpG Binding Protein 2 (MeCP2). MeCP2 is a transcriptional regulator that induces changes in the expression of thousands of genes. We previously performed an mRNA expression profiling study of the temporal cortex region from a cohort of autopsy samples from patients diagnosed with RTT and corresponding age, postmortem interval, and sex-matched controls. These studies identified a significant reduction in the expression of the histamine H_3 receptor (*HRH3*) in RTT patients compared to controls. In the current manuscript, we expand this H_3 receptor profiling to additional RTT patient brain samples representing distinct disease mutations and confirm significantly reduced levels of H_3 receptor expression in the majority of patients compared to controls. Using mouse models of RTT and MDS, we observed reciprocal changes in H_3 receptor expression across various brain areas, with *Hrh3* expression being reduced in RTT model animals and increased in a mouse model of MDS. We then tested the hypothesis that phenotypes in these mouse models would be sensitive to an H_3 receptor agonist and antagonist, respectively. This point is particularly salient for MDS, as there are no approved treatments available; encouragingly, however, the H_3 antagonist/inverse agonist pitolisant (Wakix®) has recently been approved for the treatment of narcolepsy, and we sought here to determine if there was potential to repurpose pitolisant for MDS. We evaluated both a small molecule agonist of the H_3 receptor, (*R*)-α-methylhistamine (RAMH), and pitolisant in RTT and MDS models, respectively, to determine impacts on phenotypes in these disease models. Our results show that RAMH significantly impacted an anxiety phenotype in mice modeling RTT (*Mecp*$^{Null/+}$), but pitolisant had no effect on the behaviors examined here in MDS animals (*MECP2*^{Tg1}).

40 **Introduction**

41 Rett syndrome (RTT) is caused by mutations in the X-linked gene encoding Methyl CpG Binding
42 Protein 2 (MeCP2), a transcriptional regulator of both local and global gene expression patterns (Amir, Van
43 den Veyver et al. 1999, Chahrour, Jung et al. 2008, Ben-Shachar, Chahrour et al. 2009). Although RTT can
44 occur in either sex, RTT patients are predominantly female and develop normally during their first 6 to 18
45 months of life; they then undergo rapid developmental regression, resulting in impairments in language and
46 motor skills, breathing abnormalities such as apneas, and the emergence of seizures (Neul, Kaufmann et al.
47 2010). In addition to RTT, patients can also exhibit duplication or triplication of the *MECP2* gene, resulting in
48 *MECP2* duplication syndrome (MDS), a disorder characterized by numerous abnormal symptoms such as
49 hypotonia, seizures, intellectual disability, and recurrent infections (Ramocki, Peters et al. 2009, Ramocki,
50 Tavyev et al. 2010, Collins and Neul 2022). One symptomatic treatment currently available for RTT is the
51 recently approved compound trofinetide (Daybue®) (Neul, Percy et al. 2023); other strategies are aimed
52 specifically at MeCP2 and include gene therapy, DNA and RNA editing, X-chromosome reactivation, and
53 pharmacological read-through approaches (reviewed in (Collins and Neul 2022, Palmieri, Pozzer et al. 2023);
54 NCT05898620, NCT06152237). There are currently no approved therapeutic options specifically for the
55 treatment of MDS.

56 The availability of expression profiling data from the brains of RTT patients provides an opportunity to
57 identify or confirm therapeutic targets or clarify biological mechanisms important for RTT that begin from a
58 translational perspective. We previously performed an RNA expression profiling study of the temporal cortex
59 region using autopsy samples from patients diagnosed with RTT and corresponding age, postmortem interval,
60 and sex-matched controls (Gogliotti, Fisher et al. 2018). These studies identified a number of G protein-
61 coupled receptors (GPCRs) that were changed in expression in RTT patients, including several metabotropic
62 glutamate (mGlu) and muscarinic receptor candidates (Gogliotti, Fisher et al. 2018). Further evaluation of
63 these RNA sequencing data revealed significantly reduced expression of the histamine H_3 receptor (*HRH3*) in
64 brain samples from the cerebellum of RTT patients. As shown here, expansion of this profiling set into a larger
65 cohort of temporal cortical samples from 14 control and 29 RTT patients revealed significant decreases in the
66 expression of *HRH3* mRNA in the majority of RTT patients compared to controls. Additionally, we verified
67 decreased *Hrh3* mRNA expression in several brain areas of $Mecp2^{Null/+}$ mice, a commonly used model of RTT

68 (Guy, Hendrich et al. 2001). In parallel, we also confirmed increased levels of *Hrh3* mRNA in brains from mice
69 modeling MDS (*MECP2*^{Tg1}, (Collins, Levenson et al. 2004)). These observations correlate with findings from
70 other studies showing decreases in mouse *Hrh3* expression in male mice lacking Mecp2 (*Mecp2*$^{Null/y}$), as well
71 as reciprocal increases in expression in *Mecp2Tg1* animals (Chahrour, Jung et al. 2008, Ben-Shachar,
72 Chahrour et al. 2009, Renthal, Boxer et al. 2018). Additionally, a study profiling the occipital cortex from four
73 female RTT patients demonstrated a significant decrease in the expression of *HRH3* mRNA in cells expressing
74 an MeCP2 mutant (Supplemental Table 7 in (Renthal, Boxer et al. 2018)). Recently, a profiling study of RTT
75 patients reported decreased levels of histidine, the precursor for histamine, in the plasma of RTT patients
76 compared to unaffected siblings (Neul, Skinner et al. 2020).

77 Histaminergic projections originate in the tuberomammillary nucleus of the hypothalamus and H_3
78 receptors are found throughout the brain with particular abundance in the cortex, thalamus, and nucleus
79 accumbens (Leurs, Bakker et al. 2005, Nieto-Alamilla, Marquez-Gomez et al. 2016). Activation of the
80 presynaptic H_3 receptor modulates not only the release of histamine, but also other neurotransmitters such as
81 dopamine, serotonin, GABA, and acetylcholine (Brown, Stevens et al. 2001, Galici, Boggs et al. 2009). H_3
82 receptors expressed on synapses projecting to the nucleus accumbens (NAc) regulate anxiety phenotypes and
83 repetitive behaviors by, among other mechanisms, decreasing presynaptic release of glutamate (Zhang, Peng
84 et al. 2020). Additionally, mice fed a low-histidine diet for two weeks exhibit lower levels of brain histamine and
85 release less histamine in the hypothalamus after stimulation; these animals also display increases in anxiety-
86 related behaviors in elevated maze and light-dark transition tasks (Yoshikawa et al., 2014).

87 Based on our expression profiling, we evaluated the activity of a small molecule agonist of the H_3
88 receptor, (*R*)-α-methylhistamine (RAMH), on several behaviors in *Mecp2*$^{Null/+}$ mice; additionally, we tested the
89 hypothesis that the clinically available H_3 receptor antagonist/inverse agonist pitolisant (Wakix®) might be
90 effective in correcting phenotypes in MDS animals. Our results show that RAMH induced an anxiolytic effect in
91 *Mecp2*$^{Null/+}$ animals; however, pitolisant was without effect in *MECP2*^{Tg1} animals in the behavioral assays
92 profiled here. Overall, these results suggest that there may be therapeutic utility in increasing H_3 receptor
93 activity to treat specific domains of RTT.

Materials and Methods

Human Brain Sample Profiling

Human samples were obtained from the National Institutes of Health NeuroBioBank (neurobank.nih.gov) under Public Health Service contract HHSN-271-2013-00030. The tissues were post-mortem and fully de-identified, and as such are classified as exempt from human subject research regulations. The demographics of autopsy samples used in these studies were published in (Vermudez, Freitas et al. 2025) and the 29 RTT and 14 control Brodmann Area (BA) 38 (temporal cortex) samples used for this study are shown in Table 1.

RNA-cDNA Preparation

Approximately 1 g of human tissue was impact dissociated under dry ice and then pulverized using mortar and pestle under liquid nitrogen. Total RNA was prepared from 100 to 200 mg of tissue using standard Trizol-chloroform methodology. Total RNA was purified and DNase-treated using an RNeasy kit (Qiagen, Hilden, Germany). RNA quality was confirmed using BioCube and BioAnalyzer instrumentation by the Vanderbilt Technologies for Advanced Genomics core (Vanderbilt University Medical Center). cDNA was synthesized from 2 µg total RNA with the SuperScript VILO kit (Thermo Fisher, Waltham, MA). Samples from the cortex, striatum, cerebellum, and hippocampus were prepared from mice using identical methodology.

Quantitative RT-PCR (qRT-PCR)

qRT-PCR (CFX96, Bio-Rad, Vanderbilt University Medical Center MCBR core) using 50 ng/9 µL cDNA from the cortex, hippocampus, striatum and cerebellum samples from *Mecp2*$^{Null/+}$ and *MECP2*^{Tg1} mice was run in duplicate using PowerUp™ SYBR™ Green Master Mix (ThermoFisher cat no. A25742) with the following primers (5' to 3'): human H_3 receptor (forward: TCTTCCTGCTCAACCTCGCCAT, reverse: ACTACCAGCCACAGCTTGCAGA, 127 bp), mouse H_3 receptor (forward: CTTCCTCGTGGGTGCCTTC, reverse: CAGCTCGAGTGACTGACAGG, 179 bp), and human/mouse *GAPDH*/*Gapdh* (forward: CGACTTCAACAGCAACTCCC, reverse: GCCGTATTCATTGTCATACCAGG, 106 bp). All primer sequences were designed using Primer3 (SYBR Green) and constructed by Sigma through the Vanderbilt University Medical Center MCBR core. Ct values for each sample were normalized to *GAPDH*/*Gapdh* expression and analyzed using the delta−delta Ct method (Livak and Schmittgen 2001). Values exceeding two times the

121 standard deviation were classified as outliers. Each value was compared to the average delta-Ct value
122 acquired for wild-type controls and calculated as percent-relative to the average control delta-Ct.

