Reelin deficiency contributes to long-term behavioral abnormalities induced by chronic adolescent exposure to Δ9-tetrahydrocannabinol in mice

Running Title: Influence of Reelin deficiency on THC adolescent exposure

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Abstract

Heavy and frequent use of cannabis during adolescence increases the risk of developing psychiatric disorders. However, the neurobiological mechanisms underlying this vulnerability remain largely unknown. Here, we explore whether adolescent vulnerability to long-term behavioral effects of cannabis is modulated by *Reelin*, a gene implicated in the development of the brain and of psychiatric disorders. To this aim, heterozygous Reeler (HR) mice, that express reduced level of *Reelin*, were chronically exposed during adolescence to high doses (10mg/kg) of Δ9-tetrahydrocannabinol (THC), a major psychoactive component of cannabis. Mice were tested in early adulthood with multiple behavioral assays, including working memory, social interaction, locomotor activity, anxiety-like responses, stress reactivity, and pre-pulse inhibition. Compared to wild-type (WT), HR mice treated with THC showed impaired social behaviors, elevated disinhibitory phenotypes and increased responsiveness to aversive situations, in a sex-specific manner. Independent of THC exposure, HR mice also spent more time exploring unfamiliar objects, indicating that Reelin modulates novelty seeking behavior. To identify the neuronal ensemble underlying this elevated novelty seeking in HR mice, we mapped the regional brain expression of the immediate early gene, *Fos,* in mice exposed to novel objects. HR mice exhibited reduced neuronal activation in the lateral septum, a subcortical brain structure implicated in emotions, cognition and reward processes. Overall, these findings show that (1) *Reelin* deficiency influences behavioral abnormalities caused by heavy consumption of THC

46 during adolescence, and (2) that Reelin plays a role in the neurobiological mechanisms
47 underlying disinhibitory behaviors, such as novelty seeking.
48
49 **Significant Statement**

50 The link between cannabis abuse and the development psychiatric disorders, especially in
51 adolescents, makes understanding the neurobiological mechanisms underlying cannabis effects
52 on the brain a significant biomedical problem. Reelin is a key signaling molecule in the
53 development of the adolescent brain and of psychiatric disorders, but its role in modulating the
54 behavioral changes induced by cannabis remain unknown. Here, we report an interaction
55 between *Reelin* deficiency and chronic adolescent exposure to THC, a major psychoactive
56 component of cannabis. This interaction led to cognitive deficits, disinhibitory behaviors and
57 altered emotional reactivity in mice, in a sex-specific manner. These experiments are the first to
58 establish a link between Reelin signaling and the endocannabinoid system targeted by THC.
59
60 **Introduction**
61 Heavy and frequent cannabis use by adolescents has been linked epidemiologically to increased
62 risk of developing psychiatric conditions, including schizophrenia, psychosis, and substance use
63 disorders (Volkow, 2016). Similarly, animal studies show that administration of synthetic
64 cannabinoids (e.g. THC) during adolescence perturbs a wide range of behaviors, including
65 memory, social interaction, anxiety, and sensorimotor gating by targeting the endocannabinoid
66 (eCB) system (Rubino et al., 2015). Despite the evidence of possible detrimental health
67 outcomes associated with adolescent cannabis use, a recent survey in the US revealed a
68 substantial increase in daily use of cannabis and a decreased perception of the risks associated

69 with its regular use by adolescents (Johnston, 2018). Further, the increasing legalization of
70 recreational cannabis use has led to calls to understand whether such policies put adolescents at
71 higher risk of developing psychiatric disorders. All this emphasizes the need to better understand
72 the neurobiological mechanisms associated with heavy consumption on cannabis during
73 adolescence (Wilkinson et al., 2016).

74 During adolescence, the brain undergoes continuous remodeling of its structure,
75 connectivity, and plasticity (Sturman and Moghaddam, 2011; Arain et al., 2013). In addition,
76 substantial hormonal changes during adolescence influence not only reproductive functions, but
77 also the emergence of sex differences in cognitive, social, and emotional behaviors (Schulz and
78 Sisk, 2016). Thus, the adolescence is considered a critical period wherein brain development
79 may be altered by the exposure to psychoactive drugs, which can lead to sex-specific behavioral
80 abnormalities and increased risk for psychopathology in adulthood (Cousijn et al., 2018; Lisdahl
81 et al., 2018).

82 However, the neurobiological mechanisms underlying the increased vulnerability of the
83 adolescent brain to the effects of THC remain poorly understood. The goal of this study is to
84 examine the potential role of Reelin signaling in modulating the behavioral effects of cannabis
85 on the adolescent brain.

86 Reelin is a protein of the extracellular matrix that is predominately expressed in neuronal
87 cells and plays a key role in brain development and synaptic plasticity (D'Arcangelo et al., 1995).
88 During embryonic stages, Reelin activates an extensive signaling cascade that is critical for the
89 proper migration and cell positioning of cortical neurons (Sekine et al., 2014). During
90 adolescence, Reelin signaling promotes the development of the synaptic excitation/inhibition
91 (E/I) balance within the prefrontal cortex (Iafrati et al., 2014; Bouamrane et al., 2016). In the

92 mature brain, Reelin is required for learning and memory by regulating the N-methyl-D-aspartate
93 receptors (NMDA-R) function and the expression of neuronal activity-dependent genes (Weeber
94 et al., 2002; Qiu et al., 2006; Niu et al., 2008; Rogers et al., 2011; Telese et al., 2015). In
95 humans, *Reelin* deficiency has been linked to the development of psychiatric disorders (Ishii et
96 al., 2016). Thus, the HR mice, that expressed lower level of Reelin, have been proposed as a
97 valid animal model to study neurodevelopmental psychiatric disorders (Lossi et al., 2019).
98 Whether there is a functional relationship between *Reelin* deficiency and the consequences of
99 early-life exposure to high levels of THC remains unknown.
100 To this aim, we examined the long-lasting behavioral outcomes of chronic adolescent
101 exposure to high doses of THC (10mg/kg) in female and male HR mice. We compared HR mice
102 to their WT littermate controls in a battery of behavioral tests exploring different facets of
103 cognitive and emotional responsiveness, including working memory (Sannino et al., 2012),
104 social interaction (Yang et al., 2011), anxiety-like responses (Bailey and Crawley, 2009), stress
105 reactivity (Can et al., 2012), and pre-pulse inhibition (Geyer et al., 2002). To further characterize
106 the neuronal ensembles underlying behavioral differences between WT and HR mice, we
107 examined the expression of the immediate early gene, *Fos,* in four brain regions that were
108 activated after exposure to unfamiliar objects. This is the first study to investigate the
109 relationship between *Reelin* deficiency and the effect of adolescent exposure to THC in mice.
110

111 **Materials and Methods**

112 **Animals.** All experimental procedures were approved by the institutional animal care and use
113 committee at University of California, San Diego. Mice were housed (3-4 per cage) under a 12h
114 light/12h dark cycle and provided with food and water ad libitum. HR mice were bred in house

115 using the B6C3Fe a/a-Relnrl/J line (The Jackson Laboratory, #000235), which carry a null
116 mutation in the Reelin gene (D'Arcangelo et al., 1995). HR express ~40% reduced level of
117 Reelin mRNA in brain tissues (**Suppl. Fig. 1A-B**).
118
119 **Drug treatment protocol and experimental design for behavioral analysis.** THC was
120 provided by the U.S. National Institute on Drug Abuse and was dissolved in a vehicle solution
121 consisting of ethanol, tween, and 0.9% saline (1:1:18) on the day of administration. Vehicle or
122 THC (10 mg/kg) were administered daily to adolescent mice by intraperitoneal injections from
123 post-natal day (PND) 28 to PND 48, which cover the adolescent period in mice (Laviola et al.,
124 2003) (**Suppl. Fig. 1C**). This dose was based on previous studies using low to high ranges of
125 THC in mice (Trexler et al., 2018; Kasten et al., 2019). Moreover, this dose used has been shown
126 to elevate plasma levels of THC in rodents (> 100 ng/mL) similar to those found in humans
127 smoking cannabis (Huestis, 2007; Zuurman et al., 2008; Nguyen et al., 2016). To examine the
128 long-term effects of chronic adolescent exposure to THC, mice were tested 15 days after a drug
129 abstinence period, starting at PND 63. We used 9 cohorts of mice to perform multiple behavioral
130 assays. Cohorts 1 to 5 were subjected to locomotor activity, open field (OF), six-different objects
131 (6-DOT), light-dark (LD), three-chamber social approach, and tail suspension (TS) tests. Cohorts
132 6 to 9 were subjected to locomotor activity, acoustic startle response (ASR) and pre-pulse
133 inhibition (PPI) tests. Mice were tested between 10:00 am and 5:00 pm. Behavioral assays were
134 conducted on separate days and all behavioral tests were performed once on each mouse. The
135 number of mice used in each behavioral assay is reported in Table 1. To prevent bias due to
136 olfactory cues, the behavioral apparatus was cleaned with diluted ethanol solution in between
137 mice.
138

139 **Six different objects test.** We used the 6-DOT to study short-term working memory in mice, as
140 previously described (Sannino et al., 2012). First, mice were left free to explore an empty arena
141 (60x40 x35 cm) during a 10 min habituation trial. Afterwards, mice were exposed to six different
142 objects for a total time of 10 min (familiarization trial). After an inter-trial interval of 1 minute,
143 mice were exposed to identical copies of the familiar objects, but one object was substituted with
144 a novel object (test trial). The exploration time across different trials was measured with
145 Anymaze and was used to calculate a discrimination index (DI) as follows: (time spent exploring
146 novel object – average time spent exploring familiar objects) / Total time spent exploring novel +
147 familiar objects. The time exploring the novel object and the discrimination index (DI) were used
148 as indexes of novelty-induced exploratory activity and working memory, respectively.
149
150 **Three-chamber social approach test.** The three-chamber social approach test was used to
151 measure social behaviors, as previously described (Yang et al., 2011). The apparatus comprised
152 of three-chambered box with dividing walls with small openings to allow free exploration of the
153 three chambers, such as one empty central chamber, one side chamber containing an empty small
154 wire cage (novel object) and one side chamber containing a stranger mouse inside a small wire
155 cage (novel mouse). The target mouse was first placed in the center chamber and allowed to
156 explore the apparatus for 15 min. After introducing the novel mouse and the novel object in the
157 side chambers, the mouse was allowed to explore for 10 min. The placement of the novel mouse
158 or novel object in the left or right chambers was systematically alternated in between trials. The
159 time spent in each compartment and the time spent actively sniffing the novel mouse or the novel
160 object were manually scored. Longer time spent with or exploring the novel mouse versus the
161 novel object was considered an index of sociability. Sociability index (SI) was calculated as

162 [(time spent exploring or sniffing novel mouse - time spent exploring or sniffing novel object) /
163 (total time spent exploring or sniffing novel mouse and novel object)].
164
165 **Tail suspension test.** The TS test was used to measure motor responses under aversive
166 conditions (Can et al., 2012). Mice were suspended by their tails with tape to a bar in a position
167 that they could not escape or hold on to nearby surfaces. The test lasted for 6 minutes and the
168 mobility time (sec) was manually scored for each minute. We analyzed was the mobility time
169 (sec) as the sum of the final 5 minutes.
170
171 **Open field test.** The OF test was used to measure anxiety-like behavior(Bailey and Crawley,
172 2009). Mice were placed randomly in one of the 4 corners of an open plexiglass arena (60x40x35
173 cm) for 5 minutes. The total time spent in and the latency to entry the center of the arena (s) were
174 recorded and scored using Anymaze.
175
176 **Light-dark test.** The LD test was used to measure anxiety-like behavior(Bailey and Crawley,
177 2009). The animals were tested for 10 min in a light–dark rectangular box (60x40x35 cm) in
178 which the aversive light compartment (40x40x35 cm) was illuminated by a 100 lux light. The
179 dark side (20x40x35 cm) had an opaque cover and ~ 0 lux of light. The two compartments were
180 connected by an open doorway, which allowed the subjects to move freely between the two
181 compartments. The test began by placing the animal in the dark compartment. The time spent in
182 the light compartment (s), the latency to enter in the light chamber (s), and the total number of
183 transitions, were measured using Anymaze.
184

185 **Acoustic startle response and pre-pulse inhibition test.** The ASR and PPI tests were used to
186 assess stress reactivity and sensorimotor gating functions. The tests were performed with a startle
187 reflex measuring apparatus (SR-LAB; San Diego Instruments, San Diego, CA), as previously
188 described (Toth et al., 2013). The system comprises a piezoelectric unit that transduces
189 vibrations into signals when mice startle inside the plexigas cylinder. First, mice are placed in a
190 plexiglas cylinder with background noise (65 decibel [db]) for 5 min (acclimation phase). Then,
191 mice were subjected to a total of 179 trials, for a total of 25 min, including: (a) startle trials (40
192 milliseconds [ms] with 80, 90, 100, 110 and 120 db acoustic pulses), (b) prepulse+startle trials:
193 20 ms with acoustic prepulses of 3 (68), 6 (71) and 12 (77) db above background noise followed,
194 100ms later, by a 40 ms 120-db startling pulse. Startle amplitude was measured every 1ms over a
195 65ms period beginning at the onset of the startle stimulus. Average startle amplitude over the
196 sampling period was taken as the dependent variable. Percent PPI at each pre-pulse intensity was
197 calculated as 100 - [(startle response for prepulse/startle response for startle-alone trials) x 100].
198
199 **Locomotor activity**. Locomotor activity was measured using the video tracking system
200 Anymaze (Ugo Basile, Varese, Italy). Mice were placed in an empty open field (60x40x35 cm)
201 for 20 minutes. The distance traveled (m) was recorded in 5 minutes intervals and used as an
202 index of locomotor activity in a novel environment.
203
204 **Body weight measurements**. Body weight (g) was measured through the course of the drug
205 administration protocol and at PND63 when the behavioral assays began. The change in body
206 weight was calculated as the difference between body weight at any given day and body weight
207 at PND 28.

