

1

2

3

4 **Experience-dependent plasticity in an innate social behavior is mediated by**
5 **hypothalamic LTP**

6 *Stefanos Stagkourakis^{1*}, Giada Spigolon², Grace Liu¹, David J. Anderson^{1,3*}*

7

8

9 ¹ Division of Biology and Biological Engineering 156-29, Tianqiao and Chrissy Chen Institute for
10 Neuroscience, California Institute of Technology, Pasadena, California 91125, USA

11 ² Biological Imaging Facility, California Institute of Technology, Pasadena, California 91125, USA

12 ³ Howard Hughes Medical Institute, California Institute of Technology, 1200 East California Blvd,
13 Pasadena, California 91125, USA

14 ***Corresponding author information:** Stefanos Stagkourakis, David J. Anderson

15 **Email:** stefanos.stagkourakis@caltech.edu, wuwei@caltech.edu

16 **ORCID:** Stefanos Stagkourakis ORCID: 0000-0003-1218-791X
17 Giada Spigolon ORCID: 0000-0002-1704-8372

18

19 **Classification:** Biological Sciences, Neuroscience

20 **Keywords:** innate behaviors, long term potentiation, ventromedial hypothalamus, testosterone

21 **Author Contributions:** S.S., G.S., and G.L. designed, performed and analyzed experiments. D.J.A.
22 designed experiments. S.S. and D.J.A. wrote the manuscript. All authors reviewed the manuscript.

23 **Competing interests:** The authors declare no competing financial interests.

24 **Abstract**

25 All animals can perform certain survival behaviors without prior experience, suggesting a “hard wiring” of
26 underlying neural circuits. Experience, however, can alter the expression of innate behaviors. Where in
27 the brain and how such plasticity occurs remains largely unknown. Previous studies have established the
28 phenomenon of “aggression training,” in which the repeated experience of winning successive aggressive
29 encounters across multiple days leads to increased aggressiveness. Here we show that this procedure
30 also leads to long-term potentiation (LTP) at an excitatory synapse, derived from the Anterior
31 Hippocampus/Posterior Medial amygdala (AHiPM), onto estrogen receptor 1-expressing (Esr1⁺) neurons
32 in the ventrolateral subdivision of the ventromedial hypothalamus (VMHvl). We demonstrate further that
33 the optogenetic induction of such LTP *in vivo* facilitates, while optogenetic long-term depression (LTD)
34 diminishes, the behavioral effect of aggression training, implying a causal role for potentiation at
35 AHiPM→VMHvl^{Esr1} synapses in mediating the effect of this training. Interestingly, ~25% of inbred
36 C57BL/6 mice fail to respond to aggression training. We show that these individual differences are
37 correlated both with lower levels of testosterone, relative to mice that respond to such training, and with
38 a failure to exhibit LTP *in vivo* after aggression training. Administration of exogenous testosterone to
39 such non-aggressive mice restores both behavioral and physiological plasticity *in vivo*. Together, these
40 findings reveal that LTP at a hypothalamic circuit node mediates a form of experience-dependent
41 plasticity in an innate social behavior, and a potential hormone-dependent basis for individual differences
42 in such plasticity among genetically identical mice.

43 **Significance Statement**

44 Modification of instinctive behaviors occurs through experience, yet the mechanisms through which this
45 happens have remained largely unknown. Recent studies have shown that potentiation of aggression,
46 an innate behavior, can occur through repeated winning of aggressive encounters. Here we show that
47 synaptic plasticity at a specific excitatory input to a hypothalamic cell population is correlated with, and
48 required for, the expression of increasingly higher levels of aggressive behavior following successful
49 aggressive experience. We additionally show that the amplitude and persistence of long-term potentiation
50 at this synapse are influenced by serum testosterone, administration of which can normalize individual
51 differences among genetically identical inbred mice, in the expression of intermale aggression.

52 **Introduction**

53 Brains evolved to optimize the survival of animal species by generating appropriate behavioral responses
54 to both stable and unpredictable features of the environments in which they live. Accordingly, two major
55 brain strategies for behavioral control have been selected. In the first, developmentally specified neural
56 circuits generate rapid innate responses to sensory stimuli that have remained relatively constant and
57 predictable over evolutionary timescales (1-5). In the second, neural circuits generate flexible responses
58 to stimuli that can change over an individual's lifespan, through learning and memory (6-8).

59 One prevailing view is that these two strategies are implemented largely through distinct
60 neuroanatomical structures and neurophysiological mechanisms. According to this view, in the
61 mammalian brain innate behaviors are mediated by evolutionarily ancient, deep subcortical structures,
62 such as the medial amygdala and hypothalamus, which link specific sensory inputs to evolutionarily
63 "prepared" motor outputs through relatively stable synaptic connections (9-13). In contrast, learned
64 behaviors are mediated by more recently evolved structures, such as the cortex and hippocampus, which
65 compute flexible input-output mapping responses through synaptic plasticity mechanisms (14-19).

66 The idea that innate vs. learned behaviors are mediated by largely distinct neural systems has
67 been reinforced by studies that have revealed, for example, distinct anatomical pathways through which
68 olfactory cues evoke learned vs. innate behaviors in both the mouse (20-22) and in *Drosophila* (23-25);
69 reviewed in (26). The concept of a dichotomous nervous system architecture for mediating appropriate
70 biological responses to evolutionarily ancient vs. novel stimuli is analogous to the innate vs. adaptive
71 branches of the immune system (27).

72 This view of distinct neural pathways for innate vs. learned behaviors, however, is challenged by
73 case of behaviors that, while apparently "instinctive", can nevertheless be modified by experience. For
74 example, while aggression has been considered by classical ethologists as a prototypical "released"
75 innate behavior (28, 29), studies from the 1940's onwards showed that mice could be trained to be more
76 aggressive by repeated fighting experience (30-35). Similarly, studies in rodents have shown that

77 defensive behaviors such as freezing can be elicited by both unconditional and conditional stimuli, the
78 latter via Pavlovian associative learning (reviewed in (36)).

79 These observations raise the question of where in the brain, and how, the neural circuitry that
80 mediates innate behaviors is modified by experience. In the case of defensive behaviors, such as
81 freezing, the prevailing view argues for parallel pathways: conditioned defensive behavior is mediated by
82 circuitry involving the hippocampus, thalamus and the basolateral/central amygdala, whereas innate
83 defensive responses to predators are mediate by the medial amygdala (MeA)/bed nucleus of the stria
84 terminalis (BNST) and hypothalamic structures (reviewed in (37)). Although the basolateral amygdala
85 contains representations of unconditioned aversive and appetitive stimuli, these representations are used
86 as the cellular substrate for pairing with conditioned stimuli (38, 39). Despite this segregation of learned
87 and innate defensive pathways, it remains possible that experience-dependent influences on other innate
88 behaviors may involve plasticity at synapses that directly mediate such behaviors (e.g., at inputs to
89 midbrain PAGvl neurons (40)).

90 We have investigated this issue using inter-male offensive aggression in mice as an experimental
91 paradigm. While mice housed under appropriate conditions can exhibit aggression in the absence of any
92 prior agonistic encounters with male conspecifics (31, 33, 41), an effect of repeated successful
93 aggressive experience to facilitate, or “prime”, subsequent attack behavior, considered as a form of
94 “aggression training”, has been well-documented (42, 43). The neural substrate and physiological
95 mechanisms underlying this form of experience-dependent plasticity of an innate behavior remain
96 unknown. Interestingly, inbred strains of laboratory mice exhibit individual differences in the ability to
97 manifest this form of behavioral plasticity, with up to 25% of animals failing to respond to aggression
98 training (35). The biological basis of this apparent epigenetic heterogeneity is not understood. Here we
99 provide data supporting a plausible explanation for both observations, one that links physiological
100 plasticity at hypothalamic synapses to aggressive behavior and sex hormone levels.

101 **Results**

102 **Aggression training increases VMHvl^{Esr1} neuron activity.** Aggression levels escalate following the
103 recurrent manifestation of the behavior (35, 44, 45), an effect termed here as “aggression training”. Using
104 a five consecutive-day resident-intruder assay (5cdRI, Fig. 1A), aggression training was investigated in
105 a cohort of C57BL/6 Esr1-Cre mice (n=138), which displayed increased aggression levels that remained
106 significantly elevated, relative to pre-training animals, over a prolonged period of time (maximal period
107 tested – three months, Fig. 1C-F). This assay enabled the identification of socially naïve, aggressive
108 (AGG), and non-aggressive (NON) mice, the latter of which represent ~23% of all males tested (Fig. 1B).
109 Aggression levels were found to plateau on the fourth and fifth day of the 5cdRI, suggestive of a ceiling
110 effect in the expression of aggressive behavior (Fig. 1C-E). Interestingly, aggression levels remained
111 elevated for the maximal follow-up period tested (three months) following aggression training, as
112 compared to the first instance of resident-intruder (RI) test (Fig. 1C).

113 To test whether aggression training involves plasticity in a structure that mediates the innate aspect of
114 aggression (46), we initially focused on VMHvl^{Esr1} neurons, optogenetic stimulation of which can evoke
115 attack in socially naïve, inexperienced animals (47). Using brain slice Ca²⁺ imaging, the average baseline
116 activity of VMHvl^{Esr1} neurons was found to increase in AGGs but not in NONs, following aggression
117 training (Fig. 1G-J). Voltage-clamp *ex vivo* VMHvl^{Esr1} neuron recordings revealed in AGG mice a
118 significant increase in the frequency and amplitude of spontaneous excitatory postsynaptic currents
119 (sEPSCs, Fig. 2A-C), relative to socially naïve animals. The increase in the amplitude of sEPSCs raised
120 the possibility that a synaptic potentiation mechanism may be present in VMHvl^{Esr1} neurons (48, 49). In
121 contrast, voltage-clamp *ex vivo* VMHvl^{Esr1} neuron recordings in slices from NON mice revealed an
122 increase in the frequency and amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs, Fig.
123 S1).

124 The increased activity of VMHvl^{Esr1} neurons following aggression training in AGG mice prompted us to
125 investigate whether this might involve potentiation of an excitatory input to these cells. Anatomical studies

126 have identified a strong purely excitatory input to VMHvl^{Esr1} cells, which originates anatomically from the
127 posteromedial part of the amygdalohippocampal area (AHiPM, also termed the posterior amygdala, PA)
128 (50). *In vivo* optogenetic activation of AHiPM evoked aggression (Fig. 2D-H), confirming a recent report
129 using chemogenetic activation (51). We found, moreover, that this effect is amplified in aggression-
130 experienced animals (Fig. 2D-H). Investigation of the functional connectivity at AHiPM→VMHvl^{Esr1}
131 synapses in acute slices using optogenetic activation of AHiPM inputs (Fig. 2I-K) indicated that this
132 projection is entirely excitatory, at least in part monosynaptic (Fig. S2), with an absence of any evoked
133 responses at the reversal potential for excitation (V_m hold = 0 mV, Fig. 2J – middle row) and reliable
134 photostimulation-evoked currents at the reversal potential for inhibition (V_m hold = -70 mV, Fig. 2J –
135 bottom row). These observations raised the question of whether potentiation at AHiPM→VMHvl^{Esr1}
136 synapses underlies the observed increase in the excitatory synaptic input onto VMHvl^{Esr1} neurons
137 recorded from AGG mice following aggression training.

138

139 **Plasticity at a hypothalamic synapse following aggression training.** At the postsynaptic side of
140 synapses that can undergo LTP, the response to stimulation of excitatory pre-synaptic inputs largely
141 depends on the ratio of N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methyl-4-
142 isoxazolepropionic acid (AMPA) receptors (52, 53). To test whether the AHiPM→VMHvl^{Esr1} synapse
143 undergoes potentiation following aggression training, therefore, we measured the AMPA/NMDA ratio (54-
144 56). This analysis revealed a significantly higher AMPA/NMDA ratio in AGGs following such training,
145 compared to socially naive and NON mice (Fig. 2K). As the AMPA/NMDA ratio can influence synaptic
146 integration properties (57), we also investigated synaptic integration in VMHvl^{Esr1} neurons from socially
147 naive, AGG (trained) and NON mice. Indeed, these three groups of animals exhibited distinct synaptic
148 integration properties, with depressing/static synaptic integration in socially naive and NON mice, and
149 facilitating synaptic integration in the VMHvl^{Esr1} neurons of AGG (trained) mice (Fig. 2L, M).

150 Changes in the AMPA/NMDA ratio and synaptic integration properties are often accompanied by changes
151 in neuronal morphology and dendritic spine complexity, which can be indicative of structural LTP (sLTP)
152 (58-62). To investigate this possibility, VMHvl^{Esr1} neurons which exhibited an increase in the
153 AMPA/NMDA ratio and synaptic integration in acute slice preparations were filled with neurobiotin, and
154 super-resolution images for reconstruction were obtained using the Airyscanning technique in a ZEISS
155 LSM 880 (63, 64). Analysis of second-order dendritic segments identified a prominent increase in
156 dendritic arborizations in VMHvl^{Esr1} neurons from AGG (trained) mice, in comparison to socially naive and
157 NON mice (Fig. 3A-L). These changes were reflected in most spine parameters measured, including
158 density, branching points, volume, area, length, and mean diameter (Fig. 3M-R). However, the principal
159 feature was an increase in the number of short-length spines, suggesting they were newly generated
160 during or after training. Collectively, these observations suggested the possibility that potentiation is likely
161 to occur at AHiPM→VMHvl^{Esr1} synapses, following aggression training in susceptible animals. We
162 therefore pursued this possibility using more specific electrophysiological protocols.

163

164 **Experimental induction of LTP and LTD at AHiPM→VMHvl^{Esr1} synapses.** LTP and LTD can be
165 experimentally induced in slices from brain areas typically associated with higher cognitive processing
166 (65-70), such as the hippocampus, but few studies have demonstrated that this can occur in the
167 hypothalamus (71-73), a site traditionally considered the source of instincts.