123 **Animals**

124 All animals used in the present study were group housed with food and water given ad libitum and
125 maintained on a 12hr light/dark cycle. Animals were cared for in accordance with the National Institutes of
126 Health Guide for the Care and Use of Laboratory Animals. All studies were approved by the Vanderbilt
127 Institutional Animal Care and Use Committee and took place during the light phase. *Mecp2*$^{Null/+}$ (B6.129P2(C)-
128 *Mecp2*$^{tm1.1Bird}$/J, stock no. 003890) mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA)
129 and maintained on a C57BL/6J background by breeding *Mecp2*$^{Null/+}$ animals with WT C57BL/6J mice (The
130 Jackson Laboratory, stock no. 000664). As a reflection of the predominantly female RTT patient population,
131 female *Mecp2*$^{Null/+}$ mice were utilized and aged to at least 20 weeks of age prior to experiments. *MECP2*$^{Tg1/o}$
132 mice (C57BL/6J background) were generously shared by Dr. Jeffrey Neul (Vanderbilt University Medical
133 Center) and were bred with C57BL/6J animals. Both sexes of mice were used between 6-10 weeks of age.
134

135 **Drugs**

136 RAMH, the *R*-enantiomer of α-methylhistamine dihydrobromide (H_3 receptor agonist) was purchased
137 from Tocris Bioscience (catalog number 0569, Bristol, UK) and pitolisant hydrochloride was purchased from
138 AdooQ BioScience (catalog number 15213, Irvine, CA). All drugs for behavioral experiments were diluted in
139 0.9% saline.
140

141 **Behavioral Assays**

142 All behavioral experiments were conducted at predicted symptomatic ages (20-25 week-old female
143 *Mecp2*$^{Null/+}$ mice and 8-12 weeks for male and female *MECP2*^{Tg1} animals, as well as corresponding littermate
144 controls) at the Vanderbilt Mouse Neurobehavioral Lab Core. All experiments were preceded by intraperitoneal
145 (i.p.) injections: vehicle (0.9% sterile saline), RAMH (45 mg/kg, 15 min pretreatment) or pitosilant hydrochloride
146 (10 mg/kg, 30 min pretreatment). Quantification was performed either by a researcher blinded to the genotype
147 and/or by automated software.
148

149

150 **Open Field**

151 Mice were placed in the activity chamber for 60 min. Exploratory and locomotor activity was quantified
152 using Activity software by assessing beam breaks in the X, Y and Z axis (Med Associates Inc).

153

154 **Elevated Zero Maze**

155 The elevated zero maze has two regions that are closed (contain walls) and two regions that are open
156 (no walls). Mice were allowed to freely explore the maze for five minutes under full light conditions (~ 700 lux in
157 the open regions, ~ 400 lux in the closed regions). The percentage of time spent in the open versus closed
158 regions and number of region-transitions was measured. The assay was tracked by a mounted camera and
159 analyzed by automated AnyMaze software (Stoelting, Wood Dale, IL, USA).

160

161 **Hindlimb clasping**

162 *Mecp2*$^{Null/+}$ female mice show a clasping phenotype that becomes apparent in our colony at 16 weeks
163 of age (Gogliotti, Senter et al. 2017). In mice, hind limb clasping was assessed by suspending a mouse by its
164 tail and video recording clasping dynamics for a period of 1 minute, which were scored by a blinded reviewer.

165

166 **Fear Conditioning**

167 On both the training (Day 1) and test days (Day 2), mice were habituated to the testing room for 1 hour
168 prior to the start of the behavioral assay. On Day 1, mice received either vehicle, RAMH or pitolisant. Following
169 treatment, mice were placed into the operant chamber equipped with a shock grid (Med Associates Inc.) and a
170 10% vanilla odor cue. After a 3-minute acclimation period, mice received 2x foot shocks (0.7 mA, 2 seconds
171 duration) separated by a 60 second interval, followed by an additional 30 second recovery period in the chamber.
172 On Day 2, 24 hours later, mice were returned to the same chamber for 3 minutes in the presence of 10% vanilla
173 odor cue; however, no foot shocks were administered. Freezing behavior was recorded and quantified using
174 Video Freeze software (Med Associates Inc., St. Albans, VT, USA).

175

176 **Statistical Analyses**

177 Statistics were carried out using GraphPad Prism and Excel (Microsoft). All data shown represent mean
178 ± SEM. Statistical significance between genotypes was determined using Student's t-test, or 1- or 2-way
179 ANOVA with Tukey's post-hoc tests. Sample size (denoted as "n"), statistical test and results of statistical
180 analyses are specified in each figure legend.

Results

Histamine receptor H_3 expression is reduced in brain samples from RTT patients and *Mecp2*$^{Null/+}$ mice. We previously performed an RNA sequencing study using nine cortical and six cerebellar brain samples from patients with either R168X, R255X, or R270X mutations in *MECP2,* as well as control samples matched for age, brain region, postmortem interval, and sex (Gogliotti, Fisher et al. 2018). These studies identified a significant reduction in mRNA expression of the histamine H_3 receptor (*HRH3*) in the cerebellum of RTT patients versus controls (control abundance, 8.040, RTT abundance, 4.789; fold change -0.747, *p=0.011, (Gogliotti, Fisher et al. 2018)). We confirmed that *HRH3* mRNA expression was decreased in these cerebellar samples using qRT-PCR (**Figure 1A**); although not significant in the initial RNA sequencing study, we also observed a significant decrease in *HRH3* expression in the motor cortex using qRT-PCR (**Figure 1B).** We then expanded these studies to samples from the temporal cortex of a larger cohort of RTT patients representing different *MECP2* mutations (**Table 1**). We observed a range of expression levels; across the entire cohort, expression was not significantly different between genotypes (unpaired Student's t-test, p=0.9718). However, as shown in **Figure 1C**, we observed five samples that were separated in expression from the remainder of the RTT cohort by more than 3 standard deviations; age and corresponding mutation are also shown. When these samples were removed and the expression of *HRH3* of the remaining RTT samples was compared to control, we found a significant decrease in the remaining RTT samples (**Figure 1C**).

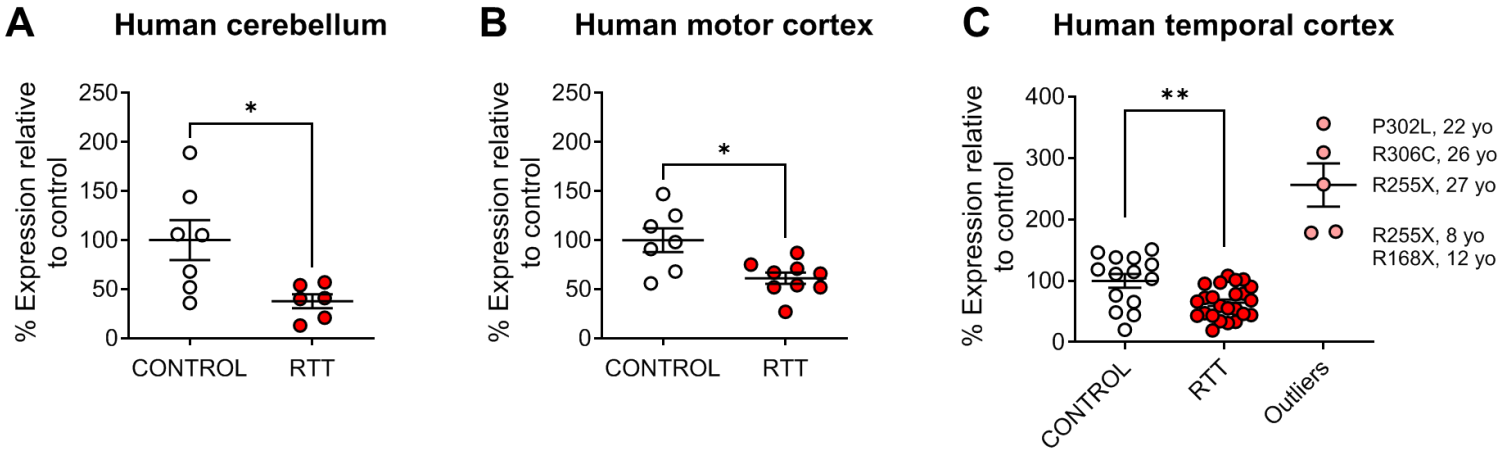


Figure 1. Among the group III mGlu receptors, only *GRM7* mRNA is decreased in cortical autopsy samples from RTT patients. RNA was prepared from human tissue from the BA38 region of the temporal cortex and quantitative RT-PCR was performed for **(A)** *GRM7*, **(B)** *GRM4*, and **(C)** *GRM8*. Data shown are mean ± SEM and were performed in duplicate and averaged. Data were normalized to *G6PD* control and

202 analyzed as unpaired t-test for each receptor (*GRM7*: control versus RTT, p=0.0012 (**); *GRM4* and *GRM8*:

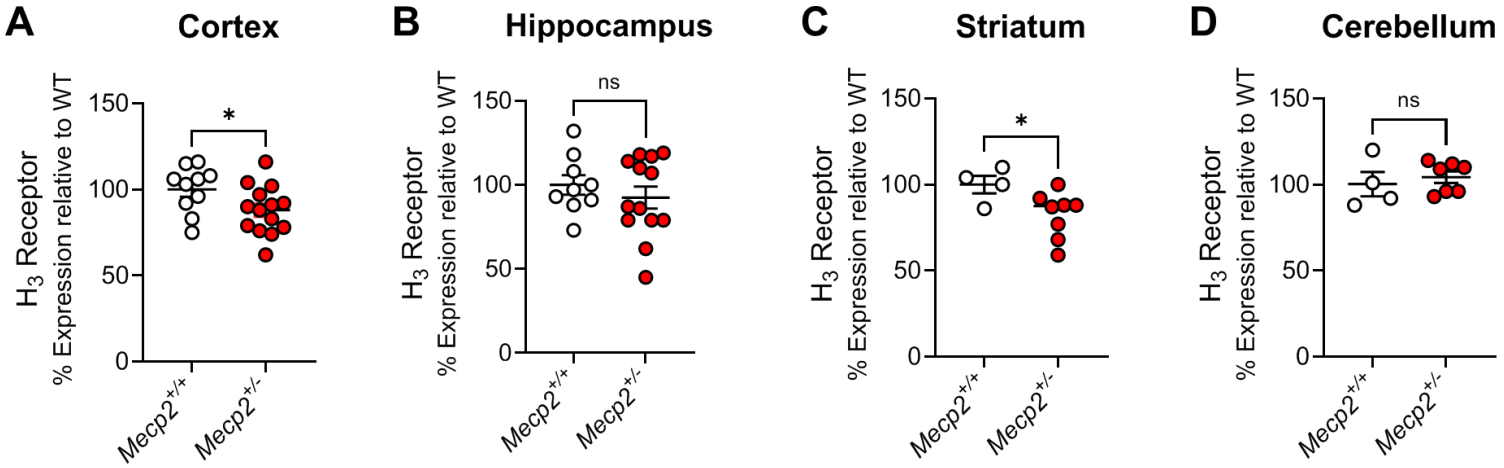
203 ns). Outliers in each dataset are noted in **Table 1**.