208 **RNA Extraction, cDNA Synthesis and qPCR.** Brain tissue from PFC *n* = 8 female WT, *n* = 6
209 male WT, *n* = 6 female HR, *n* = 5 male HR mice was homogenized in TRIzol Reagent
210 (#15596018, Thermo Fisher Scientific) and Zirconium beads (#Zr0B05-RNA, Next Advance)
211 using the Bullet Blender homogenizer (BBX24B, Next Advance,). RNA was extracted on
212 columns with the Direct-Zol RNA miniprep kit (#R2051, Zymo Research). To quantify *Reelin*
213 expression levels, equal amounts of cDNA were synthesized using the SuperScript VILO
214 MasterMix (#11755-050, Thermo Fisher Scientific) and mixed with the qPCRBIO SyGreen Blue
215 Mix (#17-507DB, PCR Biosystems) and 5 pmol of both forward (5'-
216 GGACTAAGAATGCTTATTCC -3') and reverse (5'- GGAAGTAGAATTCATCCATCAG -3')
217 *Reelin* primers. ACTB was amplified as an internal control (5'- ATGGAGGGGAATACAGCCC
218 -3') and reverse (5'- TTCTTTGCAGCTCCTTCGTT -3').
219
220 **Immunohistochemistry (IHC)**. To examine Fos expression by IHC in WT and HR mice, we
221 used *n* = 6 male WT, *n* = 6 male HR, *n* = 4 female WT, and *n* = 9 female HR littermates. Mice
222 were subjected to the 3 trials of the 6-DOT test, as described above. To confirm that the exposure
223 to the behavioral task triggers Fos expression, a separate group of 4 WT mice was used,
224 including *n* = 1 mouse for each trial and *n* = 1 mouse without any behavioral exposure (home
225 cage). Thirty minutes after the last trial, mice were anesthetized with isoflurane and fixed via
226 transcardial perfusion with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS).
227 Brain tissues were processed as previously described (Iemolo et al., 2020). Briefly, 30-µm thick
228 coronal slices were stained with a primary antibody recognizing Fos (1:1000, ABE457, EMD
229 Millipore, RRID:AB_2631318) and a secondary anti-rabbit antibody (donkey anti-rabbit Alexa
230 Fluor 488 (1:1000, #A21206, Thermo Fisher Scientific). Images were acquired with a
231 fluorescent microscope (BZX800, Keyence Corporation, Osaka, Japan). Different brain areas

232 were identified using a mouse brain atlas as a reference (Paxinos G, 2001). For the medial
233 prefrontal cortex (mPFC), 5 sections from bregma + 2.46 to + 1.10 mm, ± 340 µm apart, were
234 stained and 4 images for each section were captured for each mouse. For the CA1 and dentate
235 gyrus (DG) subregions of the hippocampus (HP), 4 sections from bregma -1.46 to - 2.46 mm, ±
236 250 µm apart, were stained and 4 images for each section were captured for each animal. For the
237 lateral septum (LS), 1 section from bregma 1.42 mm to 1.10 mm was stained and 1 image for
238 each section was captured for each mouse. The number of Fos+ cells was counted by two
239 examiners blind to the experimental conditions. All numbers are presented as mean ± SEM.
240
241 **RNA fluorescent in situ hybridization (FISH).** To examine the expression of various
242 transcripts in specific cell types of the lateral septum, we performed RNA FISH using the
243 RNAscope Multiplex Fluorescent Reagent Kit v2 (ACD, #323100) and following the
244 instructions for 'fixed-frozen tissue sample' of the user manual (ACD, USM-323100). Tissue
245 sections corresponding to the LS (bregma 0.26-0.50 mm) from n = 1 mouse exposed to the 3
246 trials of the 6-DOT, as described above, were hybridized with a mix of three probes; Reelin
247 (ACD, # 405981) + Slc32a1 (ACD, #319191-C3) + Chat (ACD, # 408731) or Fos (ACD,
248 #316921) + Slc32a1 + Chat. LSc and LSr were identified using the Allen Mouse Brain Atlas. To
249 assess both tissue RNA integrity and assay procedure, a separate group of sections were
250 incubated with probes (data not shown). Images were acquired with the Keyence fluorescent
251 microscope.
252
253 **Statistical analysis**. To examine how factors (treatment, genotype, sex) affected mice behavior
254 or Fos expression, we used linear mixed models (LMM) in JMP pro v. 15.0 (SAS Institute, Inc).

255 LMM allow modeling of both fixed and random effects (which subsume repeated measures)
256 (Quinn GP, 2002; Zuur AF, 2016). We incorporated the categorical predictor variables of interest
257 as fixed effects and included all possible interactions. We included mouse cohorts and individual
258 subjects as random effects to account for possible non-independence of the data. We ensured
259 assumptions of approximate normality and variance homogeneity were met by inspecting plots
260 of residuals versus predicted values, and by inspecting quantile-quantile plots with 95%
261 confidence limit curves. When residual plots indicated that it was appropriate for repeated
262 measures, we used a covariance structure that allowed variances to differ across the levels of the
263 repeated variable (Garrett M. Fitzmaurice, 2011). We analyzed the untransformed data in all but
264 few traits where a fourth root transformation mitigated variance heterogeneity (e.g. latency to
265 light, latency to center, Vmax of acoustic startle, body weight change). When significant
266 interactions were found, planned post-hoc pairwise comparisons were performed to identify
267 differences among specific genotype and treatment groups. We report *P*-values that remained
268 significant after controlling for multiple comparisons by holding the 'false-discovery rate' to
269 0.05 using the Benjamini-Hochberg method (Hochberg, 1995). Outliers were detected using the
270 Huber M-estimation method (Huber, 1973) in JMP pro v. 15.0 and removed when appropriate (n
271 = 3 in latency to light, n = 2 latency to center, n = 2 time spent sniffing, n = 1 sociability index, n
272 = 1 in % PPI).
273
274 **Factor analysis of behavioral assays.** Factor analysis is a data reduction method for
275 understanding underlying relationships among variables (Bartholomew, 2008). We performed
276 factor analysis using the variables from multiple behavioral assays, such as 6-DOT (DI, total
277 exploration time in test trial [T3]) , social interaction (time sniffing novel mouse, difference time

278 sniffing novel mouse vs novel object), OF (time in center), LD (time in light), TS (mobility
279 time). Factor analysis was computed in JMP 15.0 Pro and was conducted with varimax rotation
280 with a factor-loading cutoff of 0.3 (Manly and Navarro Alberto; Stephens, 1996). The number of
281 factors retained in our model was selected by inspecting the 'elbow' on the scree plot curve with
282 factors retained if their eigenvalues were greater than 1 (Cattell, 1966). With these settings, a
283 three-factor model was generated for our dataset. The loadings of the observed variables on the
284 extracted factors are shown in Fig. 6. Factor scores for individual mice were extracted and used
285 as variables for subsequent LMM analysis to identify their associations with treatment, sex, and
286 genotype.
287
288 **Results**
289 **Working memory was impaired after chronic adolescent exposure to THC, and in male HR**
290 **mice**
291 To assess the long-term effects of chronic adolescent exposure to THC on working
292 memory in WT and HR mice, we used a modified novel object recognition test that uses 6
293 instead of 2 objects (6-DOT) (Sannino et al., 2012; Olivito et al., 2016). This test evaluates
294 recognition memory under conditions of high loads of information processing, which is referred
295 to as memory span and is considered a form of working memory. A discrimination index (DI)
296 was calculated to determine the amount of time mice explored the novel object compared to the
297 familiar objects. A preference for the novel object is considered as a sign that mice remember the
298 familiar objects (Sannino et al., 2012). Higher rates of exploratory activity in response to the
299 novel object is also interpreted as a sign of novelty seeking behavior (Flagel et al., 2014).

300 Chronic THC exposure during adolescence decreased the DI (working memory) of mice
301 across all groups by ~31% compared to the vehicle-treated control group (**Fig. 1A-B**, treatment
302 effect, $F_{1,86} = 16.4$, $P < 0.0001$). HR mice also showed decreased DI, but the magnitude of the
303 genotype effect varied among sexes (**Fig. 1C**, sex x genotype interaction, $F_{1,86} = 5.7$, $P = 0.019$).
304 Compared to WT, female HR mice did not show impaired working memory, but male HR mice
305 showed a decreased DI that was similar to the level of impairment caused by THC treatment in
306 male WT mice (**Fig. 1C,** paired *t* test, $t = 2.9$, $P_{adj} = 0.019$).
307 This assay also revealed that HR mice displayed a ~25% increase in novelty-induced
308 exploratory activity compared to WT controls (**Fig. 1D-E**, genotype effect, $F_{1,84} = 6.1$, $P =$
309 0.015). This effect was triggered by the novel object, as it was revealed during the test phase
310 while the total exploratory activity toward objects did not vary among groups in the initial
311 familiarization phase (**Fig. 1F**, main effects and interactions *P*s > 0.3).
312 To further evaluate potential confounding effects of general locomotion or body weight
313 having an impact on exploratory activity in this task, we analyzed changes in spontaneous
314 locomotor activity in an empty arena or in body weight in WT and HR mice (**Suppl. Fig. 2A-B**).
315 There was no effect of THC treatment, genotype, or sex on locomotor activity or body weight at
316 the time of testing, suggesting that the deficits in working memory observed in WT and HR mice
317 were not driven by changes in locomotion or body weight.
318
319 **Social behaviors were impaired by chronic adolescent exposure to THC in HR male mice**
320 To assess the long-term effects of chronic adolescent exposure to THC on social
321 interaction behavior in WT and HR mice, we used the three-chamber social approach test in
322 which a mouse is given the choice to spend time interacting with a novel mouse or a novel

323 inanimate object, which are referred to as "exploration targets" in our analysis (Yang et al.,
324 2011). We considered two aspects of social interaction: (1) time spent in the chamber with the
325 novel mouse versus the novel object, and (2) time actively sniffing the novel mouse versus the
326 novel object, which is a more specific measure of social interaction (Yang et al., 2011). Higher
327 time spent in the chamber with or sniffing the novel mouse compared to the novel object was
328 considered an index of "sociability".

329 All mice explored the novel mouse more than the inanimate object, confirming that the
330 behavioral assay can detect sociability across all groups (**Fig. 2A-2D**, exploration target effect,
331 $F_{1,119} = 49.5$, $P < 0.0001$; $F_{1,113} = 83.3$, $P < 0.0001$, respectively). There were no significant
332 effects of treatment, genotype and sex on time spent in the chamber ($P > 0.1$). In contrast, when
333 analyzing time sniffing, the effect of chronic adolescent exposure to THC varied among the two
334 exploration targets (**Fig. 2E**, treatment x exploration target interaction, $F_{1,113} = 4.1$, $P = 0.046$).
335 Precisely, THC had no significant effect on time spent sniffing the novel inanimate object
336 compared to vehicle (**Fig. 2E**, paired *t* test, $t = 0.06$, $P_{adj} = 1$); however, THC caused a ~20%
337 decrease in time spent sniffing the novel mouse compared to vehicle across all groups (**Fig. 2E,** t
338 $= 3.1$, $P_{adj} = 0.003$). The suppressive effect of THC on social interaction depended on the
339 genotype (**Fig. 2F**, treatment x genotype interaction, $F_{1,108} = 6.1$, $P = 0.015$); a post-hoc analysis
340 revealing that only HR mice treated with THC exhibited a significant reduction in overall time
341 sniffing when compared to vehicle treated HR mice (paired *t* test, $t = 3.2$, $P_{adj} = 0.006$). However,
342 when a sociability index was calculated, we did not detect any effect of treatment or genotype
343 (all effects and interaction *Ps* >0.1, **Suppl. Fig. 3A-B**).
344

345 **Chronic adolescent exposure to THC increased anxiety-like behavior in female HR mice**

346 To evaluate how impaired Reelin signaling influences anxiety-like behavior in mice
347 chronically exposed to THC during adolescence, we compared WT and HR mice in the LD and
348 OF tests. These behavioral assays examine anxiety-like responses by which animals avoid
349 illuminated or open areas (Crawley and Goodwin, 1980; Bailey and Crawley, 2009). Decreased
350 anxiety (increased disinhibition) is represented by mice spending more time in the light or open
351 compartments, and by having a shorter latency to enter the brightly lit or center chambers.
352 In the LD test, THC increased the overall time spent in the light compartment (**Fig. 3A,**
353 treatment effect, $F_{1,102} = 8.5$, $P = 0.0043$), but the strength of these effects varied among
354 genotypes and sexes (**Fig. 3B**, treatment x genotype x sex interaction, $F_{1,97.5} = 4.4$, $P = 0.038$).
355 This effect was mainly driven by female HR mice treated with THC, which spent 43% longer
356 time in the light compared to the vehicle-treated group (**Fig. 3B,** paired *t* test, t = 3.6, P_{adj} =
357 0.004). A separate analysis revealed that the latency to enter the light chamber was also
358 influenced by genotype and sex (**Fig. 3D-E**, sex x genotype interaction, $F_{1,47} = 5$, $P = 0.0197$).
359 Female HR mice showed ~18% increase in latency compared to female WT, indicating an
360 increased baseline anxiety-like responses (paired *t* test, $t = 2.5$, $P_{adj} = 0.03$). However, the latency
361 to enter the light compartment was not significantly influenced by THC (treatment effect, $F_{1,97}$ =
362 0.02, $P = 0.88$).
363 In the open field test, HR mice spent ~18% more time in the center (**Fig. 3F-G**, genotype
364 effect, $F_{1,102} = 3.7$, $P = 0.057$ marginally significant), suggesting a reduced anxiety-like behavior.
365 Time spent in center also varied among sexes and treatment groups (treatment x genotype x sex
366 interaction, $F_{1,99} = 6.1$, $P = 0.016$); however, despite this significant overall variability, a post-
367 hoc analysis did not identify significant differences between specific pairs of groups. Latency to
368 enter the open area did not vary among groups (**Fig. 3H**, all $P > 0.2$).

369

370 **Male HR mice showed enhanced ability to strive against stress following chronic adolescent**

371 **exposure to THC**

372 To test the effect of adolescent exposure to THC on reactivity to aversive conditions in

373 WT and HR mice, we performed the TS test, an assay commonly used to screen anti-depressive

374 drugs and to measure behavioral despair in mice (Can et al., 2012). An increased mobility has

375 been associated with enhanced ability to strive against stress and is broadly related to active

376 coping, impulsive and aggressive behaviors (Strekalova et al., 2004; Brockhurst et al., 2015).

377 We quantified total mobility time in a 6-minute tail suspension assay in WT and HR

378 mice. THC treatment influenced total mobility time, but the effect varied among sexes and

379 genotypes (**Fig. 4A**, treatment x sex x genotype interaction, $F_{1,158} = 12.8$, $P = 0.0005$). While

380 THC did not significantly change the mobility time of either male or female WT mice compared

381 to the vehicle controls (paired *t* test, $t = 1.9$, $P_{adj} = 0.07$; $t = 0.5$, $P_{adj} = 0.6$, respectively), it

382 induced a 19% increase in mobility time for male HR mice (**Fig. 4B**, paired *t* test, $t = 2.6$, $P_{adj} =$

383 0.04). We also observed sex differences in baseline mobility time for which female HR mice

384 showed a 21% increase in mobility time compared to male HR mice (**Fig. 4B,** paired *t* test, $t = 3$,

385 $P_{adj} = 0.03$).