168 To determine whether LTP can be experimentally induced at AHiPM→VMHvl^{Esr1} synapses *ex vivo*, we
169 employed acute VMHvl slices, and used an optogenetics protocol composed of three bouts of
170 photostimulation of Chronos-expressing AHiPM terminals, during which the VMHvl^{Esr1} neuron (identified
171 by Cre-dependent expression of tdTomato in Esr1-Cre mice) was voltage-clamped at a depolarized
172 membrane potential (-30 mV; Fig. 4A, 4B). The choice of this Hebbian stimulation protocol was based on
173 our initial finding that combined pre- and post-synaptic depolarization were necessary for induction of

174 potentiation at AHiPM \rightarrow VMHvl $^{\text{Esr}1}$ synapses (Fig. S3) (74). The stimulation frequency of AHiPM terminals
175 used here (20 Hz) was chosen based on our previous demonstration that direct optogenetic stimulation
176 of VMHvl $^{\text{Esr}1}$ neurons at this same frequency drives action potential firing with 100% spike fidelity *ex vivo*
177 as well as *in vivo*, without depolarization block (47), and that it produces reliable synaptic integration in
178 VMHvl $^{\text{Esr}1}$ neurons (Fig. 2L, M). *Ex vivo* whole-cell voltage-clamp recordings composed of 20 min baseline
179 and 20 min follow-up, revealed that the majority of VMHvl $^{\text{Esr}1}$ neurons recorded in slices from socially
180 naive, AGG (trained) and NON mice were able to express synaptic potentiation in response to this
181 manipulation (Fig. 4C). Comparison of the responses with the animals' aggression phenotypes revealed,
182 however, that the dynamics of the response, including its maximum amplitude and persistence, differed
183 between groups, with synaptic potentiation in slices from NON mice returning to baseline levels within
184 the maximal period tested (20 min, Fig. 4D). Based on the Hebbian conditions required to evoke this form
185 of synaptic potentiation, and the similarity of its features to LTP as characterized in the hippocampus (75,
186 76), we refer to this form of plasticity as hypothalamic LTP.

187 These findings in turn raised the question of whether AHiPM \rightarrow VMHvl $^{\text{Esr}1}$ synapses can also express long-
188 term synaptic depression (LTD). This was investigated using a longer stimulation protocol for activating
189 AHiPM terminals (10 min continuous stimulation at 1Hz, Fig. 4E). Similar to the case of LTP, most
190 VMHvl $^{\text{Esr}1}$ neurons expressed LTD of varying amplitude and dynamics, in a manner that varied with the
191 animals' aggression phenotypes (Fig. 4F, 4G). Interestingly, VMHvl $^{\text{Esr}1}$ cells from NON mice expressed
192 higher amplitude LTD (Fig. 4G) than did cells from other groups.

193 An important question raised by these *ex vivo* observations was whether LTP and LTD can be induced
194 at AHiPM \rightarrow VMHvl $^{\text{Esr}1}$ synapses *in vivo*, using either optogenetic stimulation or aggression training. To
195 study the optogenetic induction of LTP *in vivo*, we used a similar paradigm to the *ex vivo* stimulation
196 protocol (Fig. 4A, 4H). However, in order to be able to simultaneously depolarize both pre- and post-
197 synaptic terminals we used spectrally segregated opsins with an overlap at 535 nm, to permit co-
198 excitation (77). As in the case of the *ex vivo* experiments, AHiPM was transduced with Chronos; in

199 addition, VMHvl^{Esr1} neurons were transduced with ChrimsonR (Fig. 4H). Chronic implantation of a silicon
200 probe optrode in the VMHvl allowed the detection of optically induced LTP or LTD in individual freely-
201 moving mice in their home cage, as a change in AHiPM stimulation-evoked local field potentials (fEPSPs;
202 Fig. 4I-4K). Application of the Hebbian protocol in socially naive mice led to the robust expression of LTP
203 in VMHvl *in vivo* (Fig. 4L), while application of the spaced protocol led to robust expression of LTD (Fig.
204 4M). Although VMHvl^{Esr1} neurons in silicon probe recordings were identified by optogenetic photo-tagging
205 of post-synaptic cells, we cannot exclude that other classes of VMHvl neurons contribute to recorded
206 fEPSPs.

207 These findings in turn raised the question of whether LTP can be induced *in vivo* by aggression training.
208 Applying the same testing method used to analyze the optogenetic induction of *in vivo* LTP and LTD, the
209 field excitatory postsynaptic potential (fEPSP) was monitored during a 10 min baseline period and then
210 following aggression training in initially socially naive mice. We used test optogenetic pulses to briefly
211 activate AHiPM terminals and ask whether the fEPSP increased in amplitude following the expression of
212 aggression. Indeed, LTP was induced in VMHvl^{Esr1} neurons immediately after the expression of social
213 behavior and aggression (Fig. 4N-P, n=4 mice tested). Notably, the behavioral induction of LTP (Fig. 4O),
214 led to a persistent change in the amplitude of the fEPSP. This might suggest a lack of an early- vs late-
215 phase distinction in the LTP at AHiPM→VMHvl^{Esr1} synapses, in contrast to LTP features observed at
216 defined synapses in the hippocampus and the amygdala (78, 79).

217 The above findings identify hypothalamic synaptic plasticity, and specifically LTP and LTD, as
218 mechanisms that can occur and can alter VMHvl^{Esr1} neuronal excitability *in vivo*. Next we sought to
219 address whether LTP and LTD have a causal role in the behavioral effect of aggression training.

220

221 **LTP facilitates and LTD inhibits potentiation of aggression following aggression training.** To
222 address whether LTP in the AHiPM→VMHvl^{Esr1} synapses can influence the expression of aggression in

223 inexperienced animals, we performed an *in vivo* optogenetic manipulation using the approach we
224 established for the experimental induction of hypothalamic LTP *in vivo*. The AHiPM of Esr1-Cre mice was
225 transduced with Chronos or YFP, while VMHvl^{Esr1} neurons were transduced with Cre-dependent
226 ChrimsonR or mCherry (Fig. S4A). The effects of these manipulations were investigated behaviorally,
227 not physiologically, therefore silicon recording probes were not implanted.

228 Following application of the LTP induction protocol over three consecutive days in socially naïve, solitary
229 mice, the RI test was performed on the fourth day, and behavior was quantified (Fig. S4B-G). The opsin
230 expressing mice (LTP group), exhibited elevated levels of aggression, indicating that the experimental
231 induction of LTP in this particular projection *in vivo* can influence the expression of aggression in the
232 absence of prior social experience (Fig. S4D-G).

233 The above paradigm was further modified to test, whether LTP and LTD can exert a causal influence on
234 aggression training. Using the approach we established for the experimental induction of hypothalamic
235 LTP or LTD *in vivo* (Fig. 4K-M), the AHiPM of Esr1-Cre mice was transduced with Chronos or YFP, while
236 VMHvl^{Esr1} neurons were transduced with Cre-dependent ChrimsonR or mCherry (Fig. 5A) and the effects
237 of these manipulations were investigated behaviorally.

238 The 5cdRI test was used to investigate the possible influence of LTP and LTD on aggression training. In
239 one experiment, to determine whether LTP could facilitate aggression training, the LTP induction protocol
240 was delivered at the end of each RI trial in both the control (YFP/mCherry-expressing) and LTP groups.
241 In a separate experiment, to determine whether LTP was necessary for aggression training, the LTD
242 induction protocol was delivered at the end of each RI trial in the control and LTD groups (Fig. 5B);
243 application of LTD is expected to override any LTP that may have occurred (80). As in Fig. 1A, smaller
244 size BALB/c intruders were introduced to Esr1-Cre residents, in which the LTP/LTD protocols were
245 optogenetically delivered (Fig. 5C). Aggression levels were recorded and analyzed on each day of the
246 5cdRI (i.e., 24 hrs following the previous LTP or LTD manipulation, with the exception of day one).

247 Applying LTP or LTD induction protocols *in vivo* facilitated or diminished the behavioral effect of
248 aggression training, respectively, as quantified by aggression/trial (% of the total trial duration occupied
249 by aggressive behavior) and attack latency (Fig. 5D-K). Interestingly, although LTP was found to enhance
250 the behavioral effect of aggression training on the second and third day of the 5cdRI assay, it did not lead
251 to ever-increasing aggression levels; rather the effect plateaued on days four and five, at a level similar
252 to the control group (see also Fig. 1C), suggesting an effect to accelerate learning. In contrast, LTD had
253 a profound inhibitory effect on aggression training, leading to similar aggression levels between day one
254 and day five of the 5cdRI test (Fig. 5D, two-tailed paired *t*-test, $P = 0.0592$ between day one and day five
255 in the LTD group).

256 We investigated next whether the observation that control and LTP-induced groups expressed similar
257 levels of aggressive behavior following training day three is due to a “ceiling effect” in the aggression
258 training paradigm. To do this, we performed further tests following completion of the 5cdRI training
259 routine. On day six, mouse social behavior was tested in a novel arena against a CD1 male conspecific
260 of larger size (Fig. 5B, 5C), under which condition aggressive resident mice are less likely to attack (81).
261 We reasoned that LTP mice that reached “ceiling” levels of aggression in the 5cdRI assays using
262 conventional, smaller subordinate intruders might nevertheless show higher aggression under these sub-
263 optimal conditions.

264 Indeed, under these conditions, the 5cdRI/LTP-treated mouse group exhibited higher aggression levels
265 than any other tested group, while the control and LTD groups expressed similar aggression levels (Fig.
266 5L-O). This finding suggests that hypothalamic LTP expressed by VMHvl^{Esr1} neurons can facilitate
267 aggression under modified conditions where resident aggressiveness is behaviorally reduced, relative to
268 that typically detected in our conventional RI assay.

269 Together these experiments demonstrate a potential role for LTP and LTD in AGG mice. We next
270 investigated the basis for individual differences in aggression training among genetically identical mice,

271 by asking whether we could identify any experimental intervention that would allow aggression and/or
272 hypothalamic LTP to be expressed in NON mice.

273

274 **Testosterone enables the expression of aggression and hypothalamic LTP in NON mice.** Levels of
275 testosterone (T) have been suggested to correlate with aggression and dominance in numerous species
276 (82-90), while administration of T following castration or ovariectomy has been shown to restore
277 aggression to the animal's behavioral repertoire (91-96). As T levels are subject to epigenetic influences
278 (97), we sought to determine whether individual differences in levels of the hormone were detectable
279 among genetically identical, inbred C57BL/6N mice, and if so whether they correlated with and were
280 responsible for individual differences in the capacity to undergo aggression training.

281 To investigate whether serum T levels differ between NON and AGG mice, we collected blood samples
282 at different time points of the 5cdRI test (Fig. 6A-D). This experiment revealed that prior to the experience
283 of aggression, a small but statistically significant ($P < .05$) difference in serum T is present between NON
284 and AGG mice (Fig. 6B). This difference between the two groups was further accentuated following
285 aggression training (Fig. 6C). This is because serum T levels remained unaltered following aggression
286 training in NON mice, whereas they increased in AGG mice following training (Fig. 6D). Interestingly the
287 increase in serum T in AGG mice occurred in the first three days, and was not further accentuated through
288 additional aggression training (Fig. 6D). Together, these data reveal a correlation between individual
289 differences in T and the ability to respond to aggression training in NON vs. AGG mice, as well as between
290 levels of aggressiveness and T levels in AGG mice during training.

291 To test whether T levels are causally responsible for the difference in aggressiveness between NON and
292 AGG mice, subcutaneous osmotic mini-pumps containing T or vehicle were implanted in NON mice (Fig.
293 6E). The serum T levels in NONs measured at seven days post T mini-pump implantation were
294 significantly higher than (but within the upper quartile of) the endogenous T levels measured in AGG mice

295 following completion of the 5cdRI training (Fig. 6D, F, serum T in NONs following T administration through
296 mini-pump 39.22 ± 2.73 ng/mL, serum T in AGGs following aggression training 16.61 ± 1.01 ng/mL, n=6
297 and 46 mice respectively, two-sided Mann–Whitney U test, $P < 0.0001$). Strikingly, the administration of
298 exogenous T induced aggression in all NON mice tested (Fig. 6G–J). We next investigated whether the
299 emergence of aggression through T administration correlated with the expression of LTP. In acute VMHvl
300 slices from vehicle vs T-treated NON mice, we investigated the induction and expression of LTP using
301 ex vivo recordings. Using the same Hebbian induction protocol, stronger LTP could be elicited from
302 VMHvl^{Esr1} neurons recorded in slices from T-treated NON mice, in comparison to those from vehicle-
303 treated NON mice (Fig. 6K–O). Thus, T implants facilitate LTP induction ex vivo in NON mice.

304 An important remaining question, however, was whether LTP was expressed in VMHvl^{Esr1} neurons *in*
305 *vivo*, following aggression training in T-treated NON mice. To address this question, we used the design
306 previously described in Fig. 4H, N, in which AHiPM was transduced with Chronos, while VMHvl^{Esr1} cells
307 were transduced with ChrimsonR. A novel BALB/c, small size male intruder was introduced into the
308 NON's home cage (Fig. 6P). In vehicle-treated mice, social interactions with intruder mice, but no
309 aggression, were observed, and LTP did not occur *in vivo*, as measured by fEPSP recordings in response
310 to optogenetic stimulation of Chronos-expressing AHiPM terminals (Fig. 6Q–T). However, T
311 administration through subcutaneous osmotic mini-pumps led to the expression of both aggressive
312 behavior and *in vivo* behaviorally induced LTP, in NON mice (Fig. 6Q–T).