204 **Table 1. Demographics of human patient samples.**

Controls	**UNMB number**	**Age**	**PMI**	***MECP2* Mutation**
CTL-1	1038	24	7	N/A
CTL-3	5161	10	22	N/A
CTL-4	5554	13	15	N/A
CTL-6	5844	42	12	N/A
CTL-7	5566	15	23	N/A
CTL-8	5309	14	8	N/A
CTL-9	4330	19	19	N/A
CTL-10	5446	18	18	N/A
CTL-11	5538	19	24	N/A
CTL-12	6544	30	23	N/A
CTL-13	5646	21	23	N/A
CTL-14	5669	25	29	N/A
CTL-15	5670	17	22	N/A
CTL-16	5751	25	24	N/A
AVE		**20.9**	**19.2**	
SEM		**2.2**	**1.7**	

RTT	**Maryland BB number**	**Age**	**PMI**	***MECP2* Mutation**
Rett-1	AN19242	41		R255X
Rett-3	AN15579	11	9.6	R255X
Rett-4	AN04573	12	5.0	R270X
Rett-5	AN08016	8	2.9	R255X
Rett-6	AN02091	24	15.8	R255X
Rett-7	AN04121	10	23.5	R270X
Rett-11	AN01508	26	28.1	R306C
Rett-13	AN13266	33	23.7	R270X
Rett-14	AN17730	22	31.8	P302L
Rett-16	AN15264	35	25.4	R168X
Rett-19	AN07799	25	31.0	T158M
Rett-21	AN06847	24	24.6	T158M
Rett-22	AN05652	41	48.8	R133C
Rett-23	AN07309	12	16.8	R255X
Rett-24	AN05180	57	16.7	CTD >398
Rett-25	AN01082	7	33.2	Y141X
Rett-26	AN17849	12	3.1	R168X
Rett-27	AN14876	26	17.1	R270X
Rett-28	AN05375	20	23.3	G269del
Rett-32	AN05266	21	19.7	R168X
Rett-33	AN09497	27	32.1	R255X
Rett-34	AN19272	16	27.6	A2V
Rett-35	AN02949	15	21.3	T158M
Rett-36	AN01220	10	24.0	M246del
Rett-37	AN05357	12	8.1	R168X
Rett-38	AN03862	35	32.5	G269del
Rett-40	AN15992	42	20.4	A202G
Rett-43	AN08774	6	23.5	P322L
Rett-45	AN07559	8	22.7	R306C
AVE		**22.0**	**21.9**	
SEM		**2.4**	**1.9**	

205 The observation that there were reductions in *HRH3* expression in the brains of many of the patients
206 replicated the work of Renthal et al. from the occipital cortex (Renthal, Boxer et al. 2018), suggesting that the
207 H_3 receptor might serve as a novel, druggable therapeutic target in RTT. To translate our findings to animals,
208 we next evaluated mRNA expression of the mouse H_3 receptor in samples from *Mecp2*$^{Null/+}$ female animals via
209 qRT-PCR in brain samples from the cortex, hippocampus, striatum and cerebellum (**Figure 2A-D**). These
210 studies revealed significant decreases in *Hrh3* receptor mRNA expression in the cortex (*p=0.0474) and
211 striatum (*p=0.0451) in 20-week-old *Mecp2*$^{Null/+}$ animals compared to wild-type controls.

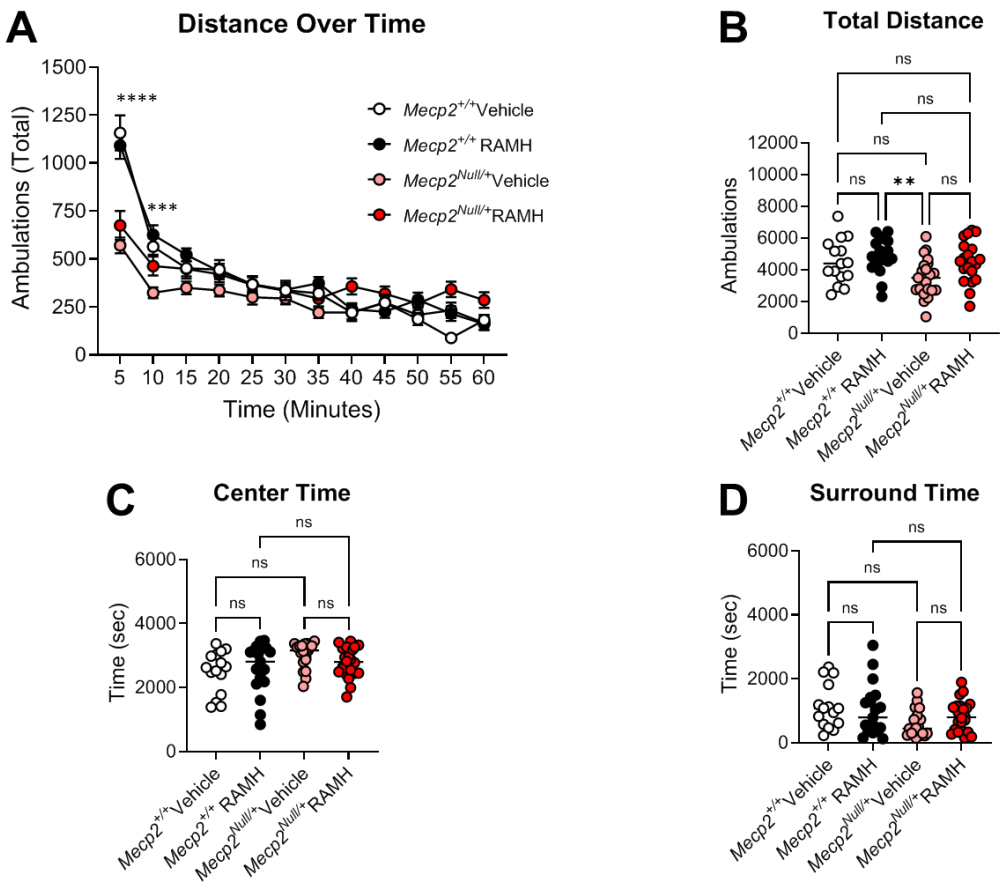


212 **Figure 2. *GRM7* expression is significantly decreased in patients with mutations that induce more**
213 **severe, but not mild, RTT symptoms**. **(A)** *GRM7* mRNA is significantly reduced in patients with severe
214 (R168X, R255X, R270X) mutations (red) and in patients with mutation-negative RTT (green), but not in
215 patients with mild mutations (R133C, T158M, and R306C). **(B)** *GRM4* and **(C)** *GRM8* are not significantly
216 different in any group versus control. Data represent mean +/- SEM and were analyzed using one-way
217 ANOVA; for *GRM7*, F (3,41) 5.928, **p=0.0019; Dunnett's post-hoc test, control versus mild, p=0.4064; control
218 versus severe, **p=0.0095, control versus mutation negative, **p=0.0013. *GRM4* one-way ANOVA,
219 F (3, 40) 1.783, p=0.1659. *GRM8* one-way ANOVA, F (3, 39) = 1.098, p=0.3617.

220

221 **The H_3 receptor agonist, RAMH, does not affect locomotion in an open field but increases time**
222 **in the open arms in an elevated zero maze in *Mecp2*$^{Null/+}$ mice.** We evaluated the effects of the compound
223 *(R)*-α-methylhistamine (RAMH, 45 mg/kg, ip), an agonist of the H_3 receptor (Arrang, Devaux et al. 1988,
224 Arrang, Garbarg et al. 1988, Rapanelli, Frick et al. 2016) on several behavioral phenotypes in symptomatic 20-
225 week-old *Mecp2*$^{+/+}$ and *Mecp2*$^{Null/+}$ female animals: locomotion in an open field, anxiety as assessed by

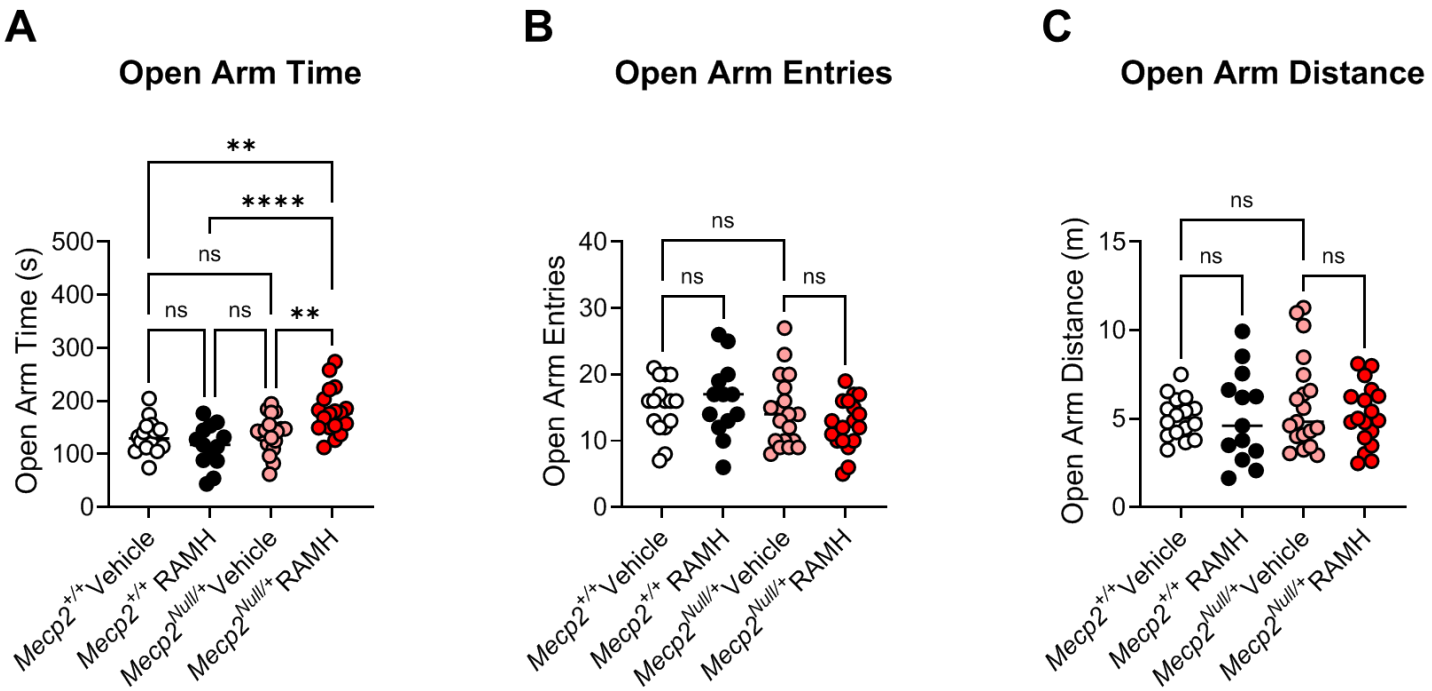
226 elevated zero maze, repetitive behavior using a hindlimb clasping measurement, and learning and memory in a
227 Pavlovian task, conditioned fear. In the open field assessment, mice were administered either RAMH or vehicle
228 15 minutes prior to testing and then monitored in the open field for 60 minutes (**Figure 3A**). Vehicle-treated
229 *Mecp2*$^{Null/+}$ animals exhibited reductions in distance traveled in the early time points of the task at 5 and 10
230 minutes compared to *Mecp2*$^{+/+}$ controls (**Figure 3A**), but this effect between vehicle-treated animals was not
231 significant once ambulation was calculated across the entire 60-minute evaluation period (**Figure 3B**).
232 *Mecp2*$^{Null/+}$ treated with RAMH also did not exhibit significantly different total distance traveled compared to
233 vehicle-treated *Mecp2*$^{Null/+}$ mice (**Figure 3B**). In *Mecp2*$^{+/+}$ controls, RAMH also had no effect. If the open field
234 arena was parsed into the duration in the center versus the surround, there were no differences between
235 genotypes and treatments (**Figure 3C, D**).