386

387 **Reelin deficiency led to enhanced startle responses and pre-pulse inhibition**

388 To evaluate psychotic-like behaviors in WT and HR mice following chronic adolescent

389 exposure to THC, we quantified the ASR and the % PPI. The ASR is a reflexive reaction to a

390 sudden acoustic stimulus (Swerdlow et al., 2001; Geyer et al., 2002). The % PPI is used as a

391 measure of sensorimotor gating, which occurs when a weak pre-pulse stimulus suppresses the

392 response to the subsequent startling stimulus. PPI is impaired in schizophrenia patients and
393 serves as an animal model of schizophrenia (Cadenhead et al., 1993).
394 We first examined the differences in ASR of WT and HR mice at various acoustic
395 intensities (65, 80, 90, 100, 110, 120 db, **Fig. 5A**). THC did not influence ASR (treatment effect,
396 $F_{1,451} = 0.7$, $P = 0.4$). Compared to WT, HR mice exhibited an overall ~3% higher startle
397 response (genotype effect, $F_{1,451} = 7.2$, $P = 0.008$), which varied by the acoustic intensity of the
398 pulse (**Fig. 5B,** genotype x db interaction, $F_{5,408} = 2.8$, $P = 0.016$). The increased startle tended to
399 be stronger at 120db (**Fig. 5B,** paired *t* test, t = 2, $P_{adj} = 0.08$ marginally significant).
400 When examining the % PPI at three pre-pulse intensities (**Fig. 5C,** 68, 71 and 77 db), HR
401 mice showed an overall ~13% increase in % PPI compared to WT mice (**Fig. 5D**, genotype
402 effect, $F_{1,482} = 8.6$, $P = 0.004$). Adolescent exposure to THC also had an effect on % PPI that
403 varied among sexes (**Fig. 5E,** treatment x sex interaction, $F_{1,482} = 8$, $P = 0.005$). Female mice
404 treated with THC tended to show ~12 % reduction in % PPI compared to vehicle-treated mice
405 (paired *t* test, t = 2, $P_{adj} = 0.05$ marginally significant).
406
407 **THC and Reelin deficiency influenced behavioral domains that were associated with**
408 **cognition and disinhibition**
409 We performed a factor analysis to determine whether the multiple behavioral responses
410 observed in our study reflect a smaller number of synthetic behavioral domains that are
411 influenced by THC and *Reelin* deficiency.
412 The factor analysis identified three unique behavioral domains (factors), which explained
413 57% of total variance (**Fig. 6A)**. The first factor explained 23.8% of the total variance and
414 contained outcomes (positive loadings) from the social interaction and working memory tests

415 (e.g. time sniffing novel mouse, discrimination index). As factor 1 largely reflected measures of
416 cognitive functions, we named it "*cognition*". The second factor (17% of the total variance) was
417 named "*disinhibition*" as it contained behaviors that reflected increased exploratory or
418 disinhibited behavior (e.g. time in the center of the open field, novel object exploration). The
419 third factor explained 15.7% of the total variance and was named "*stress reactivity*" because the
420 main outcome that contributed to this cluster was the mobility time of the TS test.

421 To determine whether these behavioral domains differed between treatments, genotypes,
422 or sexes, we analyzed the rotator factor scores for each subject (**Fig. 6B-C**). We found that the
423 behavioral domains were significantly influenced by treatment or genotype (treatment x
424 behavioral domains interaction, $F_{2,142} = 5.7$, $P = 0.004$; genotype x behavioral domains
425 interaction, $F_{2,142} = 3.4$, $P = 0.035$; respectively). Post-hoc analysis revealed that *cognition* was
426 significantly decreased by THC (**Fig. 6B,** paired *t* test, t = 3.1, $P_{adj} = 0.006$), and *disinhibition*
427 was significantly increased by *Reelin* deficiency (**Fig. 6C,** paired *t* test, t = 2.8, $P_{adj} = 0.014$).
428 Neither treatment, genotype, nor sex significantly influenced the third behavioral domain, *stress*
429 *reactivity*.

430 Overall, this exploratory data analysis is consistent with the results of the separate
431 behavioral tests by indicating that adolescent exposure to THC detrimentally affected cognitive
432 functions, and it revealed that reduced expression of *Reelin* led to disinhibitory behaviors, which
433 were intensified by THC treatment in a sex-specific manner.

434

435 **Novelty seeking in HR mice was associated with reduced neuronal activity in the lateral**
436 **septum.**

437 In response to different environmental stimuli, the expression of *Fos* is rapidly induced in
438 specific neuronal populations allowing the identification of relevant neuronal ensembles
439 encoding specific behaviors (Curran and Morgan, 1995; Chaudhuri, 1997). To explore the
440 neuronal ensemble underlying the enhanced novelty seeking behavior observed in the HR mice
441 (**Fig. 1E**), we mapped the regional brain expression of Fos protein by IHC in juvenile mice
442 subjected to the 6-DOT (**Fig. 1A**).
443 In response to this behavioral task, WT mice activated the expression of Fos in four brain
444 areas, including mPFC, LS, DG and CA1 sub-regions of the HP (**Suppl. Fig. 4**). Thus, we
445 selected these areas for measuring the number of Fos-expressing (Fos+) cells in WT and HR
446 mice, thirty minutes after the end of the task (**Fig. 7A-B**). There were no significant differences
447 in Fos expression between genotypes or sex in the mPFC, DG or CA1 (**Fig. 7F,H-I**). In contrast,
448 compared to WT, HR mice showed a ~28% reduction in number of Fos+ cells in the LS (**Fig.
449 7G,** genotype effect, $F_{1,13} = 5$, $P = 0.04$). To examine the expression of *Reelin* and *Fos* in the LS,
450 we used RNA FISH and showed that *Reelin*-expressing and *Fos*-expressing cells are
451 predominately located in the LS caudal (LSc), with fewer positive cells present in the LS rostral
452 (LSr) (**Fig. 8A,C**). Consistent with the expression of *Reelin* by GABAergic neurons (Lossi et al.,
453 2019), *Reelin*+ cells also co-expressed the vesicular GABA transporter (*Scl32a1*), a marker of
454 GABAergic neurons (**Fig. 8B**). To further characterize the *Fos*-positive ensemble, we co-labeled
455 *Fos*+ cells with markers of GABAergic (*Scl32a1*) and cholinergic neurons (*Chat*), which
456 represent the major neuronal cell types of the LS. We found that *Fos* transcript was activated in
457 both cell types and that the location of *Fos*+ cells showed a preferential location in the LSc,
458 similarly to those expressing *Reelin* (**Fig. 8D**).
459

460 **Discussion**

461 The present study revealed for the first time that reduced levels of *Reelin* influences
462 behavioral abnormalities caused by heavy consumption of THC during adolescence, and that
463 Reelin plays a role in the neurobiological mechanisms underlying novelty seeking by modulating
464 neuronal ensembles in the LS.

465 ***Influence of THC and Reelin deficiency on cognitive functions***

466 We found that chronic adolescent exposure to THC impaired working memory, in line
467 with previous reports in rodent models and clinical studies in humans (Renard et al., 2014;
468 Rubino and Parolaro, 2016; Hurd et al., 2019). Additionally, HR mice showed lower working
469 memory in comparison to the WT littermates and to a similar extent as did chronic adolescent
470 exposure to THC. Although it is the first time that HR mice are tested in a task that measures
471 memory span, such as the 6-DOT, this finding is consistent with studies demonstrating the
472 crucial role of Reelin in mechanisms underlying learning and memory (Weeber et al., 2002;
473 Rogers et al., 2013; Iafrati et al., 2014; Telese et al., 2015).

474 We found that social interaction was impaired by chronic adolescent exposure to THC
475 with the strongest effects in HR mice. These observations are in line with evidence in human and
476 preclinical studies showing social deficits induced by THC (Long et al., 2010). Only few studies
477 have examined social behaviors of the HR mice and did not report prominent social deficits
478 (Podhorna and Didriksen, 2004; Macri et al., 2010; Michetti et al., 2014). Thus, our study
479 revealed for the first time that *Reelin* deficiency exacerbates social deficits induced by THC.

480 Consistently, the factor analysis revealed that variables from both working memory and
481 social interaction tests contributed to a behavioral domain, named '*cognition*', which was
482 negatively influenced by adolescent exposure to THC.

483 ***Reelin deficiency is associated with disinhibitory phenotypes***

484 We speculate that multiple behavioral responses exhibited by HR mice may reflect general
485 behavioral disinhibition. In rodents, behavioral disinhibition is measured as a function of
486 increased exploratory activity towards unfamiliar objects or environments, and has been
487 associated with compulsive drug taking (Davis et al., 2008; Flagel et al., 2010; Belin et al.,
488 2011). In humans, behavioral disinhibition reflects personality traits that encompasses
489 impulsivity, risk-taking and novelty seeking phenotypes; these behavioral patterns are more
490 pronounced in adolescence and have been linked to addiction susceptibility (Young et al., 2009).
491 Here, we found that, compared to WT, both male and female HR mice explored the novel objects
492 for longer time in the 6-DOT. Additionally, female HR mice treated with THC showed signs of
493 reduced inhibitory control as they spent more time in the center chamber of the OF test or in the
494 brightly lit compartment of the LD test. Consistently, variables from these tests contributed to the
495 same behavioral domain ('*disinhibition'*) revealed by the factor analysis, which was positively
496 influenced by *Reelin* deficiency. These observations are also in line with a previous study
497 showing decreased anxiety of HR mice in the elevated plus maze and increased motor
498 impulsivity (Ognibene et al., 2007).

499 Collectively, these results raise the hypothesis that impairment in Reelin signaling may
500 underlie addiction traits by disrupting inhibitory control. Further supporting this hypothesis, in a
501 recent genome-wide association study (GWAS), a variant of the *Reelin* gene was associated with
502 higher likelihood to consume alcohol ($P = 4 \times 10^{-9}$, variant and risk allele rs756747-T) (Karlsson
503 Linner et al., 2019).

504 ***Reelin deficiency leads to abnormal responses to aversive conditions***

505 We observed abnormal behavior in HR mice in response to aversive conditions. First,
506 male HR mice treated with THC showed prolonged mobility time in the TS test compared to
507 vehicle group. This behavior has been linked to proactive coping in response to a threat
508 (Strekalova et al., 2004; Brockhurst et al., 2015). Second, HR mice exhibited enhanced startle
509 reactivity to acoustic stimuli compared to WT mice, which was not influenced by THC
510 treatment. In a previous study, the startling response of HR mice did not change following a
511 single acoustic pulse of 105db; however, in our study, we used five startling pulses from 80db to
512 120db that likely increased the sensitivity of the task. Elevated ASR has been observed in mental
513 conditions associated with impaired emotional reactivity, such as posttraumatic stress disorders
514 (Orr et al., 1995) and obsessive-compulsive disorders (Kumari et al., 2001). These observations
515 led us speculate that reduced expression of *Reelin* may lead to altered emotional reactivity. This
516 hypothesis deserves further examination of the HR mice using tasks specifically designed to
517 assess impulsivity and aggression.

518 ***Reelin as a susceptibility factors for psychiatric disorders***

519 The array of behavioral phenotypes exhibited by HR mice in our study, encompassing
520 memory impairments, social deficits, poor inhibitory control, and altered stress responses, is
521 reminiscent of the behaviors characterizing numerous psychiatric conditions, ranging from
522 autism spectrum disorders (ASD) to schizophrenia and substance use disorders. Consistently, a
523 role of Reelin in the development of these disorders is supported by several lines of evidence. In
524 particular, whole exome sequencing studies identified *de novo* mutations in the *Reelin* gene in
525 individuals with ASD (Neale et al., 2012; Iossifov et al., 2014; Wang et al., 2014). Further
526 support for Reelin involvement in psychiatric disorders is provided by the observation of reduced
527 expression of *Reelin* transcript or protein in postmortem brains of individuals affected by ASD

528 (Fatemi et al., 2001), and schizophrenia (Guidotti et al., 2000; Ruzicka et al., 2007; Habl et al.,
529 2012). These observations suggest that altered Reelin signaling may be a vulnerability factor for
530 psychiatric disorders and that the HR mice represent a valuable animal model for translational
531 research. However, we did not observe any PPI deficits in HR mice. In contrast, a previous study
532 showed that a single *in vivo* injection of Reelin protein increases % PPI (Rogers et al., 2013). It
533 is possible that, in our study, the elevated startle response observed in HR mice may act as a
534 confounding factor for accurately assessing the effect of *Reelin* deficiency. It is also possible that
535 impaired Reelin signaling contributes mainly to the cognitive, social and emotional deficits
536 associated with schizophrenia, which are referred to as negative symptoms, as opposed to
537 positive symptoms that include psychotic-like behaviors (Kirkpatrick et al., 2006).
538 Notably, THC reduced % PPI in female WT mice, suggesting that a history of drug
539 exposure leads to psychotic-like behaviors. Conflicting results have been reported concerning the
540 effects of adolescent chronic exposure to THC on psychotic-like behaviors in rodents (Rodriguez
541 et al., 2017; Todd et al., 2017; Ibarra-Lecue et al., 2018). Differences in THC doses, mice genetic
542 background and specific experimental conditions among different research groups may explain
543 some of these conflicts.
544 ***Sex differences in behavioral responses associated with Reelin haploinsufficiency***
545 Our study revealed numerous sex differences in the behavioral abnormalities associated
546 with *Reelin* deficiency and/or adolescent exposure to THC. Male HR mice were more sensitive
547 to working memory impairments, as well as to emotional reactivity in the TS test. In contrast,
548 female mice, showed reduced anxiety-like behaviors (HR) or reduced % PPI (WT) in response to
549 THC. Consistently, previous finding in rodents showed that chronic adolescent exposure to
550 cannabinoids is associated with numerous behavioral sex differences; however, the knowledge of

551 the underlying mechanisms remains limited (Craft et al., 2013). Given the critical role of Reelin
552 brain development, it is possible that Reelin influences the development of the endocannabinoid
553 system in a sex-specific manner, which can lead to sex differences to the behavioral effects of
554 THC.

555 ***The novelty seeking phenotype observed in HR mice is associated with reduced Fos expression***
556 ***in the LS***

557 We found that the prolonged exploratory activity of novel objects exhibited by HR mice
558 in the 6-DOT was associated with reduced neuronal activity in the LS. Although the LS has not
559 been linked directly to novelty seeking, it has been implicated in the modulation of rewarding
560 experiences that are correlated to novelty seeking (Luo et al., 2011; Harasta et al., 2015; McGlinchey
561 and Aston-Jones, 2018). Moreover, several behaviors influenced by *Reelin* deficiency in our study
562 have also been linked to the activity of the LS, such as social interaction, memory and emotional
563 processes associated with aversive situations (Singewald et al., 2011; Leroy et al., 2018) (Shin et
564 al., 2018). Notably, *Fos* and *Reelin* expression are detected in the LSc, a region implicated in the
565 regulation of drug-seeking behavior via a population of GABAergic neurons that project to the
566 ventral tegmental area (VTA) (Luo et al., 2011). We speculate that *Reelin* deficiency may alter
567 LS-VTA pathway underlying motivated behavior by disrupting GABA inhibition in the LSc and,
568 hence, disinhibiting dopaminergic neurons in the VTA. In our previous work, we showed that
569 Reelin triggers a signaling cascade via the intracellular domain of its receptor LRP8 and directly
570 activates transcriptional enhancers of immediate early genes, including *Fos* (Telese et al., 2015).
571 Thus, it is possible that Reelin signaling modulates the transcriptional and epigenomic landscape
572 of specific neuronal populations in the LS.

573 In conclusion, our study suggests that elucidating Reelin signaling will improve our
574 understanding of neurobiological mechanisms underlying behavioral traits relevant to the
575 development of psychiatric conditions.
576
577 **References**

578 Arain M, Haque M, Johal L, Mathur P, Nel W, Rais A, Sandhu R, Sharma S (2013) Maturation
579 of the adolescent brain. Neuropsychiatr Dis Treat 9:449-461.
580 Bailey KR, Crawley JN (2009) Anxiety-Related Behaviors in Mice. In: Methods of Behavior
581 Analysis in Neuroscience (nd, Buccafusco JJ, eds). Boca Raton (FL).
582 Bartholomew DJ (2008) Analysis of multivariate social science data, 2nd Edition. Boca Raton:
583 CRC Press.
584 Belin D, Berson N, Balado E, Piazza PV, Deroche-Gamonet V (2011) High-novelty-preference
585 rats are predisposed to compulsive cocaine self-administration. Neuropsychopharmacology
586 36:569-579.
587 Bouamrane L, Scheyer AF, Lassalle O, Iafrati J, Thomazeau A, Chavis P (2016) Reelin-
588 Haploinsufficiency Disrupts the Developmental Trajectory of the E/I Balance in the Prefrontal
589 Cortex. Front Cell Neurosci 10:308.
590 Brockhurst J, Cheleuitte-Nieves C, Buckmaster CL, Schatzberg AF, Lyons DM (2015) Stress
591 inoculation modeled in mice. Transl Psychiatry 5:e537.
592 Cadenhead KS, Geyer MA, Braff DL (1993) Impaired startle prepulse inhibition and habituation
593 in patients with schizotypal personality disorder. Am J Psychiatry 150:1862-1867.
594 Can A, Dao DT, Terrillion CE, Piantadosi SC, Bhat S, Gould TD (2012) The tail suspension test.
595 J Vis Exp:e3769.