313 These findings suggest that individual differences in serum T are responsible, at least in part, for
314 individual differences in the capacity for aggression training amongst inbred mice. Elevation of serum T
315 in NON mice can restore susceptibility to aggression training, as well as the capacity to express strong
316 LTP at AHiPM→VMHvl^{Esr1} synapses (both *ex vivo* and *in vivo* following aggression training). This
317 observation further strengthens the correlation between the ability to respond positively to aggression
318 training, and the expression of LTP. However, it does not distinguish whether the enhanced LTP in NON

319 mice is directly caused by T treatment, or rather is an indirect effect of the increased aggression promoted
320 by the hormone implants.

321

322 **Discussion**

323 A prevailing view in neuroscience is that anatomically distinct neural systems mediate innate vs. learned
324 behaviors: the former are thought to be processed by “labelled lines,” developmentally hard-wired circuits
325 in evolutionarily ancient, deep subcortical structures such as the hypothalamus (13, 98, 99); in contrast,
326 the latter engage synaptic plasticity mechanisms in more recently evolved brain structures, such as the
327 neocortex and hippocampus. Reflecting this view, in the mammalian brain the vast majority of studies of
328 synaptic plasticity mechanisms, such as LTP and LTD, have been performed in the latter structures (as
329 well as in the cerebellum (100-102). Whether such mechanisms also operate in deep subcortical
330 structures, and if so, what types of behavioral plasticity (if any) they might subserve, has remained
331 unclear. However, this knowledge gap reflects an absence of evidence, more than evidence of absence.

332 Here we have identified and deconstructed the neural substrate and physiological mechanism
333 underlying a form of experience-dependent plasticity in aggression, a prototypic innate social behavior.
334 We show that a training paradigm that increases aggressiveness via repeated successful agonistic
335 encounters is correlated with, enhanced by and dependent upon, LTP operating at a glutamatergic
336 synapse on a population of hypothalamic $Esr1^+$ neurons that mediates innate aggressive behavior (47).
337 The plasticity observed at $AHiPM \rightarrow VMHvl^{Esr1}$ synapses likely has both post- and pre-synaptic
338 components, as suggested by an increase in the AMPAR/NMDAR ratio (54-56) following aggression
339 training (Fig. 2J, K), and by the differential responses of $VMHvl^{Esr1}$ neurons to trains of pre-synaptic stimuli
340 (103, 104) (Fig. 2L, M), respectively. Surprisingly, the form of hypothalamic LTP studied here does not
341 exhibit “occlusion,” phenomenon observed in studies of hippocampal or amygdalar LTP (105, 106), in
342 which following *in vivo* behavioral induction of LTP in the synaptic population of interest, the magnitude
343 of LTP that can be induced subsequently *ex vivo* is markedly decreased (Fig. 4D, K). Similarly, we do

344 not observe the related phenomenon in which prior *in vivo* LTP can enhance the extent of LTD that can
345 be induced *ex vivo* in slices from such animals. The reason(s) for the failure to observe these phenomena
346 are not clear, and will require further studies to elucidate. There are a number of effects, however, which
347 could account for these observations. Firstly, it is possible that the proportion of synapses modified by
348 the *in vivo* social experience was small compared to the synapses being sampled in the slice. Another
349 possibility is that the synapses being assayed in the slice are a different population than the ones modified
350 *in vivo*, or lastly that new synapses were formed by the *in vivo* experience and they are the ones primarily
351 contributing to the LTP and LTD being measured *in vitro*. This last possibility is of particular interest,
352 given that - as presented in Fig. 3, an increase in spine density occurs in VMHvl^{Esr1} neurons of AGG mice.

353 The data on LTP presented here, blur the distinction between neural circuits mediating learned
354 vs. innate behaviors, and reinforce the concept of “learned innate behavior,” in which synaptic plasticity
355 within developmentally hardwired circuits can function to modify the strength of an instinctive behavior in
356 response to social experience. An example of the latter in an invertebrate is the post-mating response in
357 *Drosophila*, a form of memory in which female sexual receptivity is inhibited following mating (107-109).
358 Interestingly, recent studies have blurred the classic distinction between the innate and adaptive immune
359 systems as well (110, 111).

360 This idea notwithstanding, more complex forms of learning, such as classical or operant
361 conditioning, may utilize circuits that are parallel to those that mediate innate forms of the modified
362 behavior, as shown in the case of conditioned vs. unconditioned fear (112-114). In this context, it is worth
363 noting that mice can learn an instrumental, operant response using successful aggressive encounters as
364 a reinforcer (115), and that performance of this instrumental task is facilitated by optogenetic activation
365 of VMHvl neurons (116). The neural substrates and synaptic mechanisms underlying this operant
366 conditioning remain to be elucidated, although the nucleus accumbens-based reward system has been
367 implicated in recent studies (117).

368 Aggressiveness can be enhanced not only by repeated successful agonistic encounters, as
369 shown here, but also by prior mating experience (118). Recently, we showed that as little as 30 minutes
370 of free social interaction with a female was sufficient to transform a socially naïve mouse into an AGG
371 mouse within 24 hrs of the interaction (119). This effect was associated with a change in the neural
372 representation of male vs. female conspecifics among VMHvl^{Esr1} neurons, from partially overlapping to
373 largely non-overlapping (119). Whether this change in neural population coding involves synaptic
374 plasticity within VMHvl, or is inherited from upstream structures, such as the MeA (120), remains to be
375 determined. In other studies, we have shown that the effect of social isolation stress to promote
376 aggression in non-sexually experienced males is mediated by the neuropeptide Neurokinin B (NkB) and
377 its receptor Nk3R, acting in the dorso-medial hypothalamus (DMH) (121). The relationship of this form of
378 experience-dependent plasticity to VMHvl^{Esr1} neuronal activity is currently unknown.

379 Our current findings also provide insights into individual differences in the ability of genetically
380 identical animals to respond to “aggression training”. Firstly, we show here that several physiological
381 parameters in AGG mice are different from those in socially naïve mice. These include elevated baseline
382 VMHvl^{Esr1} neuron activity (Fig. 1G-J), increased spontaneous excitatory input onto VMHvl^{Esr1} neurons
383 (Fig. 2A-C), increased AMPA/NMDA ratio at AHiPM→VMHvl^{Esr1} synapses (Fig. 2I-K) and altered synaptic
384 integration properties (Fig. L, M). By contrast, in NON mice the spontaneous inhibitory inputs to VMHvl^{Esr1}
385 neurons are increased, relative to socially naïve mice (Fig. S1). In addition, NON mice exhibit shorter
386 lasting LTP and longer lasting LTD than are observed in AGG mice (Fig. 4D, G). Whether increased LTD
387 is sufficient to account for the failure of NON mice to respond to aggression training is not yet clear.
388 Another possibility, suggested by the increased spontaneous IPSCs, is that VMHvl receives stronger
389 inhibitory input from GABAergic neurons in NON mice. While there are very few GABAergic neurons
390 within VMHvl itself (122), VMHvl receives strong inhibitory input from the neighboring tuberal (TU) region.
391 It is possible that the lack of aggression in NON mice reflects potentiation of these TU GABAergic
392 neurons. Whether these TU neurons receive feed-forward input from VMHvl^{Esr1} neurons, or from another

393 source, is not known. The synaptic mechanisms responsible for the lack of aggression in NON mice will
394 clearly require further investigation.

395 We also find that NON mice – unsusceptible to aggression training, have low levels of circulating
396 T in comparison to AGG mice, and experimental administration of supplemental T can restore the
397 capacity for “aggression learning” in such animals. While the permissive role of T in promoting male
398 aggressiveness is well-established (82, 86, 123-126), our studies provide new insight into the
399 neurophysiological mechanisms that may mediate this effect in the context of aggression training.
400 Specifically, we observe that NON animals can only express LTP *in vivo* following administration of
401 exogenous T. Although LTP can be induced optogenetically *ex vivo* in slices from control NON animals,
402 LTP in slices from T-implanted NON animals exhibited higher-amplitude and persistence. Moreover, in
403 AGG mice levels of T increased during aggression training. This correlation suggests either that T acts
404 directly to enhance LTP at this synapse, which in turn promotes aggression, or that T acts indirectly, by
405 promoting aggressive behavior which in turn enhances LTP (Fig. S5). Whether T directly influences
406 synaptic plasticity, and if so the underlying molecular mechanisms involved, as well as the mechanistic
407 basis of individual differences in T levels, are interesting topics for future study.

408 Our experiments have focused on a specific glutamatergic input to VMHvl^{Esr1} neurons which have
409 a causal role in aggression. In addition to our finding, recent work reported that VMHvl-projecting Vglut⁺
410 neurons in the AHiPM exhibited elevated c-fos expression following both social investigation and attack,
411 while chemogenetic silencing of AHiPM neurons inhibited attack (51). VMHvl^{Esr1} neurons receive inputs
412 from neurons in over 30 different structures (50), raising the question of whether other inputs to these
413 cells also display plasticity. Indeed, recently published work has identified synaptic plasticity promoted
414 by foot-shock stress in a medial amygdala projection that primarily targets the central part of VMH (VMHc)
415 (127). Although a causal role in promoting aggression was not directly demonstrated for this input, and
416 the mechanism of potentiation was not established, plasticity at this synapse may regulate stress-induced
417 aggression (127). The present study demonstrates that AHiPM → VMHvl^{Esr1} synapses can undergo

418 Hebbian LTP, and that potentiation of these synapses occurs during social experience that enhances
419 offensive aggression (47, 119). Together, these data suggest that VMHvl likely provides a substrate in
420 which aggression plasticity can occur at multiple synaptic inputs, each of which may play distinct roles in
421 physiology and/or behavior. Our results also reveal striking effects of aggression training on dendritic
422 spine morphology among VMHvl^{Esr1} neurons, although we cannot be certain whether the secondary
423 dendritic branches where we observe this phenomenon receive synaptic input from AHiPM. Other recent
424 studies have identified structural plasticity among VMHvl^{PR}-derived axons innervating hypothalamic
425 targets in females, which are mediated by changes in sex steroids during estrus (128). The present work,
426 together with these other studies, begins to provide a view of the acute and dynamic changes that can
427 occur through experience and/or hormonal modulation, in a brain node that controls innate social
428 behaviors.

429 Historically, synaptic plasticity mechanisms – and in particular LTP, have been investigated
430 predominantly in hippocampal circuits that mediate spatial learning (129-132), or in thalamo-amygdalar
431 circuits that mediate Pavlovian associative conditioning (133-136). Both systems emphasize the role of
432 LTP in allowing flexible neural circuits to mediate adaptive responses on fast time-scales, as expected
433 for the recently evolved brain regions in which they operate. By contrast, studies of the hypothalamus
434 have focused primarily on identifying circuits that mediate evolutionarily ancient, innate survival
435 behaviors, with the expectation that such circuits would be comprised predominantly of relatively stable,
436 hard-wired synaptic connections (13, 98, 99). Our results and other data suggest a reconsideration of
437 this prevailing view of hypothalamic pathways as ‘hard-wired’ neural circuits. They suggest, moreover,
438 that further investigation of synaptic plasticity mechanisms within neural pathways that control
439 evolutionarily selected, robust survival behaviors, may yield new insights into both the physiological and
440 hormonal regulation of such mechanisms, as well as the forms of behavioral plasticity that they ultimately
441 subserve.

442

443

444 **Materials and Methods**

445 All experimental procedures involving the use of live animals or their tissues were carried out in
446 accordance with the NIH guidelines and approved by the Institutional Animal Care and Use Committee
447 and the Institutional Biosafety Committee at the California Institute of Technology (Caltech). *Esr1*^{Cre/+}
448 knock-in mice (47) backcrossed into the C57BL/6N background (>N10) were bred at Caltech. The
449 *Esr1*^{Cre/+} knock-in mouse line is available from the Jackson Laboratory (Stock no. 017911). Heterozygous
450 *Esr1*^{Cre/+} mice were used for all experiments and were genotyped by PCR analysis of tail DNA. Mice used
451 as residents (see five consecutive-day resident–intruder assay) were individually housed. All wild-type
452 mice used as intruders in resident–intruder assays and for behavioral experiments were of the
453 BALB/cAnNCrl or Crl:CD1 (ICR) strain (Charles River Laboratories). Health status was normal for all
454 animals. Antibodies, compounds, and the experimental procedures with the coordinates of all injection
455 sites are described in *SI Appendix*.

456

457 **Data Availability.** All data discussed in the paper are available in the main text and *SI Appendix*. We
458 used standard MATLAB functions and publicly available software indicated in the manuscript for analysis.

459 **Acknowledgments.** The authors thank Dr. B. Weissbourd and Dr. L. Li for advice on experiments, Dr.
460 Y. Ouadah and Dr. P. Williams for advice during writing of the manuscript, X. Da and J.S. Chang for
461 technical assistance, X. Da and C. Chiu for laboratory management and G. Mancuso for administrative
462 support. Members of the Anderson laboratory are thanked for discussion during the preparation of this
463 manuscript. Confocal imaging was performed in the Biological Imaging Facility, with the support of the
464 Caltech Beckman Institute and the Arnold and Mabel Beckman Foundation. This study was supported
465 by NIH Grant R01 MH070053 to D.J.A., and the EMBO ALTF 736-2018 postdoctoral fellowship to S.S.
466 D.J.A. is an investigator of the Howard Hughes Medical Institute.