236 **Figure 3. *GRM7* mRNA expression does not correlate with either *GRM4* and *GRM8*; however, *GRM4* and**
237 ***GRM8* expression exhibits a significant correlation**. mRNA expression data from the experiments in **Table**
238 **1**, **Figure 1**, and **Figure 2** were correlated by linear regression for **(A)** *GRM7* and *GRM4*, **(B)** *GRM7* and
239 *GRM8*, and *GRM4* and *GRM8* for control **(C)** and **(D)** RTT samples.

240

241 To assess anxiety, we performed an elevated zero maze assessment (EZM; **Figure 4A**), and mice
242 were again administered RAMH or vehicle 15 minutes prior to testing. In this assay, increased time spent in the
243 open arms of the maze is interpreted as anxiolytic, and *Mecp2*$^{Null/+}$ treated with RAMH exhibited significantly
244 higher amounts of time in the open arms compared to *Mecp2*$^{Null/+}$ mice treated with vehicle (**Figure 4A**). This
245 effect was not present in the *Mecp2*$^{+/+}$ animals. We also observed no differences in the number of entries into
246 the open arms or the distance traveled in this maze task based on genotype or treatment (**Figure 4B and C**).



247 **Figure 4. In mice modeling the milder R306C mutation, mGlu$_7$ expression in synaptosomes is**
248 **significantly reduced in the hippocampus, but not the cortex, cerebellum, or brainstem**. mGlu$_7$ protein
249 levels were assessed in **(A)** hippocampus, **(B)** cortex, **(C)** cerebellum, and **(D)** brainstem from *Mecp2*$^{+/+}$ (WT,
250 black) and *Mecp2*$^{R306C/+}$ (red) female mice. Mean +/- SEM shown; *p=0.0249 by unpaired Student's t-test. Full
251 Western blots are shown in Supplementary Figures 1-4. N=5 per genotype.

252

253 Patients with RTT exhibit repetitive hand motions which prevent purposeful hand movements and
254 usage (Weng, Bailey et al. 2011, Townend, Ehrhart et al. 2018, Stallworth, Dy et al. 2019). *Mecp*$^{Null/+}$ mice
255 exhibit a clasping phenotype of their hindlimbs (Guy, Hendrich et al. 2001, Guy, Gan et al. 2007). To assess
256 the effects of H_3 activation in clasping, mice were administered RAMH or vehicle 15 minutes prior to an

assessment of hindlimb clasping over one minute. This evaluation showed the expected clasping phenotype of the *Mecp2*$^{Null/+}$ mice versus controls in the presence of vehicle (***p < 0.0001), but there was no effect of RAMH on this phenotype (**Figure 5A**). Finally, we assessed activity in a contextual fear conditioning task; we did not observe impairments in contextual fear conditioning in RTT mice in this cohort and administration of RAMH did not affect the performance of mice in this task (**Figure 5B**).

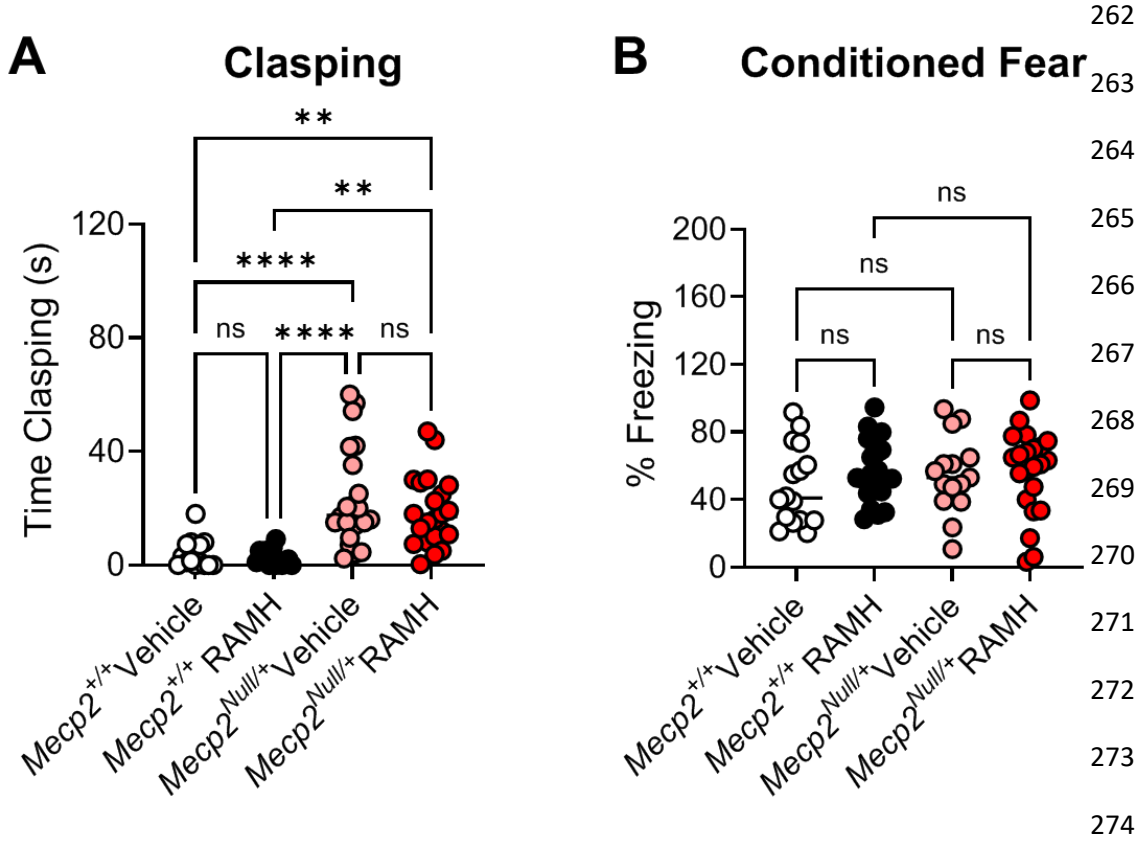


Figure 5. VU0422288 reverses apneas in *Mecp2*$^{R306C/+}$ mice. (A) Apneas were assessed using whole body plethysmography (WBP) and normalized to 10,000 breaths for *Mecp2*$^{+/+}$ (WT, black) and *Mecp2*$^{R306C/+}$ (red) female mice. Mean +/- SEM; Two way ANOVA, main effect of treatment F(1, 18) = 3.916, p=0.0613; main effect of genotype F(1, 18) = 9.514, **p=0.0064; interaction effect of treatment and genotype F(1, 18) = 5.237, *p=0.0344. Tukey's post hoc: vehicle *Mecp2*$^{+/+}$ versus *Mecp2*$^{306C/+}$, **p=0.0066; *Mecp2*$^{306C/+}$ vehicle versus *Mecp2*$^{306C/+}$ VU0422288, *p=0.0441. **(B, C)** Breath rate and minute volume were not different between genotypes. N=5-6 per genotype.

A mouse model of *MECP2* duplication syndrome (MDS) expresses elevated histamine H_3 mRNA receptor expression in cortex and striatum. The decreased expression of the histamine H_3 receptor in RTT

patients and the *Mecp2*$^{Null/+}$ mouse model of RTT, as well as previous literature, prompted us to examine *Hrh3* expression in *MECP2*^{Tg1} animals, a model of MDS (Collins, Levenson et al. 2004). We quantified mRNA expression in cortex, hippocampus, striatum, and cerebellum of male and female *Mecp2Tg1* animals (**Figure 6**). These studies showed that there was a significant increase in *Hrh3* expression in the cortex and striatum of *MECP2*^{Tg1} animals compared to wild-type (WT) littermate controls, but not in the hippocampus or cerebellum. The compound pitolisant, a histamine H_3 receptor antagonist, has recently been approved for use in humans to treat sleep disorders, specifically narcolepsy (Wakix®, (Fabara, Ortiz et al. 2021, Sarfraz, Okuampa et al. 2022)) and has been shown to exhibit efficacy in fear memory in wild-type mice (Brabant, Charlier et al. 2013). This suggested that pitolisant might have efficacy in normalizing abnormal behavioral phenotypes, particularly those requiring intact cortical and striatal circuitry, in *MECP2*^{Tg1} animals. We performed a similar battery of studies in *MECP2*^{Tg1} mice and WT controls, progressing animals through open field, EZM, and conditioned fear (*MECP2*^{Tg1} mice do not exhibit a clasping phenotype); pitolisant (10 mg/kg, ip) was administered 30 min prior to each behavioral test or prior to training in the conditioned fear assay. We used both male and female mice for these studies, and data are separated by sex in **Supplemental Figure 1**.

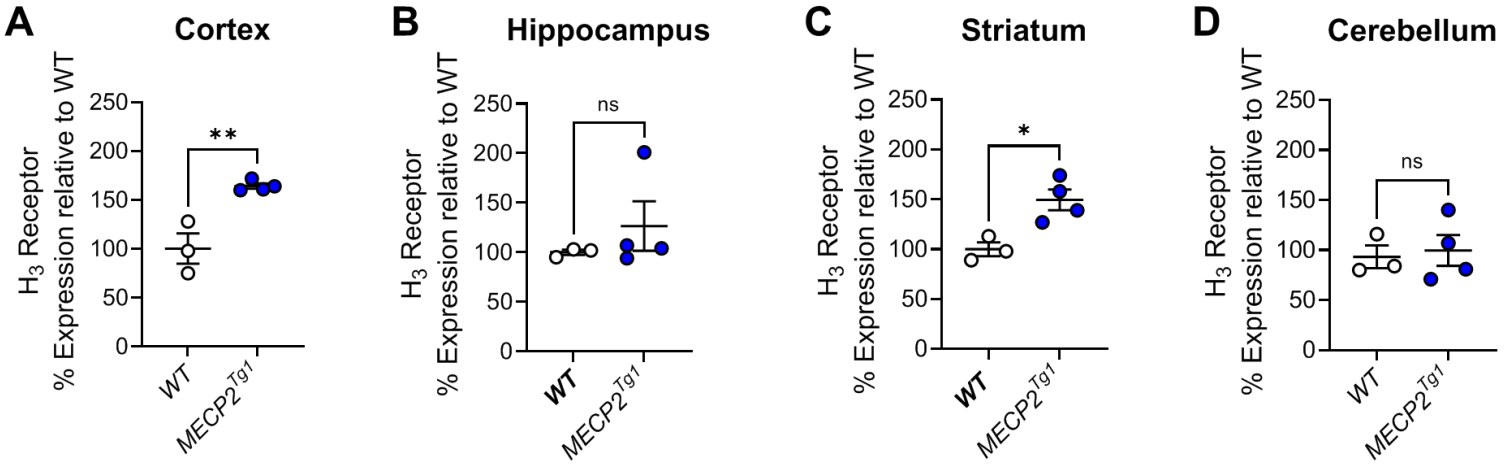


Figure 6. Mouse *Hrh3* expression is increased in the cortex and striatum of *MECP2*^{Tg1} mice compared to controls. Quantitative RT-PCR was performed on samples from various brain areas for wild-type littermate controls (WT, white) or *MECP2*^{Tg1} (blue) mice. Statistical comparisons were unpaired Student's t-tests; cortex, **p = 0.0048; hippocampus, p = 0.4123; striatum, *p = 0.0149; cerebellum, p = 0.7678; n=3-4 samples per tissue.