596 Cattell RB (1966) The Scree Test For The Number Of Factors. Multivariate Behav Res 1:245-
597 276.
598 Chaudhuri A (1997) Neural activity mapping with inducible transcription factors. Neuroreport
599 8:iii-vii.
600 Cousijn J, Luijten M, Feldstein Ewing SW (2018) Adolescent resilience to addiction: a social
601 plasticity hypothesis. Lancet Child Adolesc Health 2:69-78.
602 Craft RM, Marusich JA, Wiley JL (2013) Sex differences in cannabinoid pharmacology: a
603 reflection of differences in the endocannabinoid system? Life Sci 92:476-481.
604 Crawley J, Goodwin FK (1980) Preliminary report of a simple animal behavior model for the
605 anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 13:167-170.
606 Curran T, Morgan JI (1995) Fos: an immediate-early transcription factor in neurons. J Neurobiol
607 26:403-412.
608 D'Arcangelo G, Miao GG, Chen SC, Soares HD, Morgan JI, Curran T (1995) A protein related
609 to extracellular matrix proteins deleted in the mouse mutant reeler. Nature 374:719-723.
610 Davis BA, Clinton SM, Akil H, Becker JB (2008) The effects of novelty-seeking phenotypes and
611 sex differences on acquisition of cocaine self-administration in selectively bred High-
612 Responder and Low-Responder rats. Pharmacol Biochem Behav 90:331-338.
613 Fatemi SH, Stary JM, Halt AR, Realmuto GR (2001) Dysregulation of Reelin and Bcl-2 proteins
614 in autistic cerebellum. J Autism Dev Disord 31:529-535.
615 Flagel SB, Waselus M, Clinton SM, Watson SJ, Akil H (2014) Antecedents and consequences of
616 drug abuse in rats selectively bred for high and low response to novelty. Neuropharmacology
617 76 Pt B:425-436.

618 Flagel SB, Robinson TE, Clark JJ, Clinton SM, Watson SJ, Seeman P, Phillips PE, Akil H
619 (2010) An animal model of genetic vulnerability to behavioral disinhibition and
620 responsiveness to reward-related cues: implications for addiction. Neuropsychopharmacology
621 35:388-400.
622 Garrett M. Fitzmaurice NML, and James H. Ware (2011) Applied Longitudinal Analysis, 2nd
623 Edition.
624 Geyer MA, McIlwain KL, Paylor R (2002) Mouse genetic models for prepulse inhibition: an
625 early review. Mol Psychiatry 7:1039-1053.
626 Guidotti A, Auta J, Davis JM, Di-Giorgi-Gerevini V, Dwivedi Y, Grayson DR, Impagnatiello F,
627 Pandey G, Pesold C, Sharma R, Uzunov D, Costa E (2000) Decrease in reelin and glutamic
628 acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a
629 postmortem brain study. Arch Gen Psychiatry 57:1061-1069.
630 Habl G, Schmitt A, Zink M, von Wilmsdorff M, Yeganeh-Doost P, Jatzko A, Schneider-Axmann
631 T, Bauer M, Falkai P (2012) Decreased reelin expression in the left prefrontal cortex (BA9) in
632 chronic schizophrenia patients. Neuropsychobiology 66:57-62.
633 Harasta AE, Power JM, von Jonquieres G, Karl T, Drucker DJ, Housley GD, Schneider M,
634 Klugmann M (2015) Septal Glucagon-Like Peptide 1 Receptor Expression Determines
635 Suppression of Cocaine-Induced Behavior. Neuropsychopharmacology 40:1969-1978.
636 Hochberg Ba (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach
637 to Multiple Testing. Journal of the Royal Statistical Society Series B (Methodological)
638 57:289-300.
639 Huber PJ (1973) Robust Regression: Asymptotics, Conjecture, and Monte Carlo. Annals of
640 Statistics 1:799–821.

641 Huestis MA (2007) Human cannabinoid pharmacokinetics. Chem Biodivers 4:1770-1804.
642 Hurd YL, Manzoni OJ, Pletnikov MV, Lee FS, Bhattacharyya S, Melis M (2019) Cannabis and
643 the Developing Brain: Insights into Its Long-Lasting Effects. J Neurosci 39:8250-8258.
644 Iafrati J, Orejarena MJ, Lassalle O, Bouamrane L, Gonzalez-Campo C, Chavis P (2014) Reelin,
645 an extracellular matrix protein linked to early onset psychiatric diseases, drives postnatal
646 development of the prefrontal cortex via GluN2B-NMDARs and the mTOR pathway. Mol
647 Psychiatry 19:417-426.
648 Ibarra-Lecue I, Mollinedo-Gajate I, Meana JJ, Callado LF, Diez-Alarcia R, Uriguen L (2018)
649 Chronic cannabis promotes pro-hallucinogenic signaling of 5-HT2A receptors through
650 Akt/mTOR pathway. Neuropsychopharmacology : official publication of the American
651 College of Neuropsychopharmacology 43:2028-2035.
652 Iemolo A, Montilla-Perez P, Lai IC, Meng Y, Nolan S, Wen J, Rusu I, Dulcis D, Telese F (2020)
653 A cell type-specific expression map of NCoR1 and SMRT transcriptional co-repressors in the
654 mouse brain. The Journal of comparative neurology.
655 Iossifov I et al. (2014) The contribution of de novo coding mutations to autism spectrum
656 disorder. Nature 515:216-221.
657 Ishii K, Kubo KI, Nakajima K (2016) Reelin and Neuropsychiatric Disorders. Front Cell
658 Neurosci 10:229.
659 Johnston LD, Miech, R. A., O'Malley, P. M., Bachman, J. G., Schulenberg, J. E., & Patrick,
660 M.E. (2018) Monitoring the Future national survey results on drug use, 1975-2017: Overview,
661 key findings on adolescent drug use. . Ann Arbor: Institute for Social Research, The
662 University of Michigan

663 Karlsson Linner R et al. (2019) Genome-wide association analyses of risk tolerance and risky
664 behaviors in over 1 million individuals identify hundreds of loci and shared genetic
665 influences. Nat Genet 51:245-257.
666 Kasten CR, Zhang Y, Boehm SL, 2nd (2019) Acute Cannabinoids Produce Robust Anxiety-Like
667 and Locomotor Effects in Mice, but Long-Term Consequences Are Age- and Sex-Dependent.
668 Front Behav Neurosci 13:32.
669 Kirkpatrick B, Fenton WS, Carpenter WT, Jr., Marder SR (2006) The NIMH-MATRICS
670 consensus statement on negative symptoms. Schizophr Bull 32:214-219.
671 Kumari V, Kaviani H, Raven PW, Gray JA, Checkley SA (2001) Enhanced startle reactions to
672 acoustic stimuli in patients with obsessive-compulsive disorder. Am J Psychiatry 158:134-
673 136.
674 Laviola G, Macri S, Morley-Fletcher S, Adriani W (2003) Risk-taking behavior in adolescent
675 mice: psychobiological determinants and early epigenetic influence. Neurosci Biobehav Rev
676 27:19-31.
677 Leroy F, Park J, Asok A, Brann DH, Meira T, Boyle LM, Buss EW, Kandel ER, Siegelbaum SA
678 (2018) A circuit from hippocampal CA2 to lateral septum disinhibits social aggression.
679 Nature 564:213-218.
680 Lisdahl KM, Sher KJ, Conway KP, Gonzalez R, Feldstein Ewing SW, Nixon SJ, Tapert S,
681 Bartsch H, Goldstein RZ, Heitzeg M (2018) Adolescent brain cognitive development (ABCD)
682 study: Overview of substance use assessment methods. Dev Cogn Neurosci 32:80-96.
683 Long LE, Chesworth R, Huang XF, McGregor IS, Arnold JC, Karl T (2010) A behavioural
684 comparison of acute and chronic Delta9-tetrahydrocannabinol and cannabidiol in
685 C57BL/6JArc mice. The international journal of neuropsychopharmacology 13:861-876.

686 Lossi L, Castagna C, Granato A, Merighi A (2019) The Reeler Mouse: A Translational Model of
687 Human Neurological Conditions, or Simply a Good Tool for Better Understanding
688 Neurodevelopment? J Clin Med 8.
689 Luo AH, Tahsili-Fahadan P, Wise RA, Lupica CR, Aston-Jones G (2011) Linking context with
690 reward: a functional circuit from hippocampal CA3 to ventral tegmental area. Science
691 333:353-357.
692 Macri S, Biamonte F, Romano E, Marino R, Keller F, Laviola G (2010) Perseverative
693 responding and neuroanatomical alterations in adult heterozygous reeler mice are mitigated by
694 neonatal estrogen administration. Psychoneuroendocrinology 35:1374-1387.
695 Manly BFJ, Navarro Alberto JA Multivariate statistical methods : a primer, Fourth edition.
696 Edition.
697 McGlinchey EM, Aston-Jones G (2018) Dorsal Hippocampus Drives Context-Induced Cocaine
698 Seeking via Inputs to Lateral Septum. Neuropsychopharmacology 43:987-1000.
699 Michetti C, Romano E, Altabella L, Caruso A, Castelluccio P, Bedse G, Gaetani S, Canese R,
700 Laviola G, Scattoni ML (2014) Mapping pathological phenotypes in reelin mutant mice. Front
701 Pediatr 2:95.
702 Neale BM et al. (2012) Patterns and rates of exonic de novo mutations in autism spectrum
703 disorders. Nature 485:242-245.
704 Nguyen JD, Aarde SM, Vandewater SA, Grant Y, Stouffer DG, Parsons LH, Cole M, Taffe MA
705 (2016) Inhaled delivery of Delta(9)-tetrahydrocannabinol (THC) to rats by e-cigarette vapor
706 technology. Neuropharmacology 109:112-120.
707 Niu S, Yabut O, D'Arcangelo G (2008) The Reelin signaling pathway promotes dendritic spine
708 development in hippocampal neurons. J Neurosci 28:10339-10348.

709 Ognibene E, Adriani W, Granstrem O, Pieretti S, Laviola G (2007) Impulsivity-anxiety-related
710 behavior and profiles of morphine-induced analgesia in heterozygous reeler mice. Brain Res
711 1131:173-180.
712 Olivito L, Saccone P, Perri V, Bachman JL, Fragapane P, Mele A, Huganir RL, De Leonibus E
713 (2016) Phosphorylation of the AMPA receptor GluA1 subunit regulates memory load
714 capacity. Brain Struct Funct 221:591-603.
715 Orr SP, Lasko NB, Shalev AY, Pitman RK (1995) Physiologic responses to loud tones in
716 Vietnam veterans with posttraumatic stress disorder. J Abnorm Psychol 104:75-82.
717 Paxinos G FK (2001) The Mouse Brain in Stereotaxic Coordinates, Second Edition. San Diego:
718 Academic Press.
719 Podhorna J, Didriksen M (2004) The heterozygous reeler mouse: behavioural phenotype. Behav
720 Brain Res 153:43-54.
721 Qiu S, Zhao LF, Korwek KM, Weeber EJ (2006) Differential reelin-induced enhancement of
722 NMDA and AMPA receptor activity in the adult hippocampus. J Neurosci 26:12943-12955.
723 Quinn GP KM (2002) Experimental design and data analysis for biologists. Cambridge
724 University Press, Cambridge, UK.
725 Renard J, Krebs MO, Le Pen G, Jay TM (2014) Long-term consequences of adolescent
726 cannabinoid exposure in adult psychopathology. Front Neurosci 8:361.
727 Rodriguez G, Neugebauer NM, Yao KL, Meltzer HY, Csernansky JG, Dong H (2017) Delta9-
728 tetrahydrocannabinol (Delta9-THC) administration after neonatal exposure to phencyclidine
729 potentiates schizophrenia-related behavioral phenotypes in mice. Pharmacology,
730 biochemistry, and behavior 159:6-11.

731 Rogers JT, Rusiana I, Trotter J, Zhao L, Donaldson E, Pak DT, Babus LW, Peters M, Banko JL,
732 Chavis P, Rebeck GW, Hoe HS, Weeber EJ (2011) Reelin supplementation enhances
733 cognitive ability, synaptic plasticity, and dendritic spine density. Learn Mem 18:558-564.
734 Rogers JT, Zhao L, Trotter JH, Rusiana I, Peters MM, Li Q, Donaldson E, Banko JL, Keenoy
735 KE, Rebeck GW, Hoe HS, D'Arcangelo G, Weeber EJ (2013) Reelin supplementation
736 recovers sensorimotor gating, synaptic plasticity and associative learning deficits in the
737 heterozygous reeler mouse. J Psychopharmacol 27:386-395.
738 Rubino T, Parolaro D (2016) The Impact of Exposure to Cannabinoids in Adolescence: Insights
739 From Animal Models. Biol Psychiatry 79:578-585.
740 Rubino T, Prini P, Piscitelli F, Zamberletti E, Trusel M, Melis M, Sagheddu C, Ligresti A,
741 Tonini R, Di Marzo V, Parolaro D (2015) Adolescent exposure to THC in female rats disrupts
742 developmental changes in the prefrontal cortex. Neurobiol Dis 73:60-69.
743 Ruzicka WB, Zhubi A, Veldic M, Grayson DR, Costa E, Guidotti A (2007) Selective epigenetic
744 alteration of layer I GABAergic neurons isolated from prefrontal cortex of schizophrenia
745 patients using laser-assisted microdissection. Mol Psychiatry 12:385-397.
746 Sannino S, Russo F, Torromino G, Pendolino V, Calabresi P, De Leonibus E (2012) Role of the
747 dorsal hippocampus in object memory load. Learn Mem 19:211-218.
748 Schulz KM, Sisk CL (2016) The organizing actions of adolescent gonadal steroid hormones on
749 brain and behavioral development. Neurosci Biobehav Rev 70:148-158.
750 Sekine K, Kubo K, Nakajima K (2014) How does Reelin control neuronal migration and layer
751 formation in the developing mammalian neocortex? Neurosci Res 86:50-58.