467 **References**

- 468 1. J. Y. Kim, B. Cho, C. Moon, Timely Inhibitory Circuit Formation Controlled by Abl1 Regulates Innate
469 Olfactory Behaviors in Mouse. *Cell reports* **30**, 187-201 e184 (2020).
- 470 2. M. Yilmaz, M. Meister, Rapid innate defensive responses of mice to looming visual stimuli. *Current biology*
471 : CB **23**, 2011-2015 (2013).
- 472 3. Y. Wang *et al.*, Large-scale forward genetics screening identifies Trpa1 as a chemosensor for predator
473 odor-evoked innate fear behaviors. *Nature communications* **9**, 2041 (2018).
- 474 4. K. Sokolowski *et al.*, Specification of select hypothalamic circuits and innate behaviors by the embryonic
475 patterning gene dbx1. *Neuron* **86**, 403-416 (2015).
- 476 5. D. S. Manoli, G. W. Meissner, B. S. Baker, Blueprints for behavior: genetic specification of neural circuitry
477 for innate behaviors. *Trends Neurosci* **29**, 444-451 (2006).
- 478 6. E. B. Anderson, I. Grossrubatscher, L. Frank, Dynamic Hippocampal Circuits Support Learning- and
479 Memory-Guided Behaviors. *Cold Spring Harb Symp Quant Biol* **79**, 51-58 (2014).
- 480 7. E. Yavas, S. Gonzalez, M. S. Fanselow, Interactions between the hippocampus, prefrontal cortex, and
481 amygdala support complex learning and memory. *F1000Res* **8** (2019).
- 482 8. P. Le Merre *et al.*, Reward-Based Learning Drives Rapid Sensory Signals in Medial Prefrontal Cortex and
483 Dorsal Hippocampus Necessary for Goal-Directed Behavior. *Neuron* **97**, 83-91 e85 (2018).
- 484 9. G. B. Choi *et al.*, Lhx6 delineates a pathway mediating innate reproductive behaviors from the amygdala
485 to the hypothalamus. *Neuron* **46**, 647-660 (2005).
- 486 10. J. C. Jimenez *et al.*, Anxiety Cells in a Hippocampal-Hypothalamic Circuit. *Neuron* **97**, 670-683 e676 (2018).
- 487 11. A. Perez-Gomez *et al.*, Innate Predator Odor Aversion Driven by Parallel Olfactory Subsystems that
488 Converge in the Ventromedial Hypothalamus. *Current biology : CB* **25**, 1340-1346 (2015).
- 489 12. C. M. Root, C. A. Denny, R. Hen, R. Axel, The participation of cortical amygdala in innate, odour-driven
490 behaviour. *Nature* **515**, 269-273 (2014).
- 491 13. J. Kohl *et al.*, Functional circuit architecture underlying parental behaviour. *Nature*, 1-21 (2018).
- 492 14. S. L. Brincat, E. K. Miller, Frequency-specific hippocampal-prefrontal interactions during associative
493 learning. *Nature neuroscience* **18**, 576-581 (2015).
- 494 15. D. Huber *et al.*, Multiple dynamic representations in the motor cortex during sensorimotor learning.
495 *Nature* **484**, 473-478 (2012).
- 496 16. K. A. McDowell *et al.*, Reduced cortical BDNF expression and aberrant memory in Carf knock-out mice.
497 *The Journal of neuroscience : the official journal of the Society for Neuroscience* **30**, 7453-7465 (2010).
- 498 17. T. Wolansky, E. A. Clement, S. R. Peters, M. A. Palczak, C. T. Dickson, Hippocampal slow oscillation: a novel
499 EEG state and its coordination with ongoing neocortical activity. *The Journal of neuroscience : the official
500 journal of the Society for Neuroscience* **26**, 6213-6229 (2006).
- 501 18. Y. Goto, A. A. Grace, Dopamine-dependent interactions between limbic and prefrontal cortical plasticity
502 in the nucleus accumbens: disruption by cocaine sensitization. *Neuron* **47**, 255-266 (2005).
- 503 19. M. L. Hayashi *et al.*, Altered cortical synaptic morphology and impaired memory consolidation in
504 forebrain- specific dominant-negative PAK transgenic mice. *Neuron* **42**, 773-787 (2004).
- 505 20. D. L. Sosulski, M. L. Bloom, T. Cutforth, R. Axel, S. R. Datta, Distinct representations of olfactory
506 information in different cortical centres. *Nature* **472**, 213-216 (2011).
- 507 21. K. Miyamichi *et al.*, Cortical representations of olfactory input by trans-synaptic tracing. *Nature* **472**, 191-
508 196 (2011).
- 509 22. S. Ghosh *et al.*, Sensory maps in the olfactory cortex defined by long-range viral tracing of single neurons.
510 *Nature* **472**, 217-220 (2011).
- 511 23. M.-J. Dolan *et al.*, Communication from Learned to Innate Olfactory Processing Centers Is Required for
512 Memory Retrieval in Drosophila. *Neuron* **100**, 651-668.e658 (2018).

513 24. G. S. Jefferis *et al.*, Comprehensive maps of Drosophila higher olfactory centers: spatially segregated fruit
514 and pheromone representation. *Cell* **128**, 1187-1203 (2007).

515 25. S. J. C. Caron, V. Ruta, L. F. Abbott, R. Axel, Random convergence of olfactory inputs in the Drosophila
516 mushroom body. *Nature* **497**, 113-117 (2013).

517 26. H. Amin, A. C. Lin, Neuronal mechanisms underlying innate and learned olfactory processing in Drosophila.
518 *Curr Opin Insect Sci* **36**, 9-17 (2019).

519 27. M. Flajnik, L. Dupasquier, Evolution of innate and adaptive immunity: can we draw a line? *Trends in*
520 *Immunology* **25**, 640-644 (2004).

521 28. N. Tinbergen, *The study of instinct* (Clarendon Press, Oxford Eng., 1951), pp. 228 p.

522 29. K. Korenz, *On Aggression* (Harcourt, Brace & World, Inc., New York, 1963).

523 30. B. Ginsburg, W. Allee, Some effects of conditioning on social dominance and subordination in inbred
524 strains of mice. *Physiological Zoology* **15**, 485-506 (1942).

525 31. J. P. Scott, Incomplete Adjustment Caused by Frustration of Untrained Fighting Mice. *Journal of*
526 *comparative psychology* **39**, 379-390 (1946).

527 32. N. N. Kudryavtseva, D. A. Smagin, I. L. Kovalenko, G. B. Vishnivetskaya, Repeated positive fighting
528 experience in male inbred mice. *Nature protocols* **9**, 2705-2717 (2014).

529 33. K. Lagerspetz, Genetic and social causes of aggressive behaviour in mice. *Scandinavian Journal of*
530 *Psychology* **2**, 167-173 (1961).

531 34. N. E. Van de Poll, F. De Jonge, H. G. Van Oyen, J. Van Pelt, Aggressive behaviour in rats: Effects of winning
532 or losing on subsequent aggressive interactions. *Behav Processes* **7**, 143-155 (1982).

533 35. S. Stagkourakis *et al.*, A neural network for intermale aggression to establish social hierarchy. *Nature*
534 *neuroscience* **21**, 834-842 (2018).

535 36. J. B. Rosen, The neurobiology of conditioned and unconditioned fear: a neurobehavioral system analysis
536 of the amygdala. *Behavioral and cognitive neuroscience reviews* **3**, 23-41 (2004).

537 37. C. T. Gross, N. S. Canteras, The many paths to fear. *Nature reviews Neuroscience* **13**, 651-658 (2012).

538 38. F. Gore *et al.*, Neural Representations of Unconditioned Stimuli in Basolateral Amygdala Mediate Innate
539 and Learned Responses. *Cell* **162**, 134-145 (2015).

540 39. P. Namburi *et al.*, A circuit mechanism for differentiating positive and negative associations. *Nature* **520**,
541 675-678 (2015).

542 40. A. L. Falkner *et al.*, Hierarchical Representations of Aggression in a Hypothalamic-Midbrain Circuit. *Neuron*
543 **106**, 637-648 e636 (2020).

544 41. J. P. Scott, E. Fredericson, The causes of fighting in mice and rats. *Physiological Zoology* **24**, 273-309 (1951).

545 42. K. M. J. Lagerspetz, "Aggression and aggressiveness in laboratory mice" in Aggressive Behaviour. (1969),
546 pp. 77-85.

547 43. A. Tellegen, J. M. Horn, Primary aggressive motivation in three inbred strains of mice. *Journal of*
548 *comparative and physiological psychology* **78**, 297-304 (1972).

549 44. S. A. Golden *et al.*, Basal forebrain projections to the lateral habenula modulate aggression reward. *Nature*
550 **534**, 688-692 (2016).

551 45. S. Parmigiani, P. F. Brain, Effects of Residence, Aggressive Experience and Intruder Familiarity on Attack
552 Shown by Male-Mice. *Behav Process* **8**, 45-57 (1983).

553 46. D. Lin *et al.*, Functional identification of an aggression locus in the mouse hypothalamus. *Nature* **470**, 221-
554 226 (2011).

555 47. H. Lee *et al.*, Scalable control of mounting and attack by Esr1+ neurons in the ventromedial hypothalamus.
556 *Nature* **509**, 627-632 (2014).

557 48. H. Alle, P. Jonas, J. R. Geiger, PTP and LTP at a hippocampal mossy fiber-interneuron synapse. *Proceedings*
558 *of the National Academy of Sciences of the United States of America* **98**, 14708-14713 (2001).

559 49. Y. Perez, F. Morin, J. C. Lacaille, A hebbian form of long-term potentiation dependent on mGluR1a in
560 hippocampal inhibitory interneurons. *Proceedings of the National Academy of Sciences of the United*
561 *States of America* **98**, 9401-9406 (2001).

562 50. L. Lo *et al.*, Connectional architecture of a mouse hypothalamic circuit node controlling social behavior.
563 *Proceedings of the National Academy of Sciences of the United States of America* **116**, 7503-7512 (2019).

564 51. X. Zha *et al.*, VMHvl-Projecting Vglut1+ Neurons in the Posterior Amygdala Gate Territorial Aggression.
565 *Cell reports* **31** (2020).

566 52. C. I. Myme, K. Sugino, G. G. Turrigiano, S. B. Nelson, The NMDA-to-AMPA ratio at synapses onto layer 2/3
567 pyramidal neurons is conserved across prefrontal and visual cortices. *Journal of neurophysiology* **90**, 771-
568 779 (2003).

569 53. A. M. Thomson, Activity-dependent properties of synaptic transmission at two classes of connections
570 made by rat neocortical pyramidal axons in vitro. *The Journal of physiology* **502 (Pt 1)**, 131-147 (1997).

571 54. J. A. Kauer, R. C. Malenka, Synaptic plasticity and addiction. *Nature reviews. Neuroscience* **8**, 844-858
572 (2007).

573 55. M. A. Ungless, J. L. Whistler, R. C. Malenka, A. Bonci, Single cocaine exposure in vivo induces long-term
574 potentiation in dopamine neurons. *Nature* **411**, 583-587 (2001).

575 56. D. Saal, Y. Dong, A. Bonci, R. C. Malenka, Drugs of abuse and stress trigger a common synaptic adaptation
576 in dopamine neurons. *Neuron* **37**, 577-582 (2003).

577 57. V. Jeevakumar, S. Kroener, Ketamine Administration During the Second Postnatal Week Alters Synaptic
578 Properties of Fast-Spiking Interneurons in the Medial Prefrontal Cortex of Adult Mice. *Cerebral cortex* **26**,
579 1117-1129 (2016).

580 58. E. Harde *et al.*, EphrinB2 regulates VEGFR2 during dendritogenesis and hippocampal circuitry
581 development. *eLife* **8** (2019).

582 59. E. McClendon *et al.*, Transient Hypoxemia Disrupts Anatomical and Functional Maturation of Preterm
583 Fetal Ovine CA1 Pyramidal Neurons. *The Journal of neuroscience : the official journal of the Society for*
584 *Neuroscience* **39**, 7853-7871 (2019).

585 60. J. Ster *et al.*, Calsyntenin-1 regulates targeting of dendritic NMDA receptors and dendritic spine
586 maturation in CA1 hippocampal pyramidal cells during postnatal development. *The Journal of*
587 *neuroscience : the official journal of the Society for Neuroscience* **34**, 8716-8727 (2014).

588 61. A. S. Ivy *et al.*, Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress
589 involve excessive activation of CRH receptors. *The Journal of neuroscience : the official journal of the*
590 *Society for Neuroscience* **30**, 13005-13015 (2010).

591 62. J. W. Wang, D. J. David, J. E. Monckton, F. Battaglia, R. Hen, Chronic fluoxetine stimulates maturation and
592 synaptic plasticity of adult-born hippocampal granule cells. *The Journal of neuroscience : the official*
593 *journal of the Society for Neuroscience* **28**, 1374-1384 (2008).

594 63. J. Harmer, A. Belbelazi, M. Carr, M. L. Ginger, Airyscan Superresolution Microscopy to Study
595 Trypanosomatid Cell Biology. *Methods in molecular biology* **2116**, 449-461 (2020).

596 64. L. Scipioni, L. Lanzano, A. Diaspro, E. Gratton, Comprehensive correlation analysis for super-resolution
597 dynamic fingerprinting of cellular compartments using the Zeiss Airyscan detector. *Nature*
598 *communications* **9**, 5120 (2018).

599 65. M. Zhou *et al.*, CCR5 is a suppressor for cortical plasticity and hippocampal learning and memory. *eLife* **5**
600 (2016).

601 66. S. A. Connor *et al.*, Altered Cortical Dynamics and Cognitive Function upon Haploinsufficiency of the
602 Autism-Linked Excitatory Synaptic Suppressor MDGA2. *Neuron* **91**, 1052-1068 (2016).

603 67. A. Gruart, R. Leal-Campanario, J. C. Lopez-Ramos, J. M. Delgado-Garcia, Functional basis of associative
604 learning and its relationships with long-term potentiation evoked in the involved neural circuits: Lessons
605 from studies in behaving mammals. *Neurobiology of learning and memory* **124**, 3-18 (2015).

606 68. M. N. Quan, Y. T. Tian, K. H. Xu, T. Zhang, Z. Yang, Post weaning social isolation influences spatial cognition, 607 prefrontal cortical synaptic plasticity and hippocampal potassium ion channels in Wistar rats. *Neuroscience* **169**, 214-222 (2010).