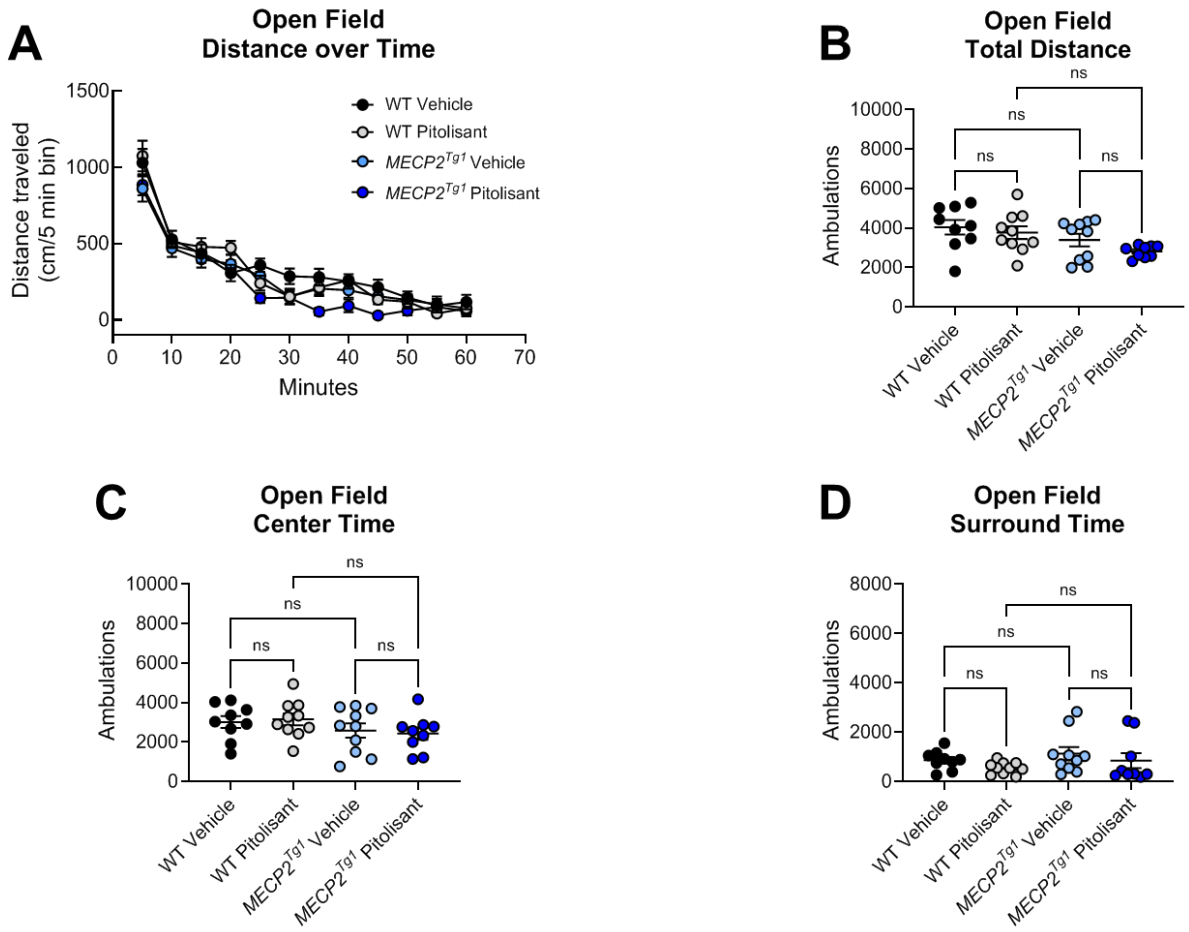
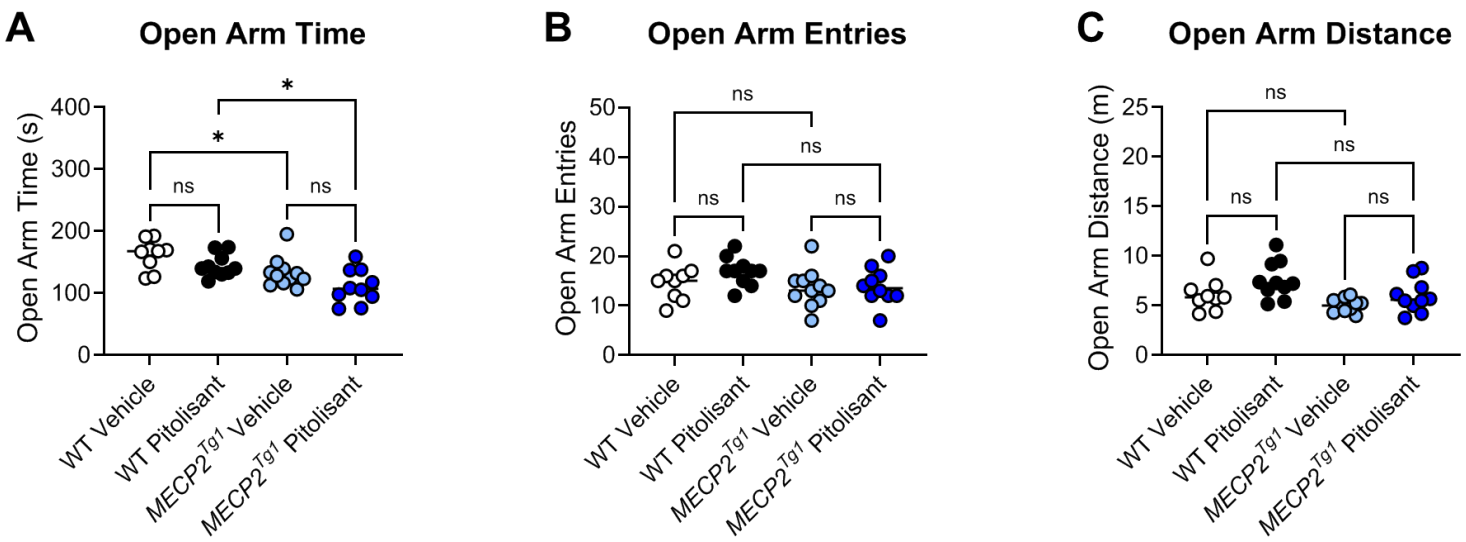


Figure 7. The H_3 receptor antagonist pitolisant does not impact open field behavior in *Mecp2*$^{+/+}$ or *MECP2*^{Tg1} mice. Wild-type (WT) littermate controls and *MECP2*^{Tg1} mice were administered vehicle or 10 mg/kg of pitolisant 30 minutes prior to placement in an open field and allowed to explore the area for 60 minutes. **A.** Time course of exploration. WT vehicle, black symbols; WT pitolisant, gray symbols; *MECP2*^{Tg1} vehicle light blue symbols; *MECP2*^{Tg1} pitolisant, dark blue symbols. **B.** Total distance traveled over 60 minutes (1-way ANOVA, F (3,34), 2.978, p = 0.0451; Tukey's post-test, WT vehicle and *MECP2*^{Tg1} pitolisant, *p=0.0393; all other comparisons were not significant). **C.** Distance in the center was not different between genotypes or treatments (1-way ANOVA, F (3, 34), 1.149, p = 0.3434). **D.** Distance in the surrounding area was not different between genotypes or treatments (1-way ANOVA, F (3, 34), 1.149, p = 0.3434).

Pitolisant does not correct behavioral deficits in *Mecp2Tg1* animals. In an open field test, we observed no differences in total distance traveled when sexes were combined (**Figure 7A, B**). Separation into male and female animals revealed that, while no significant differences were identified in the males (**Supplemental Figure 1A**), *MECP2*^{Tg1} females exhibited reduced distance traveled compared to WT females

318 (**Supplemental Figure 1B**). Additionally, WT females, but not *MECP2*^{Tg1} females, exhibited a significant
319 reduction in locomotion in response to pitolisant (**Supplemental Figure 1B**). There were no differences in time
320 spent in the center or the surrounding area of the open field (**Figure 7C, D, Supplemental Figure 1C-F**). In
321 EZM, we observed a reduction in the amount of time spent in the open arms in *MECP2*^{Tg1} animals compared to
322 WT controls; however, this phenotype was not altered by pitolisant (**Figure 8A**). When mice were separated by
323 sex, there was a significant difference in open arm time only in the male animals (*MECP2*^{Tg1} versus WT,
324 **Supplemental Figure 1G, H**), but, again, no effects of pitolisant were observed. Additionally, there were no
325 significant differences in open arm entries or open arm distance traveled (**Figure 8B**, **C, Supplemental Figure**
326 **1I-L**). Finally, similar to the findings of multiple labs, *MECP2*^{Tg1} mice treated with vehicle exhibited a strong
327 response in conditioned fear in which they froze significantly more than WT animals ((Na, Nelson et al. 2012,
328 Stansley, Fisher et al. 2018, Vermudez, Buch et al. 2022), **Figure 9)**. This was most prominently observed in
329 female animals (**Supplemental Figure 1M-N**); however, this phenotype was not impacted by pitolisant in
330 either sex, suggesting that pitolisant does not correct the behavioral deficits tested here in *MECP2*^{Tg1} animals.



331 **Figure 8. Pitolisant does not exhibit efficacy in elevated zero maze. A.** *MECP2*^{Tg1} mice exhibited a
332 significantly decreased in time in the open arms of an elevated zero maze compared to wild-type (WT)
333 littermate controls, but this response was not affected by pitolisant (1-way ANOVA, F (3, 36), 7.834, ***p =
334 0.0004; Tukey's post hoc test, WT vehicle (white) versus *MECP2*^{Tg1} vehicle (light blue), *p = 0.0447; WT
335 vehicle (white) versus *MECP2*^{Tg1} pitolisant (dark blue), ***p = 0.0002; WT pitolisant (black) versus *MECP2*^{Tg1}
336 pitolisant (dark blue), *p = 0.0162; all other comparisons were not significant). **B.** There was no significant
337 change in number of entries (1-way ANOVA, F (3, 36), 1.958, p = 0.1378). **C.** The only significant open arm

distance comparison was WT pitolisant (black) versus *MECP2*^{Tg1} vehicle (light blue) (not shown on graph, 1-way ANOVA, F (3, 35), 4.764, **p = 0.0069); Tukey's post hoc test, **p = 0.0035); all other comparisons were not significant.