752 Shin S, Pribiag H, Lilascharoen V, Knowland D, Wang XY, Lim BK (2018) Drd3 Signaling in
753 the Lateral Septum Mediates Early Life Stress-Induced Social Dysfunction. Neuron 97:195-
754 208 e196.
755 Singewald GM, Rjabokon A, Singewald N, Ebner K (2011) The modulatory role of the lateral
756 septum on neuroendocrine and behavioral stress responses. Neuropsychopharmacology
757 36:793-804.
758 Stephens D (1996) Hearing rehabilitation in a psychosocial framework. Scand Audiol Suppl
759 43:57-66.
760 Strekalova T, Spanagel R, Bartsch D, Henn FA, Gass P (2004) Stress-induced anhedonia in mice
761 is associated with deficits in forced swimming and exploration. Neuropsychopharmacology
762 29:2007-2017.
763 Sturman DA, Moghaddam B (2011) The neurobiology of adolescence: changes in brain
764 architecture, functional dynamics, and behavioral tendencies. Neurosci Biobehav Rev
765 35:1704-1712.
766 Swerdlow NR, Geyer MA, Braff DL (2001) Neural circuit regulation of prepulse inhibition of
767 startle in the rat: current knowledge and future challenges. Psychopharmacology (Berl)
768 156:194-215.
769 Telese F, Ma Q, Perez PM, Notani D, Oh S, Li W, Comoletti D, Ohgi KA, Taylor H, Rosenfeld
770 MG (2015) LRP8-Reelin-Regulated Neuronal Enhancer Signature Underlying Learning and
771 Memory Formation. Neuron 86:696-710.
772 Todd SM, Zhou C, Clarke DJ, Chohan TW, Bahceci D, Arnold JC (2017) Interactions between
773 cannabidiol and Delta(9)-THC following acute and repeated dosing: Rebound hyperactivity,
774 sensorimotor gating and epigenetic and neuroadaptive changes in the mesolimbic pathway.

775 European neuropsychopharmacology : the journal of the European College of
776 Neuropsychopharmacology 27:132-145.
777 Toth M, Ziegler M, Sun P, Gresack J, Risbrough V (2013) Impaired conditioned fear response
778 and startle reactivity in epinephrine-deficient mice. Behav Pharmacol 24:1-9.
779 Trexler KR, Nass SR, Crowe MS, Gross JD, Jones MS, McKitrick AW, Siderovski DP, Kinsey
780 SG (2018) Novel behavioral assays of spontaneous and precipitated THC withdrawal in mice.
781 Drug Alcohol Depend 191:14-24.
782 Volkow ND (2016) Effects of Cannabis Use on Human Behavior-Reply. JAMA Psychiatry
783 73:996.
784 Wang Z, Hong Y, Zou L, Zhong R, Zhu B, Shen N, Chen W, Lou J, Ke J, Zhang T, Wang W,
785 Miao X (2014) Reelin gene variants and risk of autism spectrum disorders: an integrated
786 meta-analysis. Am J Med Genet B Neuropsychiatr Genet 165B:192-200.
787 Weeber EJ, Beffert U, Jones C, Christian JM, Forster E, Sweatt JD, Herz J (2002) Reelin and
788 ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. J Biol
789 Chem 277:39944-39952.
790 Wilkinson ST, Yarnell S, Radhakrishnan R, Ball SA, D'Souza DC (2016) Marijuana
791 Legalization: Impact on Physicians and Public Health. Annu Rev Med 67:453-466.
792 Yang M, Silverman JL, Crawley JN (2011) Automated three-chambered social approach task for
793 mice. Curr Protoc Neurosci Chapter 8:Unit 8 26.
794 Young SE, Friedman NP, Miyake A, Willcutt EG, Corley RP, Haberstick BC, Hewitt JK (2009)
795 Behavioral disinhibition: liability for externalizing spectrum disorders and its genetic and
796 environmental relation to response inhibition across adolescence. J Abnorm Psychol 118:117-
797 130.

798 Zuur AF IE (2016) A protocol for conducting and presenting results of regression-type analyses.
799 . Methods in Ecology and Evolution 7:636-645.
800 Zuurman L, Roy C, Schoemaker RC, Hazekamp A, den Hartigh J, Bender JC, Verpoorte R,
801 Pinquier JL, Cohen AF, van Gerven JM (2008) Effect of intrapulmonary tetrahydrocannabinol
802 administration in humans. J Psychopharmacol 22:707-716.

803

804

805 **Figure legends**

806

807 **Figure 1: Six-different objetcs test in WT and HR after chronic adolescent exposure to**
808 **THC. (A)** 6-DOT with habituation, familiarization, and test trials. **(B)** Discrimination index (DI)
809 is shown as mean ± 95% confidence intervals. **(C)** THC reduced the DI (mean ± SEM) across all
810 groups (main treatment effect, $P < 0.0001$, LMM). **(D)** Male HR mice showed a reduced DI
811 (mean ± SEM) compared to male WT mice (sex x genotype interaction, $P = 0.02$, LMM). **(E)**
812 Exploration time (s) in test trial is expressed as mean ± SEM. **(F)** HR mice spent longer time
813 (mean ± SEM) exploring the objects in the test trial (main genotype effect, P = 0.016, LMM).
814 **(G)** Exploration time (mean ± SEM) in familiarization trial did not change across groups.
815 Adjusted $P < 0.05$ for planned post-hoc pairwise t tests are reported.

816

817 **Figure 2: Social interaction behavior in WT and HR after chronic adolescent exposure to**
818 **THC. (A)** Three-chamber interaction test diagram. Time (s) spent in chamber **(B)** or sniffing **(D)**
819 is expressed as mean ± 95% confidence intervals. **(C)** Mice spent more time (mean ± SEM) in
820 the chamber or **(D)** more time sniffing (mean ± SEM) the novel mouse compared to the novel
821 object (main exploration target effect, $P < 0.0001$, LMM). **(E)** THC reduced social investigation
822 (post-hoc pairwise *t* test $P_{adj} = 0.003$; treatment x exploration target interaction, $P = 0.05$, LMM).
823 **(G)** HR exposed to THC during adolescence showed reduced social interaction behavior (post-
824 hoc pairwise *t* test $P_{adj} = 0.006$; treatment x genotype interaction, $P = 0.015$, LMM).

825

826 **Figure 3: Anxiety-like responses in WT and HR following chronic adolescent exposure to**
827 **THC. (A)** Light-dark test schematic. **(B)** Time (s) spent in the light compartment is expressed as

828 mean ± SEM. **(C)** Female HR treated with THC during adolescence showed reduced anxiety
829 (treatment x genotype x sex interaction, $P = 0.04$, LMM). **(E)** Female HR mice showed increased
830 latency (s) to enter the light compared to WT controls (genotype x sex interaction, $P = 0.02$,
831 LMM). **(F)** Open field test schematic. **(G)** Time (s) spent in the center of the arena is expressed
832 as mean ± SEM. **(H)** HR mice showed reduced anxiety (main genotype effect, $P = 0.057$, LMM).
833 **(I)** Latency (s) to enter the center compartment is expressed as mean ± SEM and did not change
834 across groups. Adjusted $P < 0.05$ for planned post-hoc pairwise t tests are reported.
835
836 **Figure 4: Reactivity to aversive conditions in WT and HR mice following chronic**
837 **adolescent exposure to THC. (A)** Tail suspension test diagram. **(B)** Mobility time (s) is
838 expressed as mean ± SEM. **(C)** Male HR mice treated with THC during adolescence showed
839 increased mobility (treatment x genotype x sex interaction, $P = 0.0005$, LMM). Adjusted $P <$
840 0.05 for planned post-hoc pairwise t tests are reported.
841
842 **Figure 5: Acoustic startle reflex of pre-pulse inhibition in WT and HR mice following**
843 **chronic adolescent exposure to THC. (A)** Pre-pulse inhibition of acoustic startle test diagram**.**
844 **(B)** Startle amplitude is expressed as Vmax ± SEM. **(C)** HR mice show increased startle response
845 (genotype x db interaction, $P = 0.016$, LMM). **(D)** Pre-pulse inhibition is expressed as % PPI ±
846 SEM. **(E)** HM mice exhibited higher % PPI compared to WT controls (main genotype effect, $P =$
847 0.004, LMM). **(F)** Female treated with THC during adolescence showed reduced %PPI
848 compared to vehicle (treatment x sex interaction, $P = 0.005$, LMM). Adjusted $P < 0.05$ for
849 planned post-hoc pairwise t tests are reported.
850

851 **Figure 6: Factor analysis of behavioral tests. (A)** Factor loadings for each named behavioral
852 domain are reported for several behavioral variables. LMM analysis for rotated factor scores
853 revealed a **(B)** treatment x behavioral domain interaction ($P = 0.04$), and a **(C)** genotype x
854 behavioral domain interaction ($P = 0.035$). Adjusted $P < 0.05$ for planned post-hoc pairwise *t*
855 tests are reported.
856
857 **Figure 7: Fos expression is reduced in the LS of HR mice in response to novel object**
858 **exploration. (A)** Brain schematic illustrating the target areas for Fos quantification: mPFC, LS,
859 CA1, DG. (**B-E**) Schematic brain palette with bregma levels (top) and representative images
860 (bottom) of the mPFC, LS, CA1, and DG in WT ($n = 6$ male, $n = 4$ female) and HR mice ($n = 6$
861 male, $n = 9$ female). Scale bar, 50um.
862
863 **Figure 8: Distribution of different cell types of the the LS. (A)** RNA FISH showing cells
864 expressing *Reelin* and *Slc32a1* relative to Dapi-stained nuclei in the LS at bregma +0.50 mm. **(B)**
865 Higher magnification images illustrate the co-localization (white arrow) of *Reelin* and
866 *Slc32a1*mRNA. **(C)** RNA FISH showing cells expressing *Fos* and *Slc32a1* relative to Dapi-
867 stained nuclei in the LS at bregma +0.26 mm. **(D)** Higher magnification images illustrate the co-
868 localization of *Fos* and *Slc32a1* (white arrow) or *Fos* and *Chat)* mRNA. Dotted lines indicate the
869 caudal (LSc) and rostral (LSr) regions of the LS, and the fornix (fx) fibers. Scale bars are 100µm
870 in **(A)** and 20µm in **(B-C).**
871
872 **Supplementary Figure 1: HR mice are used for chronic adolescent administration of THC.**
873 **(A)** RT-PCR indicate Reelin mRNA expression in brain tissues isolated from WT and HR mice.

874 Level of Reelin expression are normalized with the housekeeping gene ACTB. **(B)** Reelin
875 mRNA is reduced in HR mice compared to WT littermates (genotype effect, $P = 0.0007$, LMM).
876 $n = 8$ female WT, $n = 6$ male WT, n = 6 female HR, $n = 5$ male HR mice. **(C)** Schematic diagram
877 illustrates the daily i.p. administration of THC (10 mg/Kg) during adolescence (PND28-48), and
878 the day when behavioral assessment began (PDN63) after 15 days of washout.
879
880 **Supplementary Figure 2: Locomotor activity and body weight for WT and HR mice. (A)**
881 Twenty-minute locomotor activity is expressed as mean distance traveled (m) ± SEM for every
882 5-minute intervals. **(B)** Body weight (g) change is expressed as mean ± SEM for the THC
883 administration course (day 1 to 14), and for day 63 when the behavioral testing started.
884
885 **Supplementary Figure 3: Sociability index in WT and HR mice following chronic**
886 **adolescent exposure to THC. (A)** Sociability index calculated using time spent in the chamber
887 with novel mouse versus novel object is shown as mean ± 95% confidence intervals. **(B)**
888 Sociability index calculated using time sniffing novel mouse versus novel object is shown as
889 mean ± 95% confidence intervals. Treatment, genotype and sex had no significant effect on both
890 sociability indices.
891
892 **Supplementary Figure 4: Fos activation in response to novelty. (A)** Schematic of behavioral
893 manipulation, including home cage (control), habituation, familiarization, and test phases of the
894 6-DOT. **(B-C)** Representative images of Fos expression by IHC in different brain regions,
895 including LS, mPFC, CA1 and DG. Dotted lines illustrate the line separating the LS from the
896 striatum (St) and the lateral ventricle (LV). Scale bars are 100μm in **(B, E)** and 50μm in **(C, D).**

Table 1. Number of mice used for behavioral analysis*

BEHAVIORAL ASSAYS	MALE WT		FEMALE WT		MALE HET		FEMALE HET	
	VEH	THC	VEH	THC	VEH	THC	VEH	THC
Locomotor	19	20	19	18	17	21	19	23
Tail suspension	24	24	20	18	18	22	20	22
Open field	18	16	13	12	10	11	16	14
Light-dark	18	16	13	12	10	11	16	14
Social	19	20	17	15	10	11	16	14
6-DOT	12	13	9	9	10	11	16	14
PPI	23	24	24	23	20	22	25	26
ASR	23	24	25	23	20	22	25	26

* The number of outliers for specific variables and removed from analysis of are reported in the method section

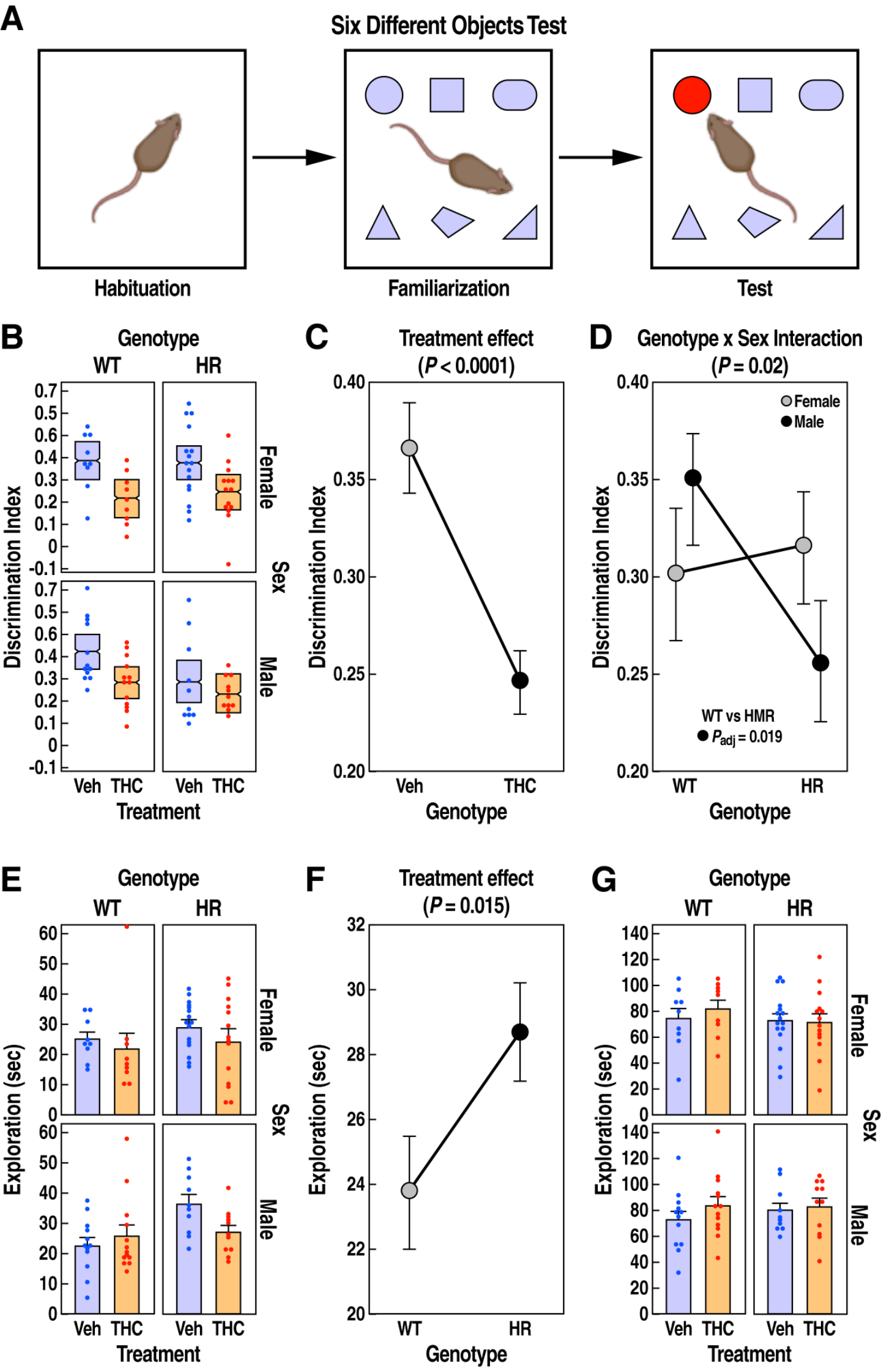

A
Six Different Objects Test
Habituation
Familiarization
Test
B
Genotype
WT
HR
Female
Male
Sex
Discrimination Index
Veh
THC
Treatment
C
Treatment effect
(P < 0.0001)
Discrimination Index
Veh
THC
Genotype
D
Genotype x Sex Interaction
(P = 0.02)
Female
Male
Discrimination Index
WT vs HMR
Padj = 0.019
WT
HR
Genotype
E
Genotype
WT
HR
Female
Male
Sex
Exploration (sec)
Veh
THC
Treatment
F
Treatment effect
(P = 0.015)
Exploration (sec)
WT
HR
Genotype
G
Genotype
WT
HR
Female
Male
Sex
Exploration (sec)
Veh
THC
Treatment