609 69. J. L. Brigman *et al.*, Loss of GluN2B-containing NMDA receptors in CA1 hippocampus and cortex impairs 610 long-term depression, reduces dendritic spine density, and disrupts learning. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **30**, 4590-4600 (2010).

612 70. T. M. Jay *et al.*, Plasticity at hippocampal to prefrontal cortex synapses is impaired by loss of dopamine 613 and stress: importance for psychiatric diseases. *Neurotoxicity research* **6**, 233-244 (2004).

614 71. Y. Qi, Y. Yang, Hunger States Control the Directions of Synaptic Plasticity via Switching Cell Type-Specific 615 Subunits of NMDA Receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **35**, 13171-13182 (2015).

617 72. Y. Rao *et al.*, Repeated *in vivo* exposure of cocaine induces long-lasting synaptic plasticity in 618 hypocretin/orexin-producing neurons in the lateral hypothalamus in mice. *The Journal of physiology* **591**, 619 1951-1966 (2013).

620 73. K. M. Crosby, W. Inoue, Q. J. Pittman, J. S. Bains, Endocannabinoids gate state-dependent plasticity of 621 synaptic inhibition in feeding circuits. *Neuron* **71**, 529-541 (2011).

622 74. G. L. Collingridge, S. J. Kehl, H. McLennan, Excitatory amino acids in synaptic transmission in the Schaffer 623 collateral-commissural pathway of the rat hippocampus. *The Journal of physiology* **334**, 33-46 (1983).

624 75. D. Jaffe, D. Johnston, Induction of long-term potentiation at hippocampal mossy-fiber synapses follows a 625 Hebbian rule. *Journal of neurophysiology* **64**, 948-960 (1990).

626 76. P. K. Stanton, T. J. Sejnowski, Associative long-term depression in the hippocampus induced by hebbian 627 covariance. *Nature* **339**, 215-218 (1989).

628 77. N. C. Klapoetke *et al.*, Independent optical excitation of distinct neural populations. *Nature methods* **11**, 629 338-346 (2014).

630 78. S. Frey, J. Bergado-Rosado, T. Seidenbecher, H. C. Pape, J. U. Frey, Reinforcement of early long-term 631 potentiation (early-LTP) in dentate gyrus by stimulation of the basolateral amygdala: heterosynaptic 632 induction mechanisms of late-LTP. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **21**, 3697-3703 (2001).

634 79. S. Maren, M. S. Fanselow, Synaptic plasticity in the basolateral amygdala induced by hippocampal 635 formation stimulation *in vivo*. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **15**, 7548-7564 (1995).

637 80. S. Nabavi *et al.*, Engineering a memory with LTD and LTP. *Nature* **511**, 348-352 (2014).

638 81. D. Natarajan, H. de Vries, D. J. Saaltink, S. F. de Boer, J. M. Koolhaas, Delineation of violence from 639 functional aggression in mice: an ethological approach. *Behavior genetics* **39**, 73-90 (2009).

640 82. E. J. Hermans, N. F. Ramsey, J. van Honk, Exogenous testosterone enhances responsiveness to social threat 641 in the neural circuitry of social aggression in humans. *Biological psychiatry* **63**, 263-270 (2008).

642 83. C. A. Frye, M. E. Rhodes, A. Walf, J. P. Harney, Testosterone enhances aggression of wild-type mice but 643 not those deficient in type I 5alpha-reductase. *Brain research* **948**, 165-170 (2002).

644 84. C. A. Logan, J. C. Wingfield, Autumnal territorial aggression is independent of plasma testosterone in 645 mockingbirds. *Hormones and behavior* **24**, 568-581 (1990).

646 85. D. J. Albert, D. M. Petrovic, M. L. Walsh, Competitive experience activates testosterone-dependent social 647 aggression toward unfamiliar males. *Physiology & behavior* **45**, 723-727 (1989).

648 86. H. D. Steklis, G. L. Brammer, M. J. Raleigh, M. T. McGuire, Serum testosterone, male dominance, and 649 aggression in captive groups of rhesus monkeys (*Cercopithecus aethiops sabaeus*). *Hormones and behavior* **19**, 154-163 (1985).

651 87. D. Olweus, A. Mattsson, D. Schalling, H. Low, Testosterone, aggression, physical, and personality 652 dimensions in normal adolescent males. *Psychosomatic medicine* **42**, 253-269 (1980).

653 88. R. P. Michael, D. Zumpe, Annual cycles of aggression and plasma testosterone in captive male rhesus
654 monkeys. *Psychoneuroendocrinology* **3**, 217-220 (1978).

655 89. C. J. Krebs, Z. T. Halpin, J. N. Smith, Aggression, testosterone, and the spring decline in populations of the
656 vole *Microtus townsendii*. *Can J Zool* **55**, 430-437 (1977).

657 90. H. Persky, K. D. Smith, G. K. Basu, Relation of psychologic measures of aggression and hostility to
658 testosterone production in man. *Psychosomatic medicine* **33**, 265-277 (1971).

659 91. D. J. Albert, R. H. Jonik, M. L. Walsh, D. M. Petrovic, Testosterone supports hormone-dependent
660 aggression in female rats. *Physiology & behavior* **46**, 185-189 (1989).

661 92. R. E. Whalen, F. Johnson, Aggression in adult female mice: chronic testosterone treatment induces attack
662 against olfactory bulbectomized male and lactating female mice. *Physiology & behavior* **43**, 17-20 (1988).

663 93. M. A. Mann, B. Svare, Prenatal testosterone exposure elevates maternal aggression in mice. *Physiology &*
664 *behavior* **30**, 503-507 (1983).

665 94. M. S. Barkley, B. D. Goldman, The effects of castration and Silastic implants of testosterone on intermale
666 aggression in the mouse. *Hormones and behavior* **9**, 32-48 (1977).

667 95. M. S. Barkley, B. D. Goldman, Testosterone-induced aggression in adult female mice. *Hormones and*
668 *behavior* **9**, 76-84 (1977).

669 96. D. A. Powell, J. Francis, N. Schneiderman, The effects of castration, neonatal injections of testosterone,
670 and previous experience with fighting on shock-elicited aggression. *Communications in behavioral biology.*
671 *Part A: [Original articles]* **5**, 371-377 (1971).

672 97. J. Archer, Testosterone and human aggression: an evaluation of the challenge hypothesis. *Neuroscience*
673 and *biobehavioral reviews* **30**, 319-345 (2006).

674 98. J. R. Moffitt *et al.*, Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic
675 region. *Science* **362** (2018).

676 99. K. K. Ishii *et al.*, A Labeled-Line Neural Circuit for Pheromone-Mediated Sexual Behaviors in Mice. *Neuron*
677 **95**, 123-137 e128 (2017).

678 100. G. Cheron, J. Marquez-Ruiz, T. Kishino, B. Dan, Disruption of the LTD dialogue between the cerebellum
679 and the cortex in Angelman syndrome model: a timing hypothesis. *Frontiers in systems neuroscience* **8**,
680 221 (2014).

681 101. C. Hansel, D. J. Linden, E. D'Angelo, Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic
682 plasticity in the cerebellum. *Nature neuroscience* **4**, 467-475 (2001).

683 102. R. Llinas, E. J. Lang, J. P. Welsh, The cerebellum, LTD, and memory: alternative views. *Learning & memory*
684 **3**, 445-455 (1997).

685 103. B. Pradier *et al.*, Persistent but Labile Synaptic Plasticity at Excitatory Synapses. *The Journal of*
686 *neuroscience : the official journal of the Society for Neuroscience* **38**, 5750-5758 (2018).

687 104. K. Koga *et al.*, Impaired presynaptic long-term potentiation in the anterior cingulate cortex of Fmr1 knock-
688 out mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **35**, 2033-2043
689 (2015).

690 105. C. A. Barnes *et al.*, LTP saturation and spatial learning disruption: effects of task variables and saturation
691 levels. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **14**, 5793-5806
692 (1994).

693 106. B. W. Schroeder, P. Shinnick-Gallagher, Fear learning induces persistent facilitation of amygdala synaptic
694 transmission. *The European journal of neuroscience* **22**, 1775-1783 (2005).

695 107. K. Keleman *et al.* (2012) Dopamine neurons modulate pheromone responses in *Drosophila* courtship
696 learning. in *Nature* (Nature Publishing Group), pp 145-149.

697 108. X. Zhao, D. Lenek, U. Dag, B. J. Dickson, K. Keleman (2018) Persistent activity in a recurrent circuit underlies
698 courtship memory in *Drosophila*. in *Elife* (eLife Sciences Publications Limited).

699 109. L. C. Griffith, A. Ejima, Courtship learning in *Drosophila melanogaster*: diverse plasticity of a reproductive
700 behavior. *Learn Mem* **16**, 743-750 (2009).

701 110. L. L. Lanier, Shades of grey — the blurring view of innate and adaptive immunity. *Nat. Rev. Immunol.* **13**,
702 73-74 (2013).

703 111. G. F. Sonnenberg, M. R. Hepworth, Functional interactions between innate lymphoid cells and adaptive
704 immunity. *Nat. Rev. Immunol.* **19**, 599-613 (2019).

705 112. M. P. Donley, J. B. Rosen, Novelty and fear conditioning induced gene expression in high and low states of
706 anxiety. *Learning & memory* **24**, 449-461 (2017).

707 113. R. C. Twining, J. E. Vantrease, S. Love, M. Padival, J. A. Rosenkranz, An intra-amygdala circuit specifically
708 regulates social fear learning. *Nature neuroscience* **20**, 459-469 (2017).

709 114. C. T. Gross, N. S. Canteras, The many paths to fear. *Nature reviews. Neuroscience* **13**, 651-658 (2012).

710 115. E. W. Fish, J. F. De Bold, K. A. Miczek, Aggressive behavior as a reinforcer in mice: activation by
711 allopregnanolone. *Psychopharmacology* **163**, 459-466 (2002).

712 116. A. L. Falkner, L. Grosenick, T. J. Davidson, K. Deisseroth, D. Lin, Hypothalamic control of male aggression-
713 seeking behavior. *Nature neuroscience* **19**, 596-604 (2016).

714 117. S. A. Golden *et al.*, Nucleus Accumbens Drd1-Expressing Neurons Control Aggression Self-Administration
715 and Aggression Seeking in Mice. *The Journal of neuroscience : the official journal of the Society for
716 Neuroscience* **39**, 2482-2496 (2019).

717 118. K. L. Gobrogge, Y. Liu, X. Jia, Z. Wang, Anterior hypothalamic neural activation and neurochemical
718 associations with aggression in pair-bonded male prairie voles. *THE JOURNAL OF COMPARATIVE
719 NEUROLOGY* **502**, 1109-1122 (2007).

720 119. R. Remedios *et al.*, Social behaviour shapes hypothalamic neural ensemble representations of conspecific
721 sex. *Nature* **550**, 388-392 (2017).

722 120. Y. Li *et al.*, Neuronal Representation of Social Information in the Medial Amygdala of Awake Behaving
723 Mice. *Cell* **171**, 1176-1190 e1117 (2017).

724 121. M. Zelikowsky *et al.*, The Neuropeptide Tac2 Controls a Distributed Brain State Induced by Chronic Social
725 Isolation Stress. *Cell* **173**, 1265-1279 e1219 (2018).

726 122. D. W. Kim *et al.*, Multimodal Analysis of Cell Types in a Hypothalamic Node Controlling Social Behavior.
727 *Cell* **179**, 713-728 e717 (2019).

728 123. K. K. Soma, Testosterone and aggression: Berthold, birds and beyond. *Journal of neuroendocrinology* **18**,
729 543-551 (2006).

730 124. J. M. Hume, K. E. Wynne-Edwards, Castration reduces male testosterone, estradiol, and territorial
731 aggression, but not paternal behavior in biparental dwarf hamsters (*Phodopus campbelli*). *Hormones and
732 behavior* **48**, 303-310 (2005).

733 125. D. J. Albert, R. H. Jonik, N. V. Watson, B. B. Gorzalka, M. L. Walsh, Hormone-dependent aggression in male
734 rats is proportional to serum testosterone concentration but sexual behavior is not. *Physiology & behavior*
735 **48**, 409-416 (1990).

736 126. D. J. Albert, E. M. Dyson, M. L. Walsh, Intermale social aggression: reinstatement in castrated rats by
737 implants of testosterone propionate in the medial hypothalamus. *Physiology & behavior* **39**, 555-560
738 (1987).

739 127. J. Nordman *et al.*, Potentiation of divergent medial amygdala pathways drives experience-dependent
740 aggression escalation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*
741 10.1523/JNEUROSCI.0370-20.2020 (2020).

742 128. S. Inoue *et al.*, Periodic Remodeling in a Neural Circuit Governs Timing of Female Sexual Behavior. *Cell*
743 **179**, 1393-+ (2019).

744 129. G. D'Agostino *et al.*, Prolyl endopeptidase-deficient mice have reduced synaptic spine density in the CA1
745 region of the hippocampus, impaired LTP, and spatial learning and memory. *Cerebral cortex* **23**, 2007-
746 2014 (2013).

747 130. H. Eichenbaum, Spatial learning. The LTP-memory connection. *Nature* **378**, 131-132 (1995).

748 131. K. Sakimura *et al.*, Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor epsilon
749 1 subunit. *Nature* **373**, 151-155 (1995).

750 132. S. Davis, S. P. Butcher, R. G. Morris, The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate
751 (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that
752 block LTP in vitro. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **12**, 21-
753 34 (1992).

754 133. J. T. Kwon, J. S. Choi, Cornering the fear engram: long-term synaptic changes in the lateral nucleus of the
755 amygdala after fear conditioning. *The Journal of neuroscience : the official journal of the Society for
756 Neuroscience* **29**, 9700-9703 (2009).