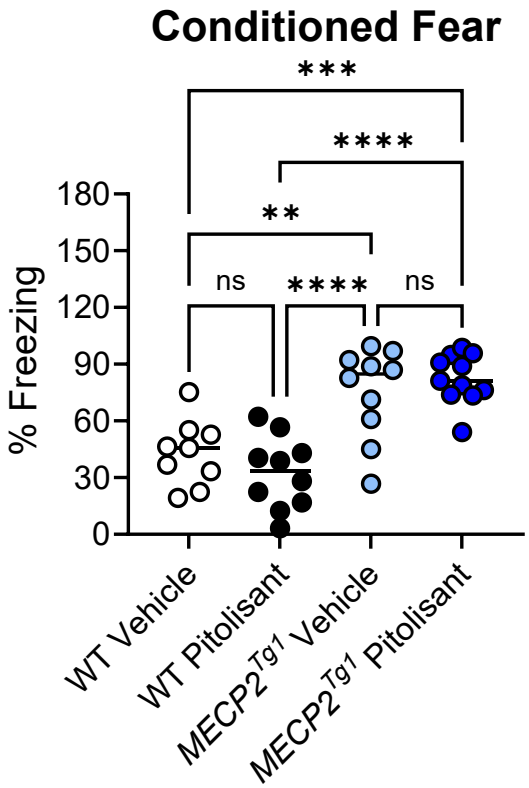


Figure 9. Pitolisant does not change the elevated contextual fear response characteristic of *MECP2*^{Tg1} animals. Wild-type (WT) littermate controls and *MECP2*^{Tg1} mice were treated with vehicle or pitolisant 30 minutes before training in a contextual fear conditioning task; percent freezing was assessed 24 hours later when the mice were returned to the same context. 1-way ANOVA, F (3.36), 17.09m ****p< 0.0001).Tukey's post-test, WT Vehicle versus WT pitolisant, p = 0.6119; WT Vehicle versus *MECP2*^{Tg1} Vehicle, **p = 0.0035; WT Vehicle versus *MECP2*^{Tg1} pitolisant, ***p = 0/0002; WT pitolisant versus *MECP2*^{Tg1} Vehicle, **** p < 0.0001; WT pitolisant versus *MECP2*^{Tg1} pitolisant, ****p < 0.0001; *MECP2*^{Tg1} Vehicle versus *MECP2*^{Tg1} pitolisant, p = 0.8106.

349 **Discussion**

350 RTT is a disorder characterized by a constellation of symptoms that appear in girls following a
351 regression period, including the development of intellectual disability, seizures, repetitive behaviors, and
352 anxiety. The related disorder, MDS, also results in abnormal symptoms in patients. We and others have taken
353 a "bedside-to-bench" approach to identify targets with clinically relevant changes in expression using 'omics
354 profiling of samples from RTT patients as well as RTT and MDS model mice. These strategies have resulted in
355 our exploration of the therapeutic potential of several mGlu and muscarinic targets (Gogliotti, Senter et al.
356 2016, Gogliotti, Senter et al. 2017, Gogliotti, Fisher et al. 2018, Cikowski, Holt et al. 2022, Smith, Arthur et al.
357 2022, Vermudez, Buch et al. 2022). We have now turned our attention to the H_3 histamine receptor, based on
358 findings that expression of the receptor is reduced in autopsy samples from a cohort of RTT patients,
359 correlating with previous data published by (Renthal, Boxer et al. 2018), and we validated this decrease in
360 expression across a larger cohort of RTT samples. These findings reveal that the majority of RTT patients can
361 be clustered into a group with significantly reduced *HRH3* expression (**Figure 1C**). Interestingly, there appear
362 to be several individuals who diverge from this pattern; with the exception of potentially the P302L/R306C
363 samples (2 patients expressed *HRH3* levels at 309 and 356% of control, one patient expressed 101% of
364 control), this does not appear to correlate with specific *MECP2* mutation, age, or postmortem interval.

365 The *Mecp2*$^{Null/+}$ mouse model of RTT syndrome provides strong face and construct validity for the study
366 of potential treatments for RTT (Guy, Hendrich et al. 2001, Vashi and Justice 2019). These mice exhibit
367 numerous behavioral phenotypes that are similar to those observed in the human disorder, notably,
368 impairments in cognition, motor impairments, seizures, repetitive behaviors, and anxiety (Guy, Hendrich et al.
369 2001). Additionally, replacement of Mecp2 corrects behavioral deficits and restores normal lifespan (Guy, Gan
370 et al. 2007) suggesting that the disease is not degenerative in mice. Therapeutic strategies for RTT have now
371 taken several avenues: those aimed at correcting MeCP2 itself using strategies such as gene therapy, DNA
372 and RNA editing, and X chromosome reactivation, and strategies that exploit changes in targets that occur
373 upon the loss of MeCP2. While only the former strategies would be considered curative, symptomatic
374 treatments may still be of benefit in the daily lives of RTT patients, and the overlap of RTT symptoms with other
375 neurodevelopmental disorders suggests that treatments that impact RTT symptoms may extend to other
376 disorders. After determining that there are reduced levels of *HRH3* mRNA in postmortem brain samples of RTT

377 patients, we also found here that 20-week-old, symptomatic *Mecp2*$^{Null/+}$ mice express reduced levels of *Hrh3*
378 mRNA in the cortex and striatum when compared to *Mecp2*$^{+/+}$ controls, but normal levels in the hippocampus
379 and cerebellum. The finding that *Hrh3* mRNA level is not reduced in the cerebellum of *Mecp2*$^{Null/+}$ mice
380 compared to *Mecp2*$^{+/+}$ controls contrasts with our original RNA sequencing data from human RTT patients,
381 where we observed decreased expression of *HRH3* in the cerebellum. This discrepancy might be attributed to
382 species-specific differences in the expression and distribution of histamine H_3 receptor mRNA in the
383 cerebellum (Li, Zhu et al. 2014). Indeed, we and others have previously observed low overlap of gene
384 expression changes between mice modeling RTT and RTT patients (Gogliotti, Fisher et al. 2018, Renthal,
385 Boxer et al. 2018). We would also note that our findings of reductions in *HRH3* mRNA in the RTT cortex align
386 with those previously reported in other RNA sequencing studies (Ben-Shachar, Chahrour et al. 2009, Chao,
387 Chen et al. 2010)

388 RTT patients exhibit a variety of repetitive behaviors and anxiety phenotypes (Stallworth, Dy et al.
389 2019, Buchanan, Stallworth et al. 2022). These include the repetitive hand wringing/clasping that is a hallmark
390 of the disorder and a core diagnostic criterion for RTT (Gold, Percy et al. 2024). Anxiety symptoms range from
391 self-injurious behavior, screaming and crying, and mood changes which are common among RTT patients
392 (Barnes, Coughlin et al. 2015, Gold, Percy et al. 2024). In mouse models of RTT, specific behavioral
393 phenotypes can be separated when MeCP2 is lost from or replaced within specific neuronal subtypes (Chao,
394 Chen et al. 2010, Ito-Ishida, Ure et al. 2015, Meng, Wang et al. 2016, Ure, Lu et al. 2016). Interestingly,
395 histamine H_3 receptors have been shown to be involved in the manifestation of anxiety behaviors (Yokoyama,
396 Yamauchi et al. 2009, Mohsen, Yoshikawa et al. 2014, Zhang, Peng et al. 2020). Mechanistic studies revealed
397 that activation of the *Hrh3* system in the nucleus accumbens (NAc) core inhibits glutamatergic
398 neurotransmission from the prelimbic to the NAc circuit and inhibits anxiety-like and obsessive-compulsive-like
399 behavior induced by exposure to stress (Zhang, Peng et al. 2020). These studies indicate that the histamine H_3
400 receptor might be a target for developing potential therapeutic strategies for anxiety and repetitive behaviors.
401 Additionally, mice fed a low histidine diet exhibit reductions in histamine levels and release in the brain
402 (Yoshikawa, Nakamura et al. 2014) and develop anxiety-relevant behaviors. RTT patients have also been
403 shown to have lower levels of histidine in plasma (Neul, Skinner et al. 2020) and experience multiple sleep-
404 related disorders (Tascini, Dell'Isola et al. 2022). Together, these results suggest a strong link between H_3

405 expression and histamine levels with anxiety-like phenotype in RTT model mice, which provided a rationale to
406 test 20-week-old *Mecp2*$^{Null/+}$ mice in a behavior relevant to anxiety. While much attention has been focused on
407 the development of antagonists/inverse agonists for the H_3 receptor (Fabara, Ortiz et al. 2021, Keam 2023),
408 several compounds that increase the activity of H_3 receptors have also been tested clinically for indications
409 such as asthma and migraine (O'Connor, Lecomte et al. 1993, Fozard 2000, Leurs, Bakker et al. 2005, Millan-
410 Guerrero, Isais-Millan et al. 2006). Using a pharmacological treatment scheme comparing the effects of 45
411 mg/kg of the histamine H_3 receptor agonist, RAMH, with vehicle, we observed that administration of RAMH
412 significantly increased the time spent by *Mecp2*$^{Null/+}$ mice in open arms of the elevated zero maze, suggesting
413 that increasing H_3 receptor signaling may alleviate anxiety-like behaviors. This effect was not accompanied by
414 significant reductions in arm entries or in total distance traveled, suggesting that the effects on open arm time
415 are not confounded by decreases in movement. It should be noted that effects in the open field in various lines
416 of RTT mice in general are conflicting, with some reports showing that RTT model mice spend less time in the
417 open arms of the maze (interpreted as higher anxiety) and other studies showing that RTT mice spend more
418 time in the open arms (interpreted as lower anxiety) at baseline (Gogliotti, Senter et al. 2017). Here, we did not
419 observe a significant difference in open arm time between *Mecp2*$^{+/+}$ and *Mecp2*$^{Null/+}$ mice with vehicle
420 treatment, but, as a group, *Mecp2*$^{Null/+}$ animals treated with RAMH exhibited a robust preference for the open
421 arms of the maze. This suggests a clear effect of RAMH that is only present in the *Mecp2*$^{Null/+}$ animals.

422 Activation of H_3 has been shown to ameliorate obsessive-compulsive-like behaviors induced by
423 activation of cortico-accumbal glutamatergic afferents (Zhang, Peng et al. 2020). Based on these findings and
424 due to the robust hindlimb clasping phenotype observed in *Mecp2*$^{Null/+}$ mice (Patterson, Hawkins et al. 2016),
425 we anticipated that this might be a domain in which *Mecp2*$^{Null/+}$ females might be sensitive to RAMH treatment.
426 In contrast to this hypothesis, we did not observe efficacy of RAMH in reversing hindlimb clasping. This
427 observation fits with the findings of the Zoghbi lab in which anxiety and hindlimb clasping were separated when
428 Mecp2 was restored in glutamatergic versus GABAergic neurons in mice, respectively (Meng, Wang et al.
429 2016, Ure, Lu et al. 2016). Future studies in this area could include testing the efficacy of RAMH in mice with
430 loss of Mecp2 selectively from each type of neuron; if the above hypothesis is correct, it would be anticipated
431 that the effects of RAMH on anxiety phenotypes would be lost in the mice lacking Mecp2 in glutamatergic, but
432 not GABAergic, neurons. Indeed, RAMH-induced activation of presynaptic H_3 receptors has been shown to

433 selectively inhibit glutamatergic, but not GABAergic, neurotransmission in the NAc core and activation of these
434 receptors alleviates anxiety-like behaviors induced by optogenetic activation of glutamatergic signaling from
435 the prelimbic cortex to the NAc core circuitry (Zhang, Peng et al. 2020). Since *Mecp2* is also expressed in
436 cortical areas and in the NAc, studies aiming at determining whether loss of Mecp2 expressed in this circuitry
437 mediates anxiety-like behavior observed in *Mecp2*$^{Null/+}$ mice is an exciting future direction.