Figure 1

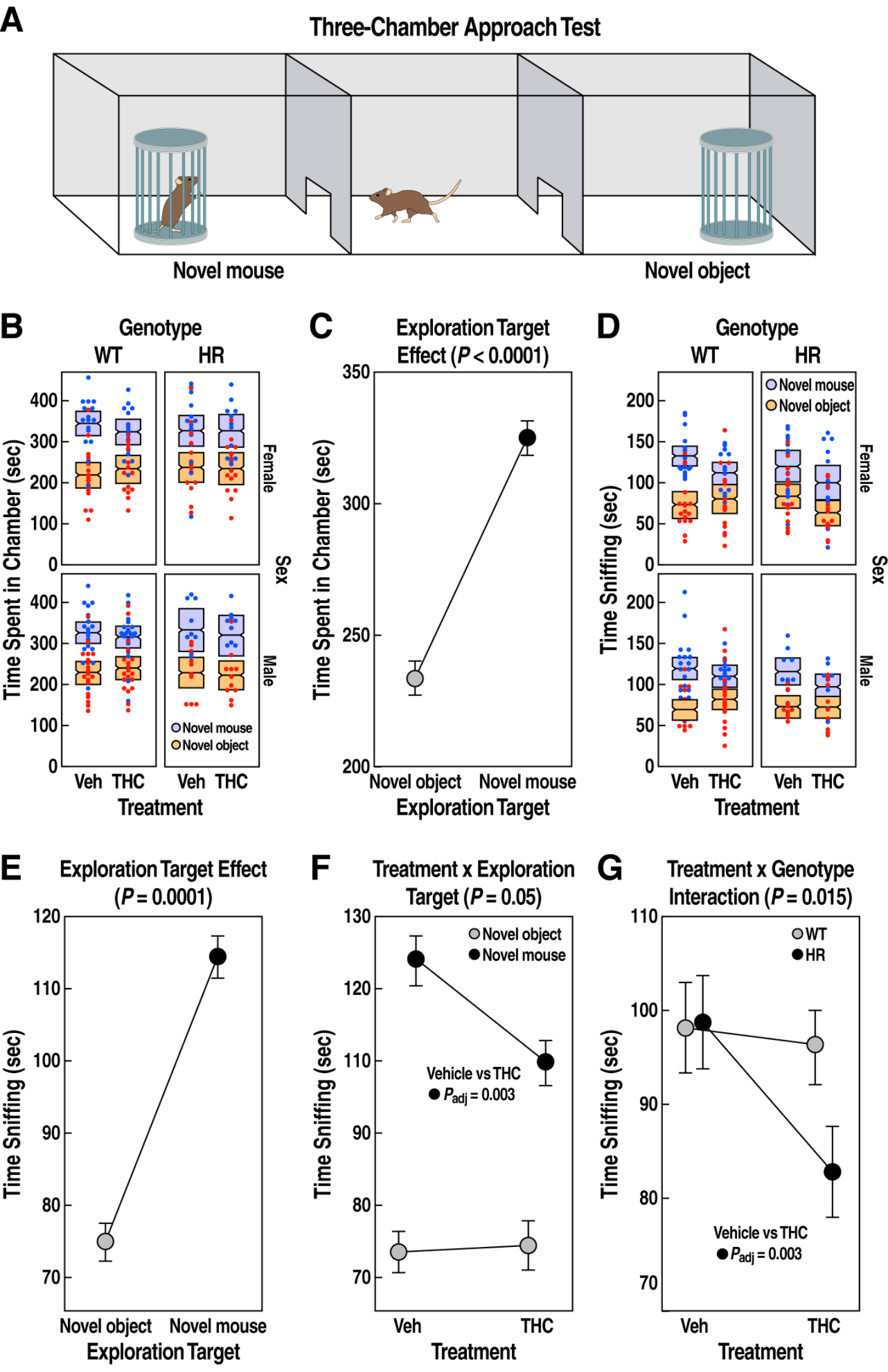

A
Three-Chamber Approach Test
Novel mouse
Novel object
B
Genotype
WT
HR
Time Spent in Chamber (sec)
Female
Male
Sex
Novel mouse
Novel object
Veh
THC
Treatment
C
Exploration Target Effect (P < 0.0001)
Time Spent in Chamber (sec)
Novel object
Novel mouse
Exploration Target
D
Genotype
WT
HR
Novel mouse
Novel object
Time Sniffing (sec)
Female
Male
Sex
Veh
THC
Treatment
E
Exploration Target Effect (P = 0.0001)
Time Sniffing (sec)
Novel object
Novel mouse
Exploration Target
F
Treatment x Exploration Target (P = 0.05)
Novel object
Novel mouse
Time Sniffing (sec)
Vehicle vs THC
Padj = 0.003
Veh
THC
Treatment
G
Treatment x Genotype Interaction (P = 0.015)
WT
HR
Time Sniffing (sec)
Vehicle vs THC
Padj = 0.003
Veh
THC
Treatment

Figure 2

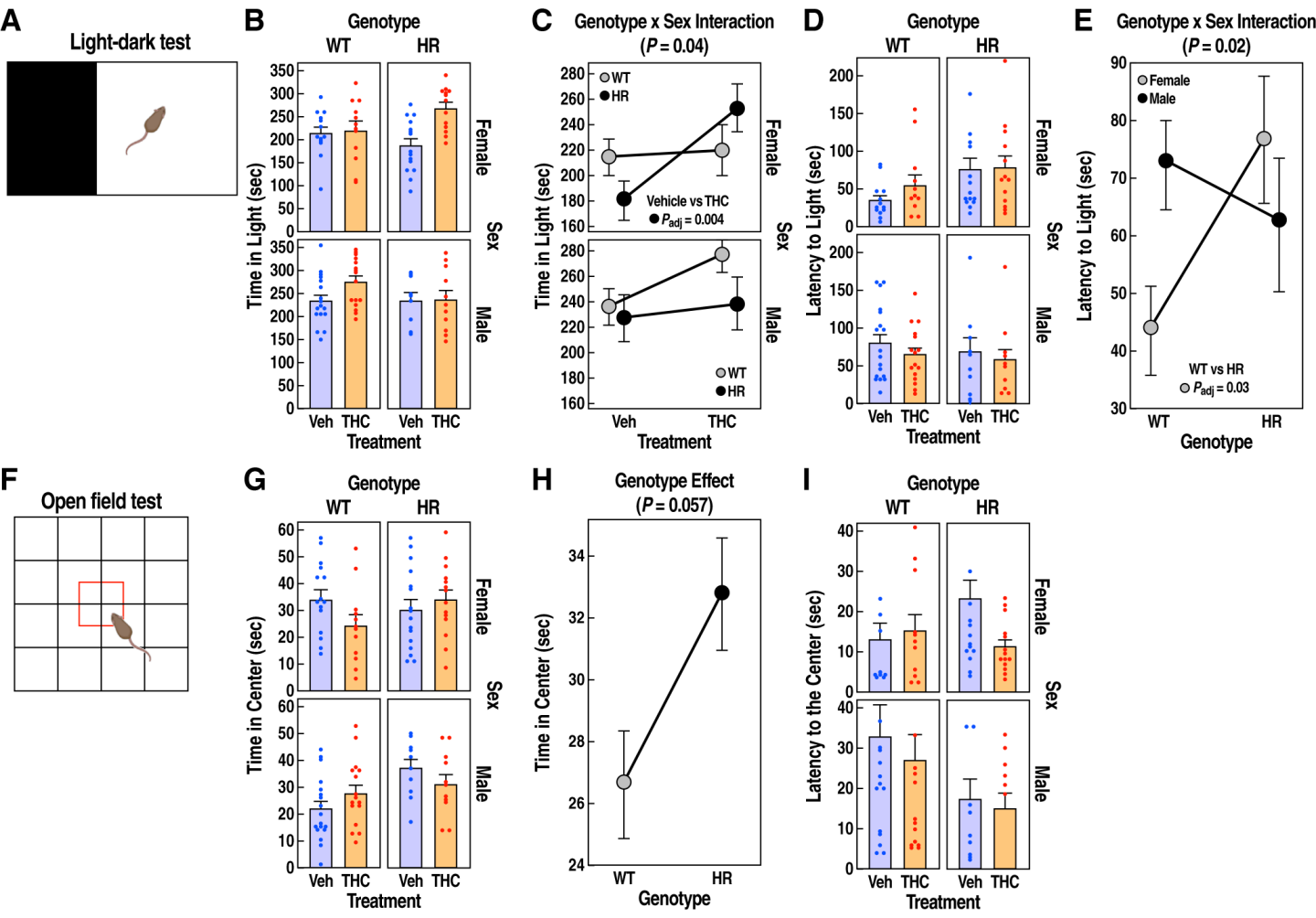

A
Light-dark test
B
Genotype
WT
HR
Time in Light (sec)
Female
Male
Sex
Veh
THC
Treatment
C
Genotype x Sex Interaction
(P = 0.04)
WT
HR
Vehicle vs THC
Padj = 0.004
D
Latency to Light (sec)
E
Genotype x Sex Interaction
(P = 0.02)
Female
Male
WT vs HR
Padj = 0.03
Genotype
F
Open field test
G
Time in Center (sec)
H
Genotype Effect
(P = 0.057)
I
Latency to the Center (sec)

Figure 3

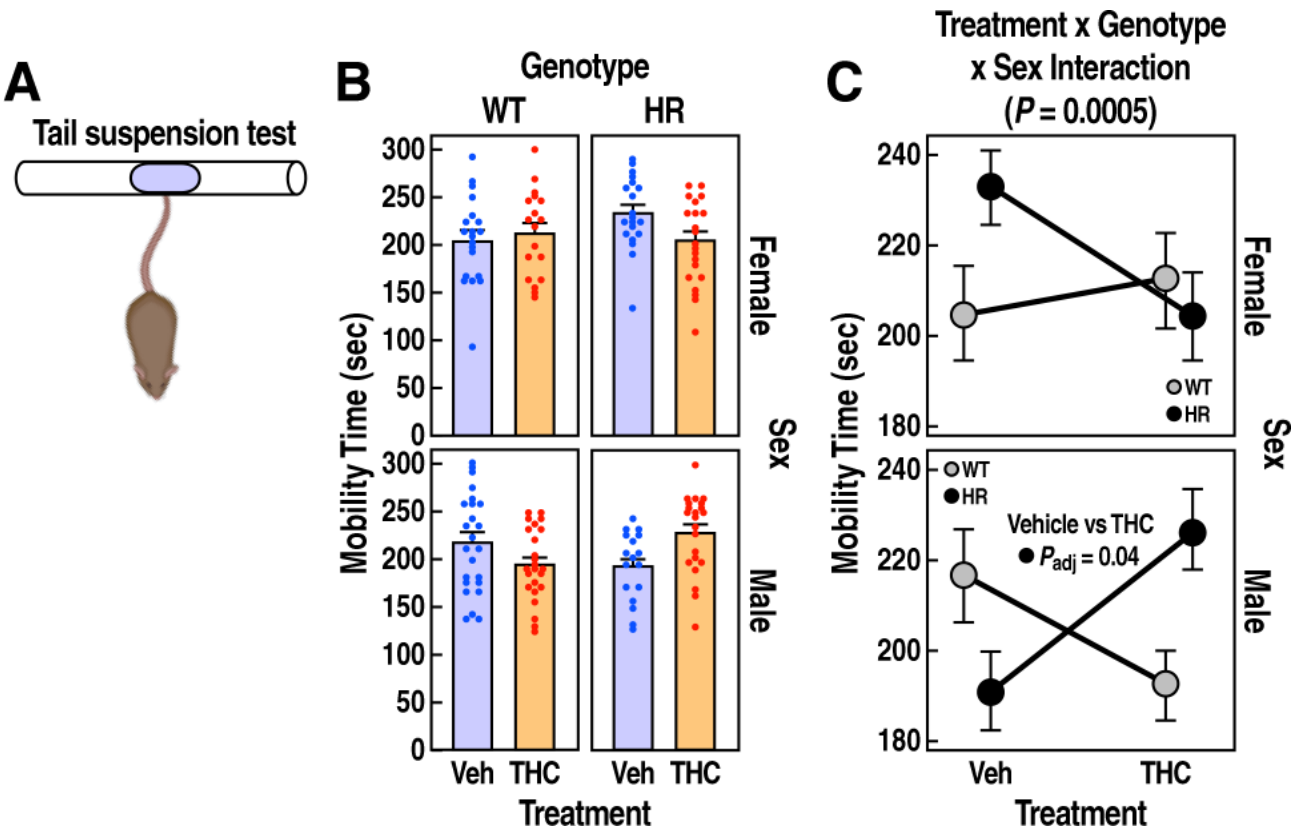

A
Tail suspension test
B
Genotype
WT
HR
Mobility Time (sec)
300
250
200
150
100
50
0
Female
Male
Sex
Veh
THC
Treatment
C
Treatment x Genotype
x Sex Interaction
(P = 0.0005)
240
220
200
180
WT
HR
Vehicle vs THC
Padj = 0.04