757 134. H. C. Bergstrom, The neurocircuitry of remote cued fear memory. *Neuroscience and biobehavioral reviews*
758 **71**, 409-417 (2016).

759 135. A. M. Apergis-Schoute, J. Debiec, V. Doyere, J. E. LeDoux, G. E. Schafe, Auditory fear conditioning and long-
760 term potentiation in the lateral amygdala require ERK/MAP kinase signaling in the auditory thalamus: a
761 role for presynaptic plasticity in the fear system. *The Journal of neuroscience : the official journal of the
762 Society for Neuroscience* **25**, 5730-5739 (2005).

763 136. X. B. Li, T. Inoue, S. Nakagawa, T. Koyama, Effect of mediodorsal thalamic nucleus lesion on contextual
764 fear conditioning in rats. *Brain research* **1008**, 261-272 (2004).

765 137. L. Petreanu, T. Mao, S. M. Sternson, K. Svoboda, The subcellular organization of neocortical excitatory
766 connections. *Nature* **457**, 1142-1145 (2009).

767 138. J. Lu *et al.*, MIN1PIPE: A Miniscope 1-Photon-Based Calcium Imaging Signal Extraction Pipeline. *Cell reports*
768 **23**, 3673-3684 (2018).

769 139. J. I. Sanders, A. Kepecs, A low-cost programmable pulse generator for physiology and behavior. *Frontiers
770 in neuroengineering* **7**, 43 (2014).

771 140. J. H. Siegle *et al.*, Open Ephys: an open-source, plugin-based platform for multichannel electrophysiology.
772 *J Neural Eng* **14**, 045003 (2017).

773 141. K. B. J. Franklin, G. Paxinos, *THE MOUSE BRAIN IN STEREOTACTIC COORDINATES* (2008), vol. Third edition.

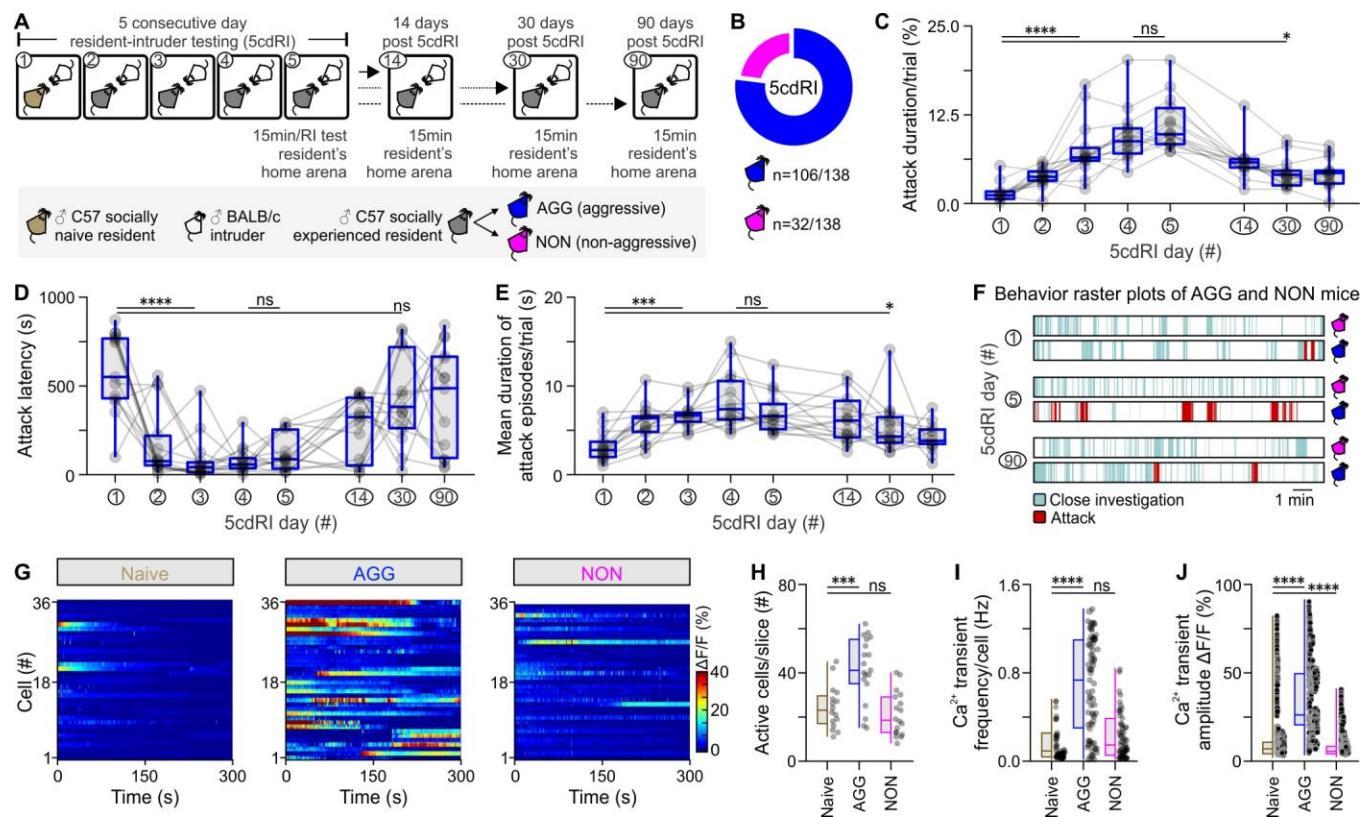
774 142. G. Lee, K. A. Goosens, Sampling Blood from the Lateral Tail Vein of the Rat. *Jove-J Vis Exp* ARTN e52766
775 10.3791/52766 (2015).

776 143. C. W. Chua *et al.*, Differential requirements of androgen receptor in luminal progenitors during prostate
777 regeneration and tumor initiation. *eLife* **7** (2018).

778 144. Q. Xie *et al.*, Dissecting cell-type-specific roles of androgen receptor in prostate homeostasis and
779 regeneration through lineage tracing. *Nature communications* **8**, 14284 (2017).

780 145. R. E. Sorge *et al.*, Different immune cells mediate mechanical pain hypersensitivity in male and female
781 mice. *Nature neuroscience* **18**, 1081-1083 (2015).

782



783

784 **Fig. 1. Aggression learning alters baseline activity dynamics in VMHvl^{Esr1} neurons.**

785 (A) Schematic of the experimental design - five consecutive day resident-intruder test (5cdRI), with three
786 follow-up dates, used for the study of the behavioral effect of aggression training.

787 (B) Summary indicative of the number of male animals exhibiting the two distinct aggression phenotypes
788 (n=138).

789 (C) Quantification of the cumulative duration (in %) of aggression per trial (n=15 AGG mice per group,
790 Kruskal-Wallis one-way ANOVA with uncorrected Dunn's post hoc test, $P < 0.0001$ between day 1 and
791 day 3 of the 5cdRI, $P = 0.4819$ between day 4 and day 5 of the 5cdRI, $P = 0.0149$ between day 1 and
792 day 30).

793 (D) Quantification of attack latency (in seconds) of aggression per trial (n=15 AGG mice per group,
794 Kruskal-Wallis one-way ANOVA with uncorrected Dunn's post hoc test, $P < 0.0001$ between day 1 and

795 day 3 of the 5cdRI, $P = 0.5602$ between day 4 and day 5 of the 5cdRI, $P = 0.2184$ between day 1 and
796 day 30).

797 (E) Quantification of average attack episode duration (in seconds) per trial (n=15 AGG mice per group,
798 Kruskal-Wallis one-way ANOVA with uncorrected Dunn's post hoc test, $P < 0.0001$ between day 1 and
799 day 3 of the 5cdRI, $P = 0.3326$ between day 4 and day 5 of the 5cdRI, $P = 0.0209$ between day 1 and
800 day 30).

801 (F) Behavior raster plots from AGG and NON mice, at different days of the 5cdRI test.

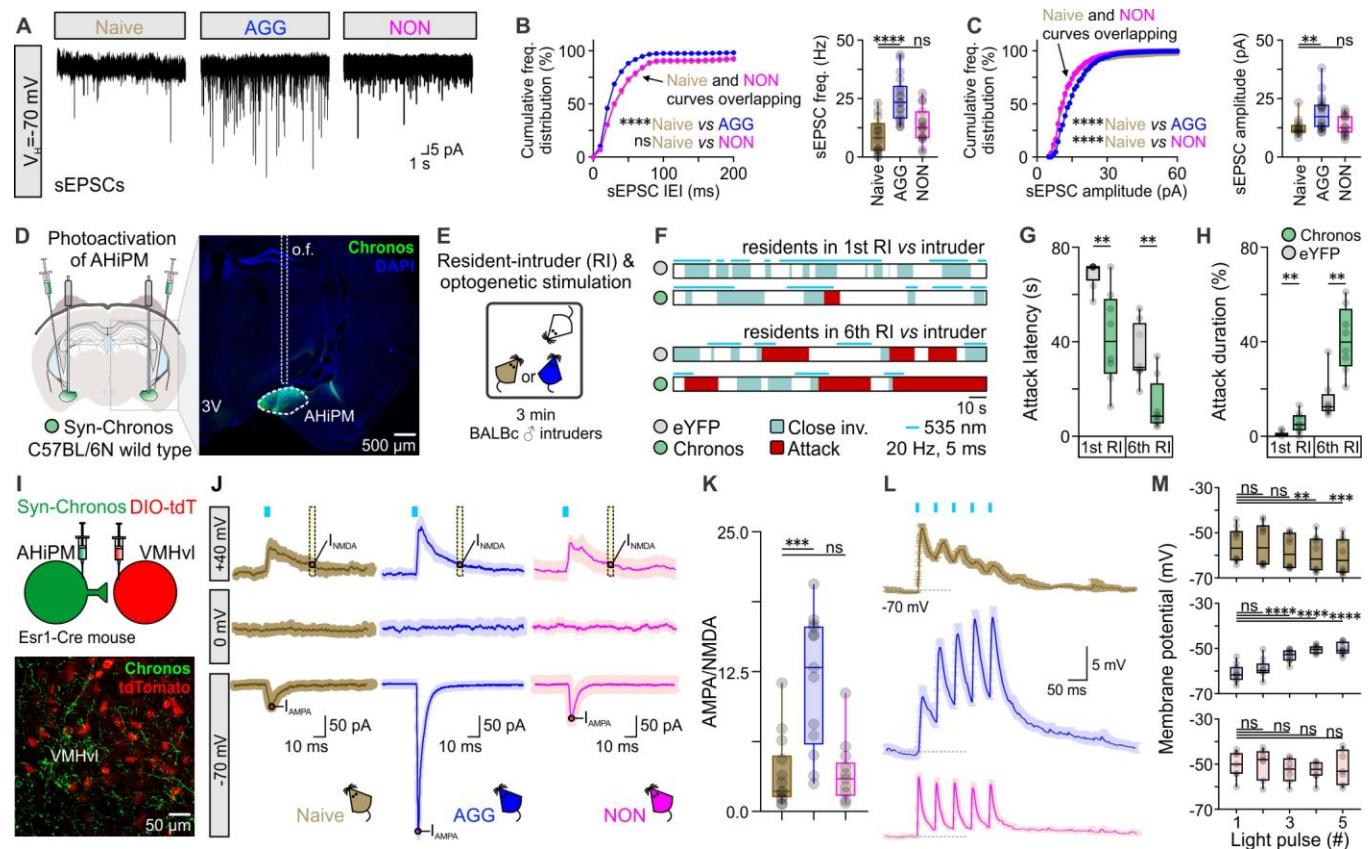
802 (G) Baseline Ca^{2+} activity of VMHvl^{Esr1} neurons recorded *ex vivo*, in brain slices of socially naive,
803 aggressive (AGG), and non-aggressive (NON) males.

804 (H) Quantification of active cells/slice (n=16-19 brain slices, collected from n=7-9 mice, one-way ANOVA
805 with Dunnett's post hoc test, $P = 0.0002$ between socially naive and AGG mouse brain slices, $P = 0.6358$
806 between socially naive and NON mouse brain slices).

807 (I) Quantification of Ca^{2+} spike frequency per cell (n=16-19 brain slices, collected from n=7-9 mice,
808 Kruskal-Wallis one-way ANOVA with Dunn's post hoc test, $P < 0.0001$ between socially naive and AGG
809 mice, $P = 0.3331$ between socially naive and NON mice).

810 (J) Quantification of Ca^{2+} spike amplitude (n=16-19 brain slices, collected from n=7-9 mice, Kruskal-Wallis
811 one-way ANOVA with Dunn's post hoc test, $P < 0.0001$ between socially naive and AGG mice, $P < 0.0001$
812 between socially naive and NON mice).

813 ns; not significant, * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$. In box-and-whisker plots, center lines indicate
814 medians, box edges represent the interquartile range, and whiskers extend to the minimal and maximal
815 values.



816

817 **Fig. 2. AHiPM → VMHvl^{Esr1} synapses become potentiated following aggression training.**

818 (A) Representative recordings of spontaneous excitatory post-synaptic currents (sEPSCs) from VMHvl^{Esr1}
819 neurons, from socially naive, AGG and NON mice.
820 (B) Left – cumulative frequency distribution plot of sEPSC IEI in voltage-clamp recordings collected from
821 VMHvl^{Esr1} neurons from socially naive, AGG and NON mice (n=14-18 VMHvl^{Esr1} neuron recording per
822 group, collected from 8-10 mice per group, Kolmogorov-Smirnov test, $P < 0.0001$ between socially naive
823 and AGG mice, $P = 0.3454$ between socially naive and NON mice). Right – comparison of sEPSC
824 frequency from voltage-clamp recordings collected from VMHvl^{Esr1} neurons from socially naive, AGG and
825 NON mice (n=14-18 VMHvl^{Esr1} neuron recording per group, collected from 8-10 mice per group, one-way
826 ANOVA with Dunnett's post hoc test, $P < 0.0001$ between socially naive and AGG mouse brain slices, P
827 = 0.2576 between socially naive and NON mouse brain slices).