438 Tight regulation of MeCP2 expression is important as duplication of the gene causes MDS with
439 considerable overlap with classical RTT syndrome patients in terms of the presence of stereotypic behavior,
440 anxiety, and social avoidance (Moretti and Zoghbi 2006, Ramocki, Peters et al. 2009). Therefore, similar to our
441 studies in *Mecp2*$^{Null/+}$ mice, we also profiled the levels of histamine H_3 receptors in the cortex, hippocampus,
442 striatum and cerebellum of *MECP2*^{Tg1} mice. In contrast to the *Mecp2*$^{Null/+}$ animals, we observed an elevated
443 expression of histamine H_3 receptors mRNA in the cortex and striatum of *MECP2*^{Tg1} mice, suggesting elevated
444 histamine H_3 receptor signaling in these areas. This finding prompted us to test the activity of the histamine H_3
445 receptor antagonist, pitolisant (Fabara, Ortiz et al. 2021, Sarfraz, Okuampa et al. 2022), in correcting observed
446 behavioral deficits in a mouse model of MDS. In contrast to our hypothesis, pitolisant did not correct anxiety-
447 like behavior and enhanced freezing behavior in *MECP2*^{Tg1} mouse model of MDS, suggesting that inhibition of
448 histamine H_3 receptor signaling with pitolisant was not sufficient to correct these behaviors in MDS mice. It
449 should be noted that the main indication for pitolisant in the clinic is narcolepsy and sleep disorders, which
450 were not examined here. Patients with MDS have been reported to exhibit sleep disturbances (Pehlivan,
451 Huang et al. 2025) and 80% of patients with RTT exhibit sleep problems (Tascini, Dell'Isola et al. 2022, Gold,
452 Percy et al. 2024). Future studies examining H_3 receptor modulation in sleep in RTT and MDS mice, therefore,
453 may be areas that deserve future study.

454 In summary, our findings reveal that administration of histamine H_3 receptor agonist, RAMH, can
455 increase time spent by *Mecp2*$^{Null/+}$ mice in the open arms of an elevated zero maze test of anxiety. Future
456 studies designed to evaluate the performance of mice in a battery of anxiety tests like elevated plus maze and
457 light/dark transition task will provide more information about the role of histamine H_3 receptors in regulating
458 anxiety in RTT mice, as these test measure anxiety under different conditions (van Gaalen and Steckler 2000).
459 Additionally, future studies could explore the effect of chronic dosing of an H_3 agonist. Overall, these studies

provide important information regarding the physiological role of histamine H_3 receptor in the regulation of anxiety behavior in RTT syndrome.

Authorship contribution statement

Kelly Weiss: Writing – original draft, Visualization, Methodology, Investigation. **Sheryl Anne D. Vermudez:** Writing – original draft, Visualization, Methodology, Investigation. **Geanne A. Freitas:** Writing – review & editing, Visualization, Methodology, Investigation. **Mackenzie Meadows:** Visualization, Methodology, Investigation. **Shalini Dogra:** Writing – review & editing, Visualization. **Rocco Gogliotti:** Writing – review and editing, Visualization, Methodology, Investigation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Colleen M. Niswender:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Data availability

All mentioned data are presented in this published article or the supplementary information or are available from the corresponding author upon reasonable request.

Ethics statement

This manuscript is an original work and is not under consideration for publication elsewhere. All authors have reviewed and approved of the submission.

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485 Maryland Brain Bank is a tissue repository of the National Institutes of Health (NIH) NeuroBioBank.

486

487 **Declaration of Competing Interest**

488 The authors declare that the research was conducted in the absence of any commercial or financial
489 relationships that could be construed as a potential conflict of interest.

References

Amir, R. E., I. B. Van den Veyver, M. Wan, C. Q. Tran, U. Francke and H. Y. Zoghbi (1999). "Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2." Nat Genet **23**(2): 185-188.

Arrang, J. M., B. Devaux, J. P. Chodkiewicz and J. C. Schwartz (1988). "H3-receptors control histamine release in human brain." J Neurochem **51**(1): 105-108.

Arrang, J. M., M. Garbarg, J. C. Lancelot, J. M. Lecomte, H. Pollard, M. Robba, W. Schunack and J. C. Schwartz (1988). "Highly potent and selective ligands for a new class H3 of histamine receptor." Invest Radiol **23 Suppl 1**: S130-132.

Barnes, K. V., F. R. Coughlin, H. M. O'Leary, N. Bruck, G. A. Bazin, E. B. Beinecke, A. C. Walco, N. G. Cantwell and W. E. Kaufmann (2015). "Anxiety-like behavior in Rett syndrome: characteristics and assessment by anxiety scales." J Neurodev Disord **7**(1): 30.

Ben-Shachar, S., M. Chahrour, C. Thaller, C. A. Shaw and H. Y. Zoghbi (2009). "Mouse models of MeCP2 disorders share gene expression changes in the cerebellum and hypothalamus." Hum Mol Genet **18**(13): 2431-2442.

Brabant, C., Y. Charlier and E. Tirelli (2013). "The histamine H(3)-receptor inverse agonist pitolisant improves fear memory in mice." Behav Brain Res **243**: 199-204.

Brown, R. E., D. R. Stevens and H. L. Haas (2001). "The physiology of brain histamine." Prog Neurobiol **63**(6): 637-672.

Buchanan, C. B., J. L. Stallworth, A. E. Joy, R. E. Dixon, A. E. Scott, A. A. Beisang, T. A. Benke, D. G. Glaze, R. H. Haas, P. T. Heydemann, M. D. Jones, J. B. Lane, D. N. Lieberman, E. D. Marsh, J. L. Neul, S. U. Peters, R. C. Ryther, S. A. Skinner, S. M. Standridge, W. E. Kaufmann and A. K. Percy (2022). "Anxiety-like behavior and anxiolytic treatment in the Rett syndrome natural history study." J Neurodev Disord **14**(1): 31.

Chahrour, M., S. Y. Jung, C. Shaw, X. Zhou, S. T. Wong, J. Qin and H. Y. Zoghbi (2008). "MeCP2, a key contributor to neurological disease, activates and represses transcription." Science **320**(5880): 1224-1229.

Chao, H. T., H. Chen, R. C. Samaco, M. Xue, M. Chahrour, J. Yoo, J. L. Neul, S. Gong, H. C. Lu, N. Heintz, M. Ekker, J. L. Rubenstein, J. L. Noebels, C. Rosenmund and H. Y. Zoghbi (2010). "Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes." Nature **468**(7321): 263-269.

Cikowski, J., C. Holt, B. Arthur, M. Smith, S. Gonzalez, C. W. Lindsley, C. M. Niswender and R. G. Gogliotti (2022). "Optimized Administration of the M4 PAM VU0467154 Demonstrates Broad Efficacy, but Limited Effective Concentrations in Mecp2(+/-) Mice." ACS Chem Neurosci **13**(13): 1891-1901.

Collins, A. L., J. M. Levenson, A. P. Vilaythong, R. Richman, D. L. Armstrong, J. L. Noebels, J. David Sweatt and H. Y. Zoghbi (2004). "Mild overexpression of MeCP2 causes a progressive neurological disorder in mice." Hum Mol Genet **13**(21): 2679-2689.

Collins, B. E. and J. L. Neul (2022). "Rett Syndrome and MECP2 Duplication Syndrome: Disorders of MeCP2 Dosage." Neuropsychiatr Dis Treat **18**: 2813-2835.

Fabara, S. P., J. F. Ortiz, A. Anas Sohail, J. Hidalgo, A. Altamimi, B. Tama and U. K. Patel (2021). "Efficacy of Pitolisant on the Treatment of Narcolepsy: A Systematic Review." Cureus **13**(7): e16095.

Fozard, J. R. (2000). "BP-294 Ste Civile Bioprojet." Curr Opin Investig Drugs **1**(1): 86-89.

Galici, R., J. D. Boggs, L. Aluisio, I. C. Fraser, P. Bonaventure, B. Lord and T. W. Lovenberg (2009). "JNJ-10181457, a selective non-imidazole histamine H(3) receptor antagonist, normalizes acetylcholine neurotransmission and has efficacy in translational rat models of cognition." Neuropharmacology **56**(8): 1131-1137.

Gogliotti, R. G., N. M. Fisher, B. J. Stansley, C. K. Jones, C. W. Lindsley, P. J. Conn and C. M. Niswender (2018). "Total RNA Sequencing of Rett Syndrome Autopsy Samples Identifies the M4 Muscarinic Receptor as a Novel Therapeutic Target." J Pharmacol Exp Ther **365**(2): 291-300.

Gogliotti, R. G., R. K. Senter, N. M. Fisher, J. Adams, R. Zamorano, A. G. Walker, A. L. Blobaum, D. W. Engers, C. R. Hopkins, J. S. Daniels, C. K. Jones, C. W. Lindsley, Z. Xiang, P. J. Conn and C. M. Niswender (2017). "mGlu7 potentiation rescues cognitive, social, and respiratory phenotypes in a mouse model of Rett syndrome." Sci Transl Med **9**(403): eaai7459 [pii] 7410.1126/scitranslmed.aai74599/74403/eaai77459 [pii].

Gogliotti, R. G., R. K. Senter, J. M. Rook, A. Ghoshal, R. Zamorano, C. Malosh, S. R. Stauffer, T. M. Bridges, J. M. Bartolome, J. S. Daniels, C. K. Jones, C. W. Lindsley, P. J. Conn and C. M. Niswender (2016). "mGlu5 positive allosteric modulation normalizes synaptic plasticity defects and motor phenotypes in a mouse model of Rett syndrome." Hum Mol Genet.

Gold, W. A., A. K. Percy, J. L. Neul, S. R. Cobb, L. Pozzo-Miller, J. K. Issar, B. Ben-Zeev, A. Vignoli and W. E. Kaufmann (2024). "Rett syndrome." Nat Rev Dis Primers **10**(1): 84.