Figure 4

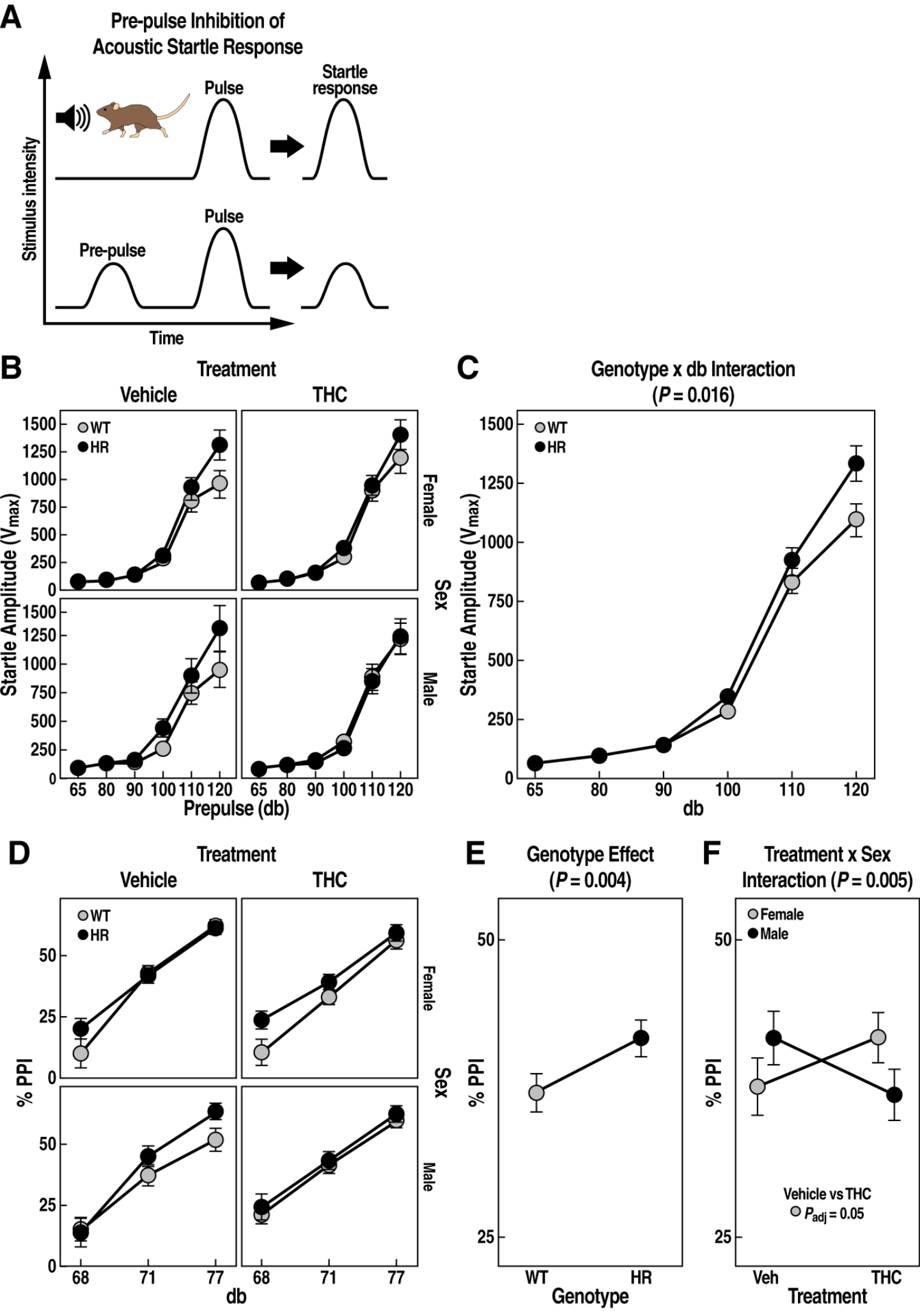

A
Pre-pulse Inhibition of
Acoustic Startle Response
Stimulus intensity
Pulse
Startle response
Pre-pulse
Pulse
Time
B
Treatment
Vehicle
THC
WT
HR
Startle Amplitude (V_{max})
Female
Male
Sex
Prepulse (db)
C
Genotype x db Interaction
(P = 0.016)
db
D
% PPI
db
E
Genotype Effect
(P = 0.004)
Genotype
F
Treatment x Sex
Interaction (P = 0.005)
Female
Male
Vehicle vs THC
P_{adj} = 0.05
Veh
Treatment

Figure 5

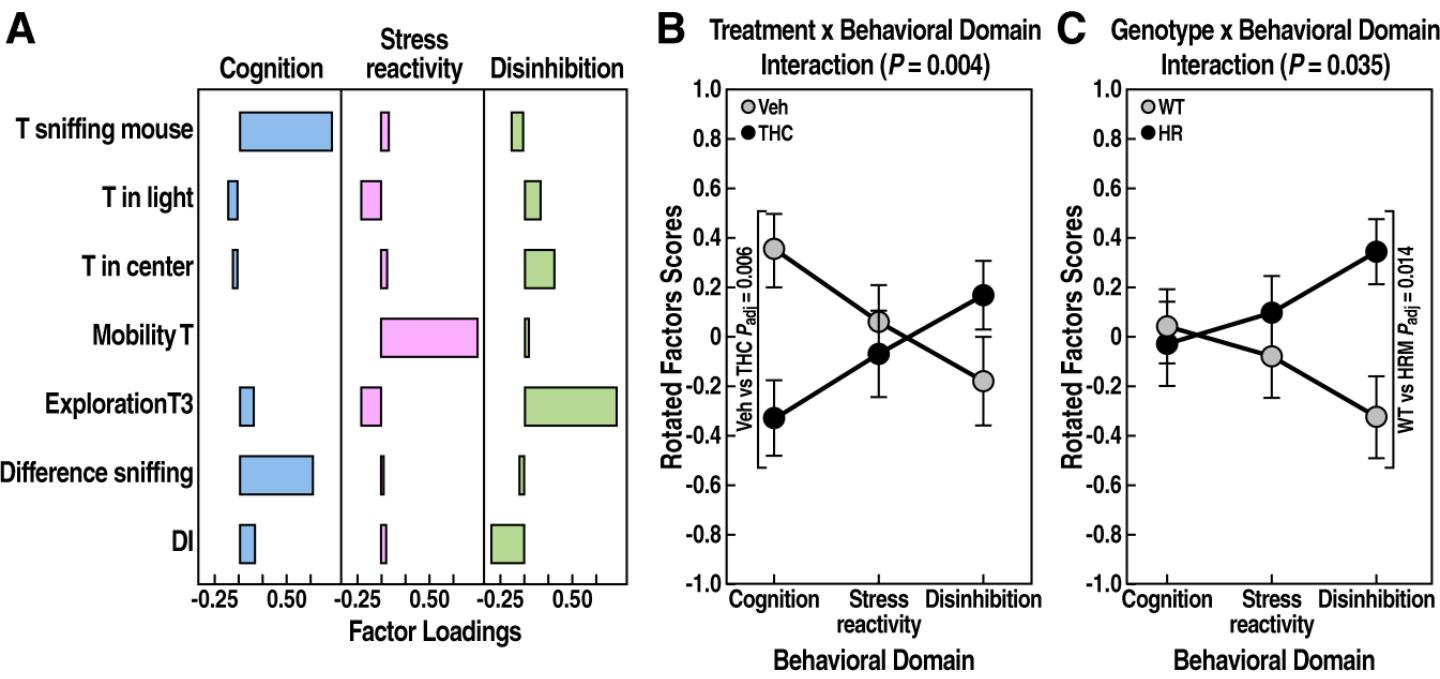

A
Cognition
Stress reactivity
Disinhibition
T sniffing mouse
T in light
T in center
Mobility T
ExplorationT3
Difference sniffing
DI
-0.25
0.50
Factor Loadings
B
Treatment x Behavioral Domain Interaction (P = 0.004)
Veh
THC
Veh vs THC Padj = 0.006
Rotated Factors Scores
Cognition
Stress reactivity
Disinhibition
Behavioral Domain
C
Genotype x Behavioral Domain Interaction (P = 0.035)
WT
HR
WT vs HRM Padj = 0.014
Rotated Factors Scores
Cognition
Stress reactivity
Disinhibition
Behavioral Domain

Figure 6

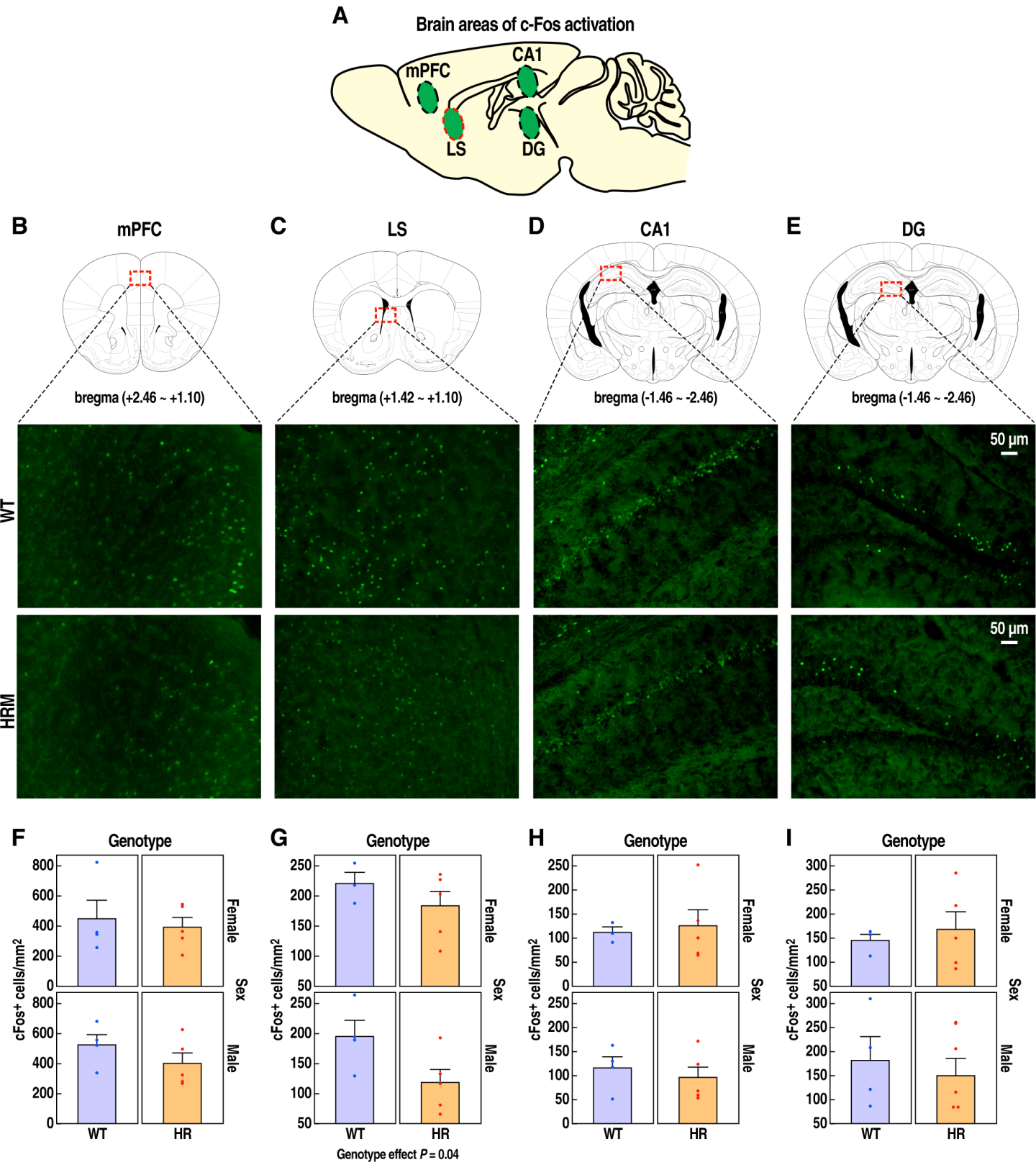

A
Brain areas of c-Fos activation
mPFC
CA1
LS
DG
B
mPFC
bregma (+2.46 ~ +1.10)
C
LS
bregma (+1.42 ~ +1.10)
D
CA1
bregma (-1.46 ~ -2.46)
E
DG
bregma (-1.46 ~ -2.46)
50 μm
WT
HRM
F
G
H
I
Genotype
cFos+ cells/mm2
Female
Male
Sex
WT
HR
Genotype effect P = 0.04