828 (C) Left – cumulative frequency distribution plot of sEPSC amplitude in voltage-clamp recordings
829 collected from VMHvl^{Esr1} neurons from socially naive, AGG and NON mice (n=14-18 VMHvl^{Esr1} neuron
830 recording per group, collected from 8-10 mice per group, Kolmogorov-Smirnov test, $P < 0.0001$ between
831 socially naive and AGG mice, $P < 0.0001$ between socially naive and NON mice). Right – comparison of
832 sEPSC frequency from voltage-clamp recordings collected from VMHvl^{Esr1} neurons from socially naive,
833 AGG and NON mice (n=14-18 VMHvl^{Esr1} neuron recording per group, collected from 8-10 mice per group,
834 Kruskal-Wallis one-way ANOVA with uncorrected Dunn's post hoc test, $P = 0.0041$ between socially
835 naive and AGG mouse brain slices, $P = 0.6712$ between socially naive and NON mouse brain slices).

836 (D) Left – schematic of the experimental design used for optogenetic studies of aggression following
837 photoactivation of AHiPM, and right – confocal image indicative of Chronos-eYFP expression in AHiPM.

838 (E) Schematic illustration of the experimental protocol used in AHiPM^{Chronos} stimulation experiments.

839 (F) Sample behavior raster plots with *in vivo* optogenetics and social behavior in the resident intruder (RI)
840 assay, of socially naive and AGG mice.

841 (G) Quantification of attack latency, in the first and sixth RI trial (n=8 mice per group, first RI, two-sided
842 Mann-Whitney U test, $P = 0.0033$ between YFP and Chronos groups, sixth RI, two-tailed unpaired *t*-test,
843 $P = 0.0022$ between YFP and Chronos groups).

844 (H) Quantification of attack duration, in the first and sixth RI trial (n=8 mice per group, first RI, two-sided
845 Mann-Whitney U test, $P = 0.0079$ between YFP and Chronos groups, sixth RI, $P = 0.0011$ between YFP
846 and Chronos groups).

847 (I) Top – schematic of the experimental design used for the study of the AHiPM \square VMHvl synapse and
848 bottom – confocal image indicative of AHiPM originating processes in VMHvl.

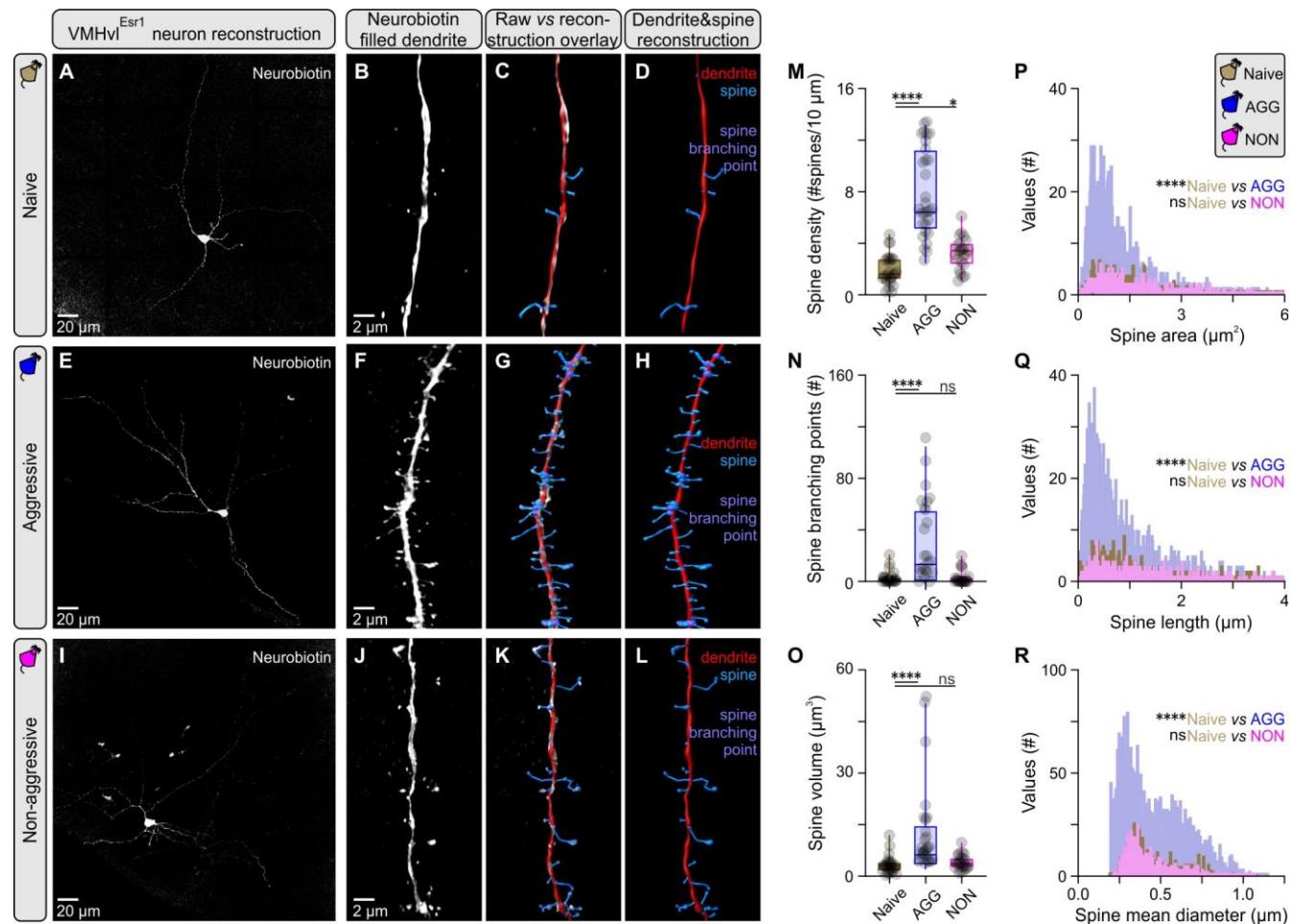
849 (J) Identification of the AHiPM \rightarrow VMHvl synapse as purely excitatory, and extraction of the AMPA to
850 NMDA ratio in socially naive, AGG and NON mice (average of n=13-14 neuron recordings from 8-9
851 socially naive, AGG and NON mice respectively).

852 (K) Quantification of the AMPA/NMDA ratio (n=13-14, Kruskal-Wallis one-way ANOVA with Dunn's post
853 hoc test, $P = 0.0005$ between socially naive and AGG mice, $P > 0.9999$ between socially naive and NON
854 mice).

855 (L) Synaptic integration in VMHvl Esr1 neurons from socially naive, AGG and NON mice (average traces
856 of n=7-10 neuron recordings from 7-9 mice respectively).

857 (M) Quantification of the five optically-evoked excitatory post-synaptic potentials (oEPSP) peak amplitude
858 presented in (O). Top - oEPSP amplitude quantification in VMHvl Esr1 neurons recorded from socially naive
859 mice (n=10 neurons from 9 mice, Friedman one-way ANOVA with Dunn's post hoc test, $P > 0.9999$
860 between 1st and 2nd pulse, $P = 0.3587$ between 1st and 3rd pulse, $P = 0.0028$ between 1st and 4th pulse,
861 and $P = 0.0009$ between 1st and 5th pulse). Middle - oEPSP amplitude quantification in VMHvl Esr1 neurons
862 recorded from AGG mice (n=10 neurons from 9 mice, one-way ANOVA with Dunnett's post hoc test, $P =$
863 0.2935 between 1st and 2nd pulse, $P < 0.0001$ between 1st and 3rd pulse, $P < 0.0001$ between 1st and 4th
864 pulse, and $P < 0.0001$ between 1st and 5th pulse). Bottom - oEPSP amplitude quantification in VMHvl Esr1
865 neurons recorded from NON mice (n=7 neurons from 7 mice, one-way ANOVA with Dunnett's post hoc
866 test, $P = 0.9865$ between 1st and 2nd pulse, $P = 0.5704$ between 1st and 3rd pulse, $P = 0.0751$ between 1st
867 and 4th pulse, and $P = 0.9803$ between 1st and 5th pulse).

868 ns; not significant, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. In box-and-whisker plots, center lines indicate
869 medians, box edges represent the interquartile range, and whiskers extend to the minimal and maximal
870 values.



871

872 **Fig. 3. Increased dendritic spine complexity in VMHvl^{Esr1} neurons following aggression training.**

873 (A) Maximum projection confocal image of a VMHvl^{Esr1} neuron from a socially naive mouse recorded ex
874 vivo, and filled with Neurobiotin.

875 (B) 3D rendering of a second order dendritic segment Airyscan image from the neuron presented in (A).

876 (C) Overlay of reconstruction data generated in Imaris against 3D rendering for the dendritic segment
877 presented in (B).

878 (D) Reconstructed dendritic segment of a VMHvl^{Esr1} neuron from a socially naive mouse, with color coding
879 for the dendrite and spines.

880 (E) Maximum projection confocal image of a VMHvl^{Esr1} neuron from an aggressive (AGG) mouse
881 recorded *ex vivo*, and filled with Neurobiotin.

882 (F) 3D rendering of a second order dendritic segment Airyscan image from the neuron presented in (E).

883 (G) Overlay of reconstruction data generated in Imaris against 3D rendering for the dendritic segment
884 presented in (F).

885 (H) Reconstructed dendritic segment of a VMHvl^{Esr1} neuron from an AGG mouse, with color coding for
886 the dendrite and spines.

887 (I) Maximum projection confocal image of a VMHvl^{Esr1} neuron from a non-aggressive (NON) mouse
888 recorded *ex vivo*, and filled with Neurobiotin.

889 (J) 3D rendering of a second order dendritic segment Airyscan image from the neuron presented in (I).

890 (K) Overlay of reconstruction data generated in Imaris against 3D rendering for the dendritic segment
891 presented in (J).

892 (L) Reconstructed dendritic segment of a VMHvl^{Esr1} neuron from a NON mouse, with color coding for the
893 dendrite and spines.

894 (M) Quantification of spine density in second order dendrites of VMHvl^{Esr1} neurons from socially naive,
895 AGG and NON mice (n=3-5 cells per group, n=1 cell/brain slice/animal, n=23-26 segments analyzed per
896 group, Kruskal-Wallis one-way ANOVA with Dunn's post hoc test, $P < 0.0001$ between socially naive and
897 AGG mice, $P = 0.0432$ between socially naive and NON mice).

898 (N) Quantification of branching points in second order dendrites of VMHvl^{Esr1} neurons from socially naive,
899 AGG and NON mice (n=23-26 segments analyzed per group, Kruskal-Wallis one-way ANOVA with
900 Dunn's post hoc test, $P < 0.0001$ between socially naive and AGG mice, $P = 0.9969$ between socially
901 naive and NON mice).

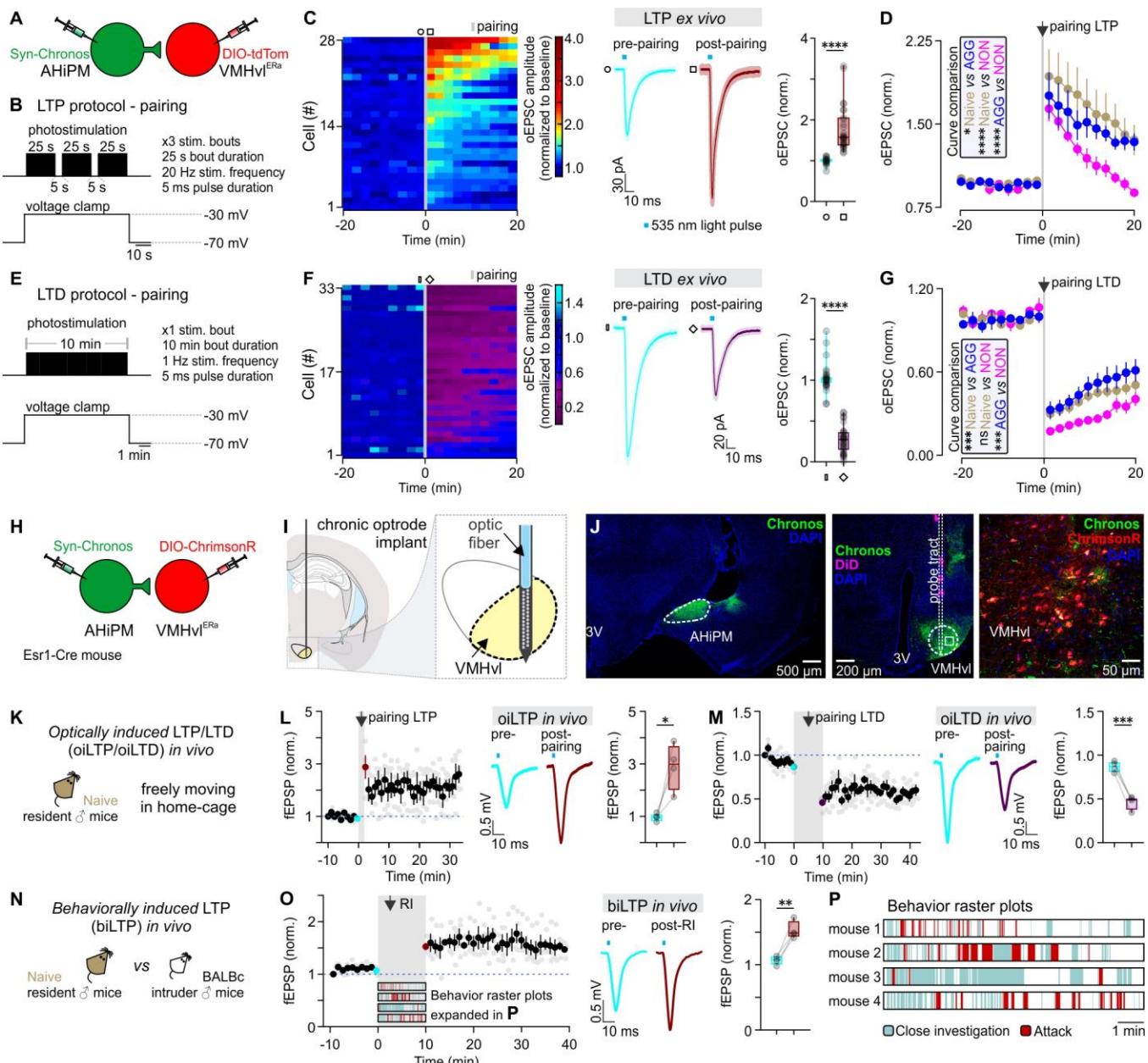
902 (O) Quantification of spine volume in second order dendrites of VMHvl^{Esr1} neurons from socially naive,
903 AGG and NON mice (n=23-26 segments analyzed per group, Kruskal-Wallis one-way ANOVA with
904 Dunn's post hoc test, $P < 0.0001$ between socially naive and AGG mice, $P = 0.4640$ between socially
905 naive and NON mice).