Guy, J., J. Gan, J. Selfridge, S. Cobb and A. Bird (2007). "Reversal of neurological defects in a mouse model of Rett syndrome." Science **315**(5815): 1143-1147.
Guy, J., B. Hendrich, M. Holmes, J. E. Martin and A. Bird (2001). "A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome." Nat Genet **27**(3): 322-326.
Ito-Ishida, A., K. Ure, H. Chen, J. W. Swann and H. Y. Zoghbi (2015). "Loss of MeCP2 in Parvalbumin-and Somatostatin-Expressing Neurons in Mice Leads to Distinct Rett Syndrome-like Phenotypes." Neuron **88**(4): 651-658.
Keam, S. J. (2023). "Pitolisant: Pediatric First Approval." Paediatr Drugs **25**(4): 483-488.
Leurs, R., R. A. Bakker, H. Timmerman and I. J. de Esch (2005). "The histamine H3 receptor: from gene cloning to H3 receptor drugs." Nat Rev Drug Discov **4**(2): 107-120.
Li, B., J. N. Zhu and J. J. Wang (2014). "Histaminergic afferent system in the cerebellum: structure and function." Cerebellum Ataxias **1**: 5.
Livak, K. J. and T. D. Schmittgen (2001). "Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method." Methods **25**(4): 402-408.
Meng, X., W. Wang, H. Lu, L. J. He, W. Chen, E. S. Chao, M. L. Fiorotto, B. Tang, J. A. Herrera, M. L. Seymour, J. L. Neul, F. A. Pereira, J. Tang, M. Xue and H. Y. Zoghbi (2016). "Manipulations of MeCP2 in glutamatergic neurons highlight their contributions to Rett and other neurological disorders." Elife **5**.
Millan-Guerrero, R. O., R. Isais-Millan, T. H. Benjamin and C. E. Tene (2006). "Nalpha-methyl histamine safety and efficacy in migraine prophylaxis: phase III study." Can J Neurol Sci **33**(2): 195-199.
Mohsen, A., T. Yoshikawa, Y. Miura, T. Nakamura, F. Naganuma, K. Shibuya, T. Iida, R. Harada, N. Okamura, T. Watanabe and K. Yanai (2014). "Mechanism of the histamine H(3) receptor-mediated increase in exploratory locomotor activity and anxiety-like behaviours in mice." Neuropharmacology **81**: 188-194.
Moretti, P. and H. Y. Zoghbi (2006). "MeCP2 dysfunction in Rett syndrome and related disorders." Curr Opin Genet Dev **16**(3): 276-281.
Na, E. S., E. D. Nelson, M. Adachi, A. E. Autry, M. A. Mahgoub, E. T. Kavalali and L. M. Monteggia (2012). "A mouse model for MeCP2 duplication syndrome: MeCP2 overexpression impairs learning and memory and synaptic transmission." J Neurosci **32**(9): 3109-3117.
Neul, J. L., W. E. Kaufmann, D. G. Glaze, J. Christodoulou, A. J. Clarke, N. Bahi-Buisson, H. Leonard, M. E. Bailey, N. C. Schanen, M. Zappella, A. Renieri, P. Huppke, A. K. Percy and C. RettSearch (2010). "Rett syndrome: revised diagnostic criteria and nomenclature." Ann Neurol **68**(6): 944-950.
Neul, J. L., A. K. Percy, T. A. Benke, E. M. Berry-Kravis, D. G. Glaze, E. D. Marsh, T. Lin, S. Stankovic, K. M. Bishop and J. M. Youakim (2023). "Trofinetide for the treatment of Rett syndrome: a randomized phase 3 study." Nat Med **29**(6): 1468-1475.
Neul, J. L., S. A. Skinner, F. Annese, J. Lane, P. Heydemann, M. Jones, W. E. Kaufmann, D. G. Glaze and A. K. Percy (2020). "Metabolic Signatures Differentiate Rett Syndrome From Unaffected Siblings." Front Integr Neurosci **14**: 7.
Nieto-Alamilla, G., R. Marquez-Gomez, A. M. Garcia-Galvez, G. E. Morales-Figueroa and J. A. Arias-Montano (2016). "The Histamine H3 Receptor: Structure, Pharmacology, and Function." Mol Pharmacol **90**(5): 649-673.
O'Connor, B. J., J. M. Lecomte and P. J. Barnes (1993). "Effect of an inhaled histamine H3-receptor agonist on airway responses to sodium metabisulphite in asthma." Br J Clin Pharmacol **35**(1): 55-57.
Palmieri, M., D. Pozzer and N. Landsberger (2023). "Advanced genetic therapies for the treatment of Rett syndrome: state of the art and future perspectives." Front Neurosci **17**: 1172805.
Patterson, K. C., V. E. Hawkins, K. M. Arps, D. K. Mulkey and M. L. Olsen (2016). "MeCP2 deficiency results in robust Rett-like behavioural and motor deficits in male and female rats." Hum Mol Genet **25**(15): 3303-3320.
Pehlivan, D., C. Huang, H. K. Harris, C. Coquery, A. Mahat, M. Maletic-Savatic, L. Mignon, S. Aras, D. G. Glaze, C. S. Layne, L. Sahelijo, H. Y. Zoghbi, M. J. McGinley and B. Suter (2025). "Comprehensive assessment reveals numerous clinical and neurophysiological differences between MECP2-allelic disorders." Ann Clin Transl Neurol **12**(2): 433-447.
Ramocki, M. B., S. U. Peters, Y. J. Tavyev, F. Zhang, C. M. Carvalho, C. P. Schaaf, R. Richman, P. Fang, D. G. Glaze, J. R. Lupski and H. Y. Zoghbi (2009). "Autism and other neuropsychiatric symptoms are prevalent in individuals with MeCP2 duplication syndrome." Ann Neurol **66**(6): 771-782.
Ramocki, M. B., Y. J. Tavyev and S. U. Peters (2010). "The MECP2 duplication syndrome." Am J Med Genet A **152A**(5): 1079-1088.
Rapanelli, M., L. R. Frick, K. D. Horn, R. C. Schwarcz, V. Pogorelov, A. C. Nairn and C. Pittenger (2016). "The Histamine H3 Receptor Differentially Modulates Mitogen-activated Protein Kinase (MAPK) and Akt Signaling in Striatonigral and Striatopallidal Neurons." J Biol Chem **291**(40): 21042-21052.

601 Renthal, W., L. D. Boxer, S. Hrvatin, E. Li, A. Silberfeld, M. A. Nagy, E. C. Griffith, T. Vierbuchen and M. E.
602 Greenberg (2018). "Characterization of human mosaic Rett syndrome brain tissue by single-nucleus RNA
603 sequencing." Nat Neurosci **21**(12): 1670-1679.
604 Sarfraz, N., D. Okuampa, H. Hansen, M. Alvarez, E. M. Cornett, J. Kakazu, A. M. Kaye and A. D. Kaye (2022).
605 "pitolisant, a novel histamine-3 receptor competitive antagonist, and inverse agonist, in the treatment of
606 excessive daytime sleepiness in adult patients with narcolepsy." Health Psychol Res **10**(3): 34222.
607 Smith, M., B. Arthur, J. Cikowski, C. Holt, S. Gonzalez, N. M. Fisher, S. A. D. Vermudez, C. W. Lindsley, C. M.
608 Niswender and R. G. Gogliotti (2022). "Clinical and Preclinical Evidence for M1 Muscarinic Acetylcholine
609 Receptor Potentiation as a Therapeutic Approach for Rett Syndrome." Neurotherapeutics.
610 Stallworth, J. L., M. E. Dy, C. B. Buchanan, C. F. Chen, A. E. Scott, D. G. Glaze, J. B. Lane, D. N. Lieberman,
611 L. M. Oberman, S. A. Skinner, A. E. Tierney, G. R. Cutter, A. K. Percy, J. L. Neul and W. E. Kaufmann (2019).
612 "Hand stereotypies: Lessons from the Rett Syndrome Natural History Study." Neurology **92**(22): e2594-e2603.
613 Stansley, B. J., N. M. Fisher, R. G. Gogliotti, C. W. Lindsley, P. J. Conn and C. M. Niswender (2018).
614 "Contextual Fear Extinction Induces Hippocampal Metaplasticity Mediated by Metabotropic Glutamate
615 Receptor 5." Cereb Cortex **28**(12): 4291-4304.
616 Tascini, G., G. B. Dell'Isola, E. Mencaroni, G. Di Cara, P. Striano and A. Verrotti (2022). "Sleep Disorders in
617 Rett Syndrome and Rett-Related Disorders: A Narrative Review." Front Neurol **13**: 817195.
618 Townend, G. S., F. Ehrhart, H. J. van Kranen, M. Wilkinson, A. Jacobsen, M. Roos, E. L. Willighagen, D. van
619 Enckevort, C. T. Evelo and L. M. G. Curfs (2018). "MECP2 variation in Rett syndrome-An overview of current
620 coverage of genetic and phenotype data within existing databases." Hum Mutat **39**(7): 914-924.
621 Ure, K., H. Lu, W. Wang, A. Ito-Ishida, Z. Wu, L. J. He, Y. Sztainberg, W. Chen, J. Tang and H. Y. Zoghbi
622 (2016). "Restoration of Mecp2 expression in GABAergic neurons is sufficient to rescue multiple disease
623 features in a mouse model of Rett syndrome." Elife **5**.
624 van Gaalen, M. M. and T. Steckler (2000). "Behavioural analysis of four mouse strains in an anxiety test
625 battery." Behav Brain Res **115**(1): 95-106.
626 Vashi, N. and M. J. Justice (2019). "Treating Rett syndrome: from mouse models to human therapies." Mamm
627 Genome **30**(5-6): 90-110.
628 Vermudez, S. A. D., A. Buch, K. Weiss, R. G. Gogliotti and C. M. Niswender (2022). "Exploration of group II
629 metabotropic glutamate receptor modulation in mouse models of Rett syndrome and MECP2 Duplication
630 syndrome." Neuropharmacology **209**: 109022.
631 Vermudez, S. A. D., G. A. Freitas, M. Smith, R. Gogliotti and C. M. Niswender (2025). "Profiling metabotropic
632 glutamate receptor 7 expression in Rett syndrome: consequences for pharmacotherapy." bioRxiv.
633 Weng, S. M., M. E. Bailey and S. R. Cobb (2011). "Rett syndrome: from bed to bench." Pediatr Neonatol **52**(6):
634 309-316.
635 Yokoyama, F., M. Yamauchi, M. Oyama, K. Okuma, K. Onozawa, T. Nagayama, R. Shinei, M. Ishikawa, Y.
636 Sato and N. Kakui (2009). "Anxiolytic-like profiles of histamine H3 receptor agonists in animal models of
637 anxiety: a comparative study with antidepressants and benzodiazepine anxiolytic." Psychopharmacology (Berl)
638 **205**(2): 177-187.
639 Yoshikawa, T., T. Nakamura, T. Shibakusa, M. Sugita, F. Naganuma, T. Iida, Y. Miura, A. Mohsen, R. Harada
640 and K. Yanai (2014). "Insufficient intake of L-histidine reduces brain histamine and causes anxiety-like
641 behaviors in male mice." J Nutr **144**(10): 1637-1641.
642 Zhang, X. Y., S. Y. Peng, L. P. Shen, Q. X. Zhuang, B. Li, S. T. Xie, Q. X. Li, M. R. Shi, T. Y. Ma, Q. Zhang, J.
643 J. Wang and J. N. Zhu (2020). "Targeting presynaptic H3 heteroreceptor in nucleus accumbens to improve
644 anxiety and obsessive-compulsive-like behaviors." Proc Natl Acad Sci U S A **117**(50): 32155-32164.