Figure 7

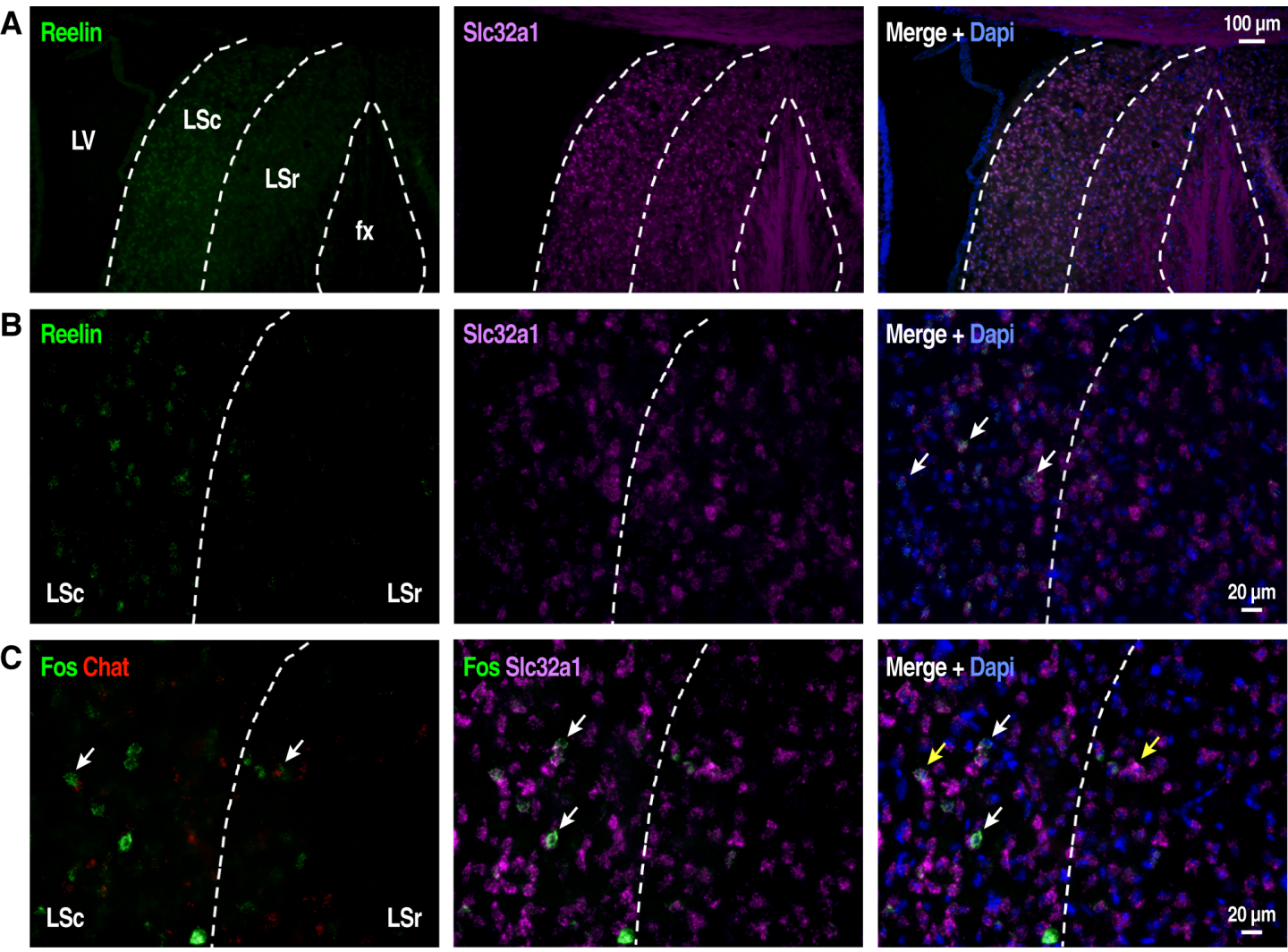
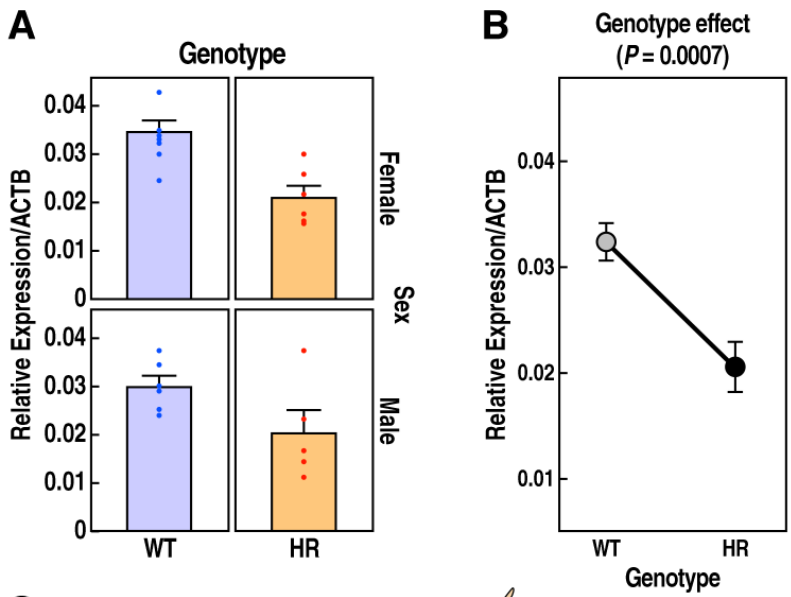
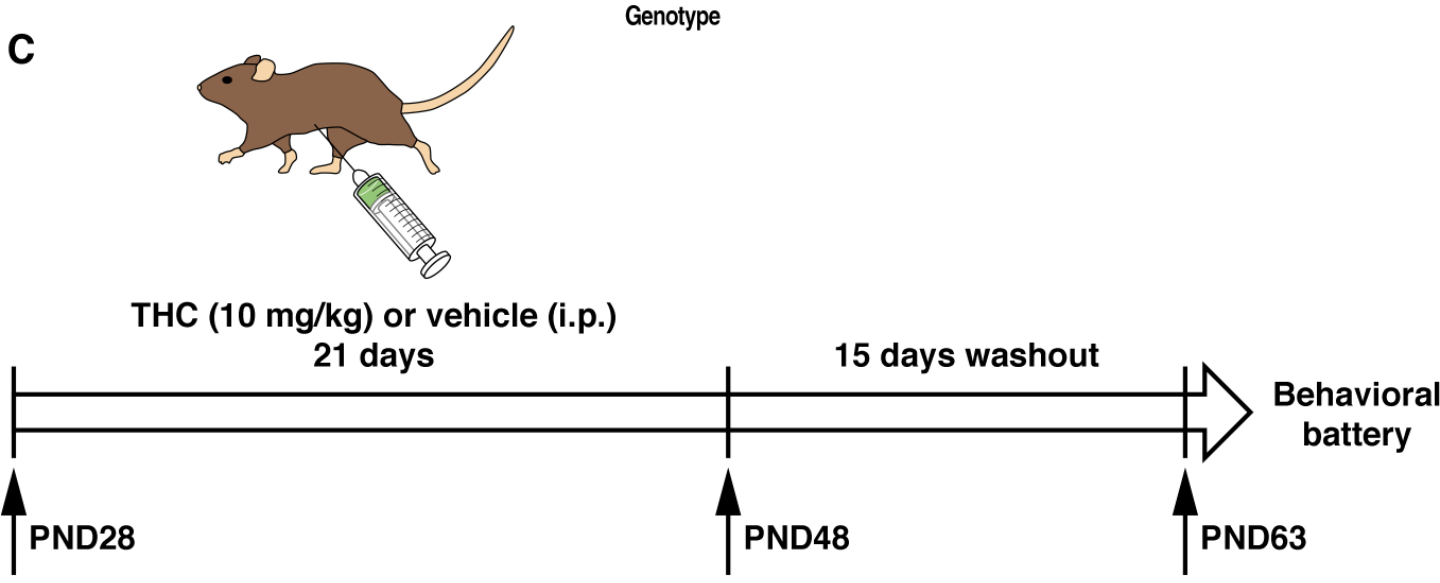
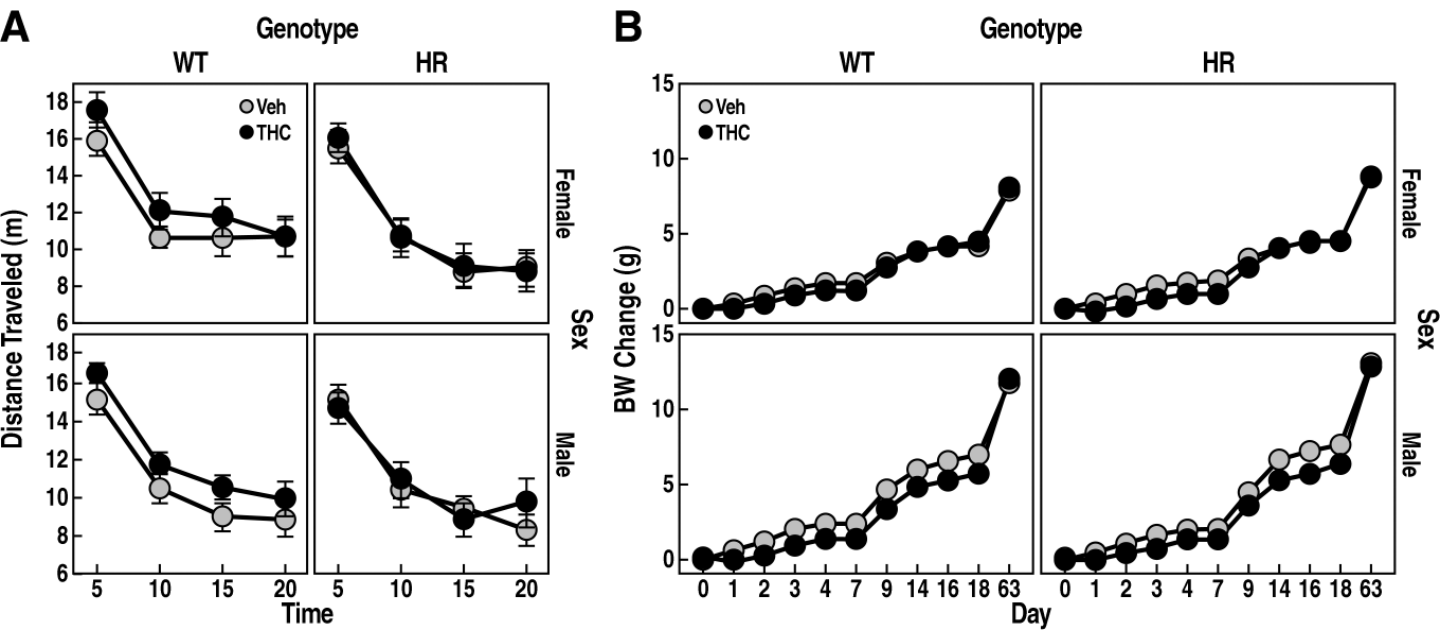

A
Reelin
LV
LSc
LSr
fx
Slc32a1
Merge + Dapi
100 µm
B
Reelin
LSc
LSr
Slc32a1
Merge + Dapi
20 µm
C
Fos Chat
LSc
LSr
Fos Slc32a1
Merge + Dapi
20 µm

Figure 8

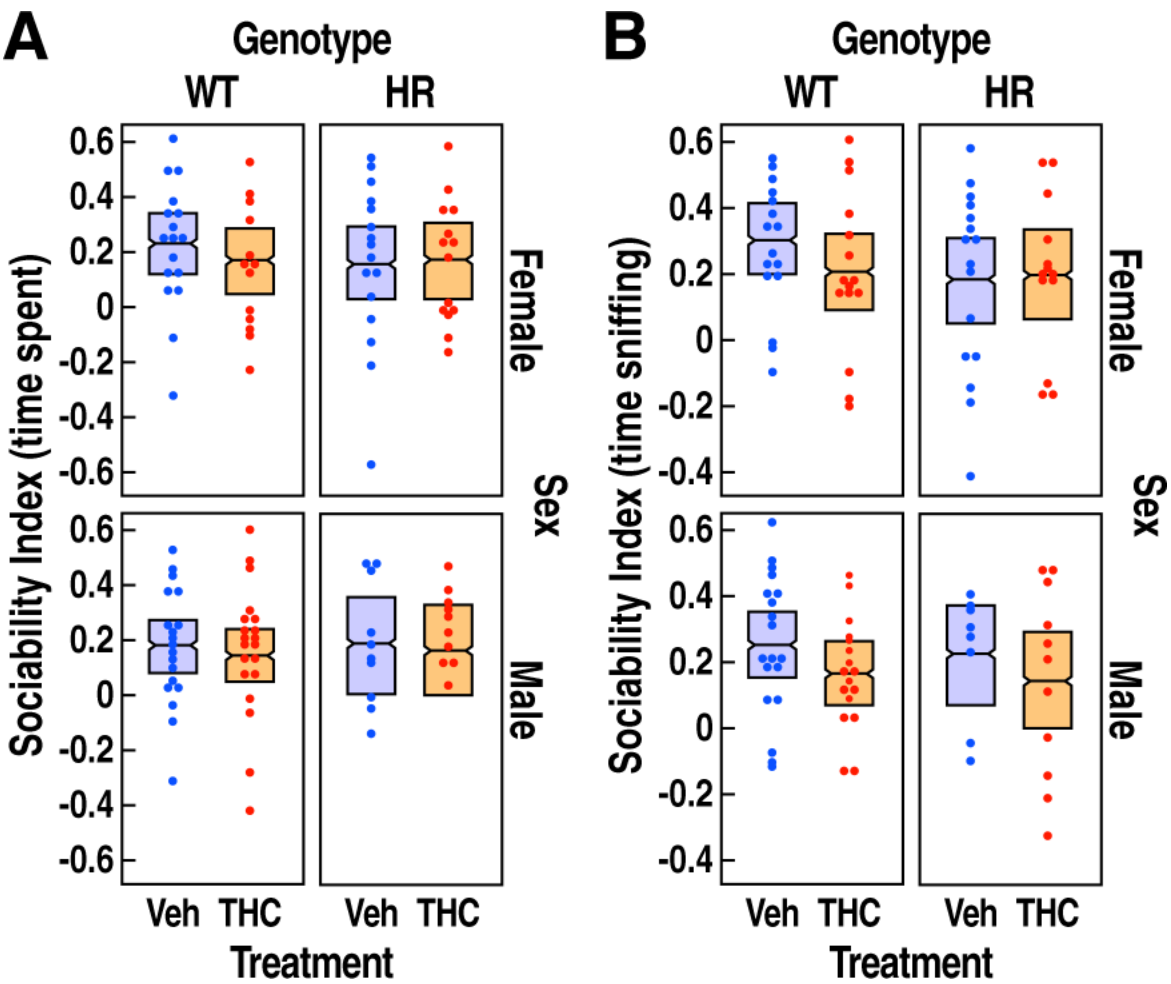




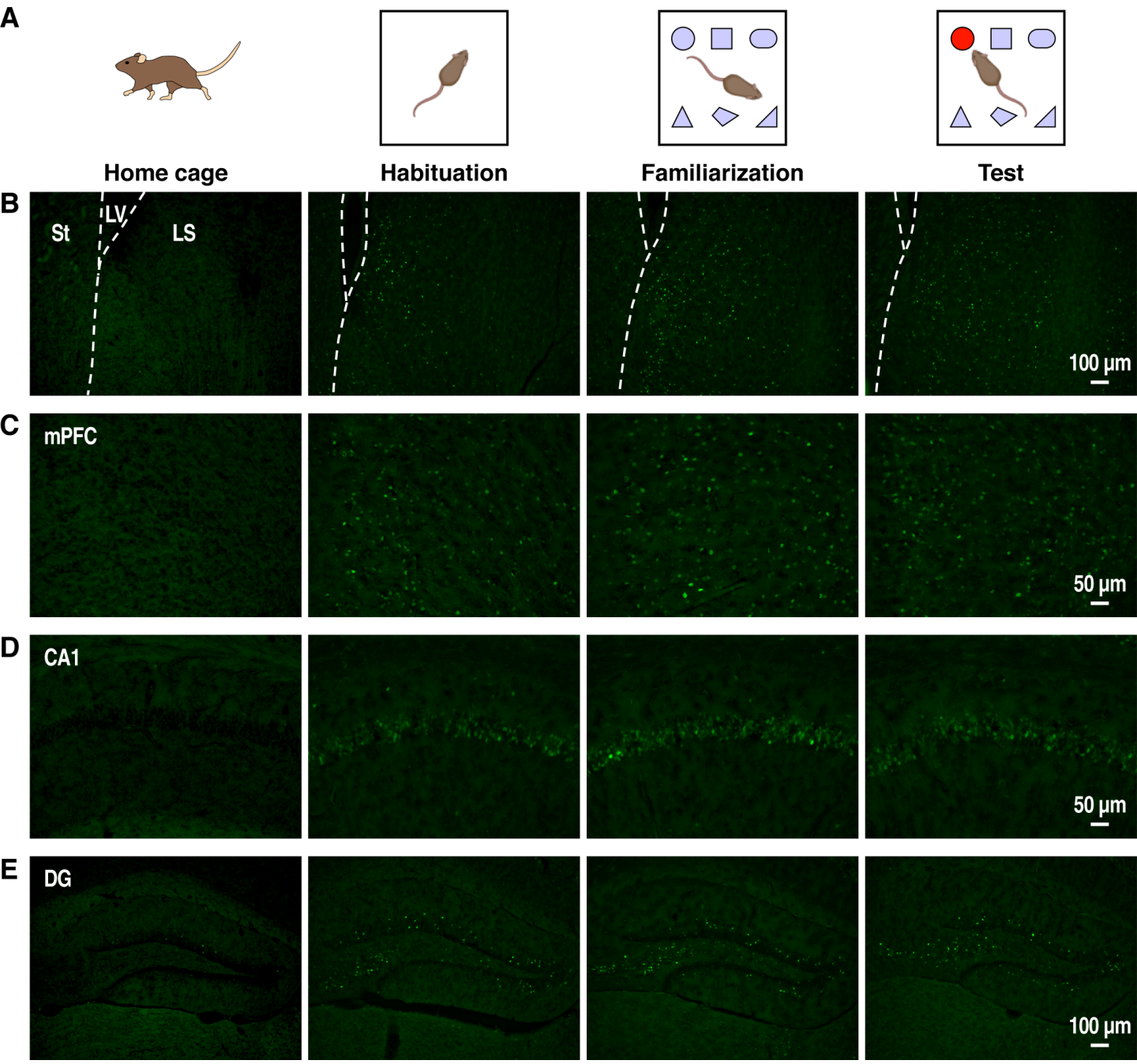
Suppl. Figure 1



Suppl. Figure 2



Suppl. Figure 3



Suppl. Figure 4