906 (P) Frequency distribution plot of spine area, of spines present in second order dendrites in VMHvl^{Esr1}
907 neurons from socially naive, AGG and NON mice (n=3-5 cells per group, n = 1 cell/brain slice/animal,
908 n=402-2365 spines per group).

909 (Q) Frequency distribution plot of spine length, of spines present in second order dendrites in VMHvl^{Esr1}
910 neurons from socially naive, AGG and NON mice (n=402-2365 spines per group).

911 (R) Frequency distribution plot of spine mean diameter, of spines present in second order dendrites in
912 VMHvl^{Esr1} neurons from socially naive, AGG and NON mice (n=402-2365 spines per group).

913 ns; not significant, * $P < 0.05$, **** $P < 0.0001$. In box-and-whisker plots, center lines indicate medians, box
914 edges represent the interquartile range, and whiskers extend to the minimal and maximal values.



915

916 **Fig. 4. Induction of LTP and LTD at AHIPM → VMHvl^{Esr1} synapses *ex vivo* and *in vivo*.**

917 (A) Schematic of the experimental design used to study the induction of LTP and LTD *ex vivo* in socially
918 naive, aggressive (AGG) and non-aggressive (NON) mice.

919 (B) Illustration of the experimental protocol used to induce LTP in the AHIPM → VMHvl synapse.

920 (C) Left – heat map illustrating the magnitude of LTP induction in all recorded VMHvl^{Esr1} neurons. Middle
921 – average current immediately prior to and following the induction of LTP (middle, n=28 neurons collected
922 from the three groups – socially naive, AGG and NON, with n=6-8 mice per group, light color envelope is
923 the standard error). Right – quantification of the optically evoked excitatory post-synaptic current
924 (oEPSC), prior to and following the induction of LTP (pre- vs post-pairing, n=28 neurons collected from
925 the three groups – socially naive, AGG and NON, with n=6-8 mice per group, two-tailed Wilcoxon signed-
926 rank test, $P < 0.0001$).

927 (D) Identification of differences in amplitude and persistence of LTP in socially naive, AGG, and NON
928 mice (n=9 neurons for 6 socially naive mice, n=10 neurons from 8 AGGs, and n=9 neurons from 6 NONs,
929 Kolmogorov-Smirnov test for curve comparison, $P = 0.0183$ between socially naive and AGG mice, $P <$
930 0.0001 between socially naive and NON mice, and $P < 0.0001$ between AGG and NON mice).

931 (E) Illustration of the experimental protocol used to induce LTD in the AHiPM → VMHvl synapse.

932 (F) Left – heat map illustrating the magnitude of LTD induction in all recorded VMHvl^{Esr1} neurons. Middle
933 – average current immediately prior to and following the induction of LTD (middle, n=33 neurons collected
934 from the three groups – socially naive, AGG and NON, with n=8 mice per group, light color envelope is
935 the standard error). Right – quantification of the oEPSC, prior to and following the induction of LTD (pre-
936 vs post-pairing, n=33 neurons collected from the three groups – socially naive, AGG and NON, with n=8
937 mice per group, two-tailed Wilcoxon signed-rank test, $P < 0.0001$).

938 (G) LTD dynamics in the three groups (n=12 neurons for 8 socially naive mice, n=10 neurons from 8
939 AGGs, and n=11 neurons from 8 NONs, Kolmogorov-Smirnov test for curve comparison, $P = 0.0002$
940 between socially naive and AGG mice, $P > 0.9999$ between socially naive and NON mice, and $P = 0.0008$
941 between AGG and NON mice).

942 (H) Schematic of the experimental design used to study the induction of LTP and LTD *in vivo* in socially
943 naive mice.

944 (I) Schematic illustration of the target coordinates of the optrode used to record local field potentials in
945 VMHvl.

946 (J) Left – representative confocal image of Chronos-eYFP expression in AHiPM. Middle - representative
947 confocal image of the silicon probe tract targeted to VMHvl. Right – high magnification confocal image of
948 VMHvl.

949 (K) Illustration of the experimental design used to induce LTP or LTD in the AHiPM→VMHvl synapse *in*
950 *vivo*.

951 (L) Left – plot of average of four experiments from four mice of field EPSP slope (normalized to baseline
952 period) before and after optically-induced LTP (oiLTP). Middle – *in vivo* average field response prior to
953 and following the induction of LTP. Right – quantification of optically induced field EPSPs (fEPSP), prior
954 to and following the induction of LTP (pre- vs post-pairing, n=4 mice per group, two-tailed paired *t*-test, *P*
955 = 0.0283).

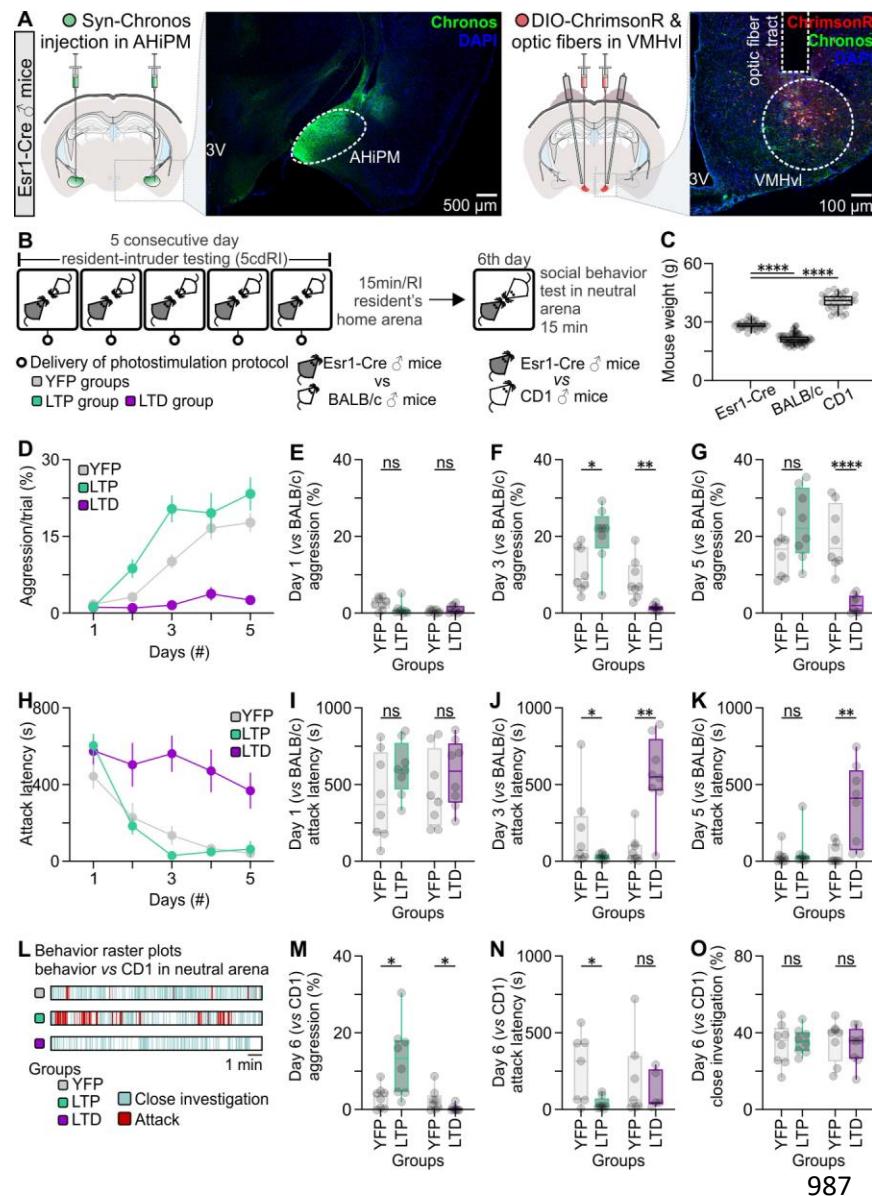
956 (M) Left – plot of average of four experiments from four mice of field EPSP slope (normalized to baseline
957 period) before and after optically-induced LTD (oiLTD). Middle – *in vivo* average field response prior to
958 and following the induction of LTD. Right – quantification of optically induced field EPSPs (fEPSP), prior
959 to and following the induction of LTD (pre- vs post-pairing, n=4 mice per group, two-tailed paired *t*-test,
960 *P* = 0.0007).

961 (N) Illustration of the experimental design used to test the behavioral induction of LTP.

962 (O) Left – plot of average of four experiments from four mice of field EPSP slope (normalized to baseline
963 period) before and after behaviorally-induced LTP (biLTP). Middle – *in vivo* average field response prior
964 to and following the behavioral induction of LTP. Right – quantification of optically induced field EPSPs
965 (fEPSP), prior to and following social behavior experience in a socially naive mouse (pre- vs post-pairing,
966 n=4 mice per group, two-tailed paired *t*-test, *P* = 0.0071).

967 (P) Illustration of the behaviors expressed in the resident-intruder assay from socially naive mice used
968 for the *in vivo* study of hypothalamic LTP.

969 ns; not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$. In box-and-whisker plots, center
970 lines indicate medians, box edges represent the interquartile range, and whiskers extend to the minimal
971 and maximal values.



987

988 **Fig. 5. Optogenetic induction of LTP or LTD at AHiPM \rightarrow VMHvl Esr1 synapses *in vivo* facilitates or**
 989 **abolishes, respectively, the effect of aggression training.**

990 (A) Left- representative confocal image and schematic indicative of ChrimsonR expression in VMHvl Esr1
 991 neurons, eYFP terminals of the AHiPM \rightarrow VMHvl projection, and the optic fiber tract terminating above
 992 VMHvl. Right - representative confocal image and schematic indicative of Chronos-eYFP expression in
 993 AHiPM.

994 (B) Schematic of the experimental design used to identify whether LTP and LTD have an impact on
995 aggression training.

996 (C) Weight measurements of the mice which were used in the protocol; specifically, the Esr1-Cre mice
997 were used as residents, the BALB/c as intruders, and the CD1 as novel conspecifics in a novel/neutral
998 arena (n=32-64 mice per group, one-way ANOVA with Tukey's test, $P < 0.0001$ between Esr1-Cre and
999 BALB/c mice, $P < 0.0001$ between Esr1-Cre and CD1 mice).

1000 (D) Quantification of aggression levels expressed during a trial throughout the 5 consecutive day RI test
1001 (5cdRI) in the YFP (control), LTP and LTD groups.

1002 (E) Quantification of aggression levels on the first day of the 5cdRI test (n=8 mice per group, two-tailed
1003 unpaired *t*-test, $P = 0.1049$ between YFP and LTP groups, $P = 0.2304$ between YFP and LTD groups).

1004 (F) Quantification of aggression levels on the third day of the 5cdRI test (n=8 mice per group, two-tailed
1005 unpaired *t*-test, $P = 0.0162$ [observed power=0.989, Cohen's D=0.7979, difference between
1006 means=9.13±3.34%, 95% CI =1.966 to 16.29] between YFP [lower 95% CI=6.452, higher 95% CI=16.06]
1007 and LTP [lower 95% CI=14.12, higher 95% CI=26.65] groups, $P = 0.0017$ between YFP and LTD groups).

1008 (G) Quantification of aggression levels on the fifth day of the 5cdRI test (n=8 mice per group, two-tailed
1009 unpaired *t*-test, $P = 0.0777$ between YFP and LTP groups, $P < 0.0001$ between YFP and LTD groups).

1010 (H) Quantification of attack latency throughout the 5cdRI in the YFP (control), LTP and LTD groups.

1011 (I) Quantification of attack latency on the first day of the 5cdRI test (n=8 mice per group, two-tailed
1012 unpaired *t*-test, $P = 0.1406$ between YFP and LTP groups, $P = 0.3688$ between YFP and LTD groups).

1013 (J) Quantification of attack latency on the third day of the 5cdRI test (n=8 mice per group, two-sided
1014 Mann-Whitney U test, $P = 0.0415$ [observed power=0.999, Cohen's D=0.6072, difference between
1015 means=159.40±90.79 sec, 95% CI =-378.8 to 60.04] between YFP [lower 95% CI=-21.05, higher 95%

1016 CI=407.0] and LTP [lower 95% CI=16.54, higher 95% CI=50.56] groups, $P = 0.0019$ between YFP and
1017 LTD groups).

1018 (K) Quantification of attack latency on the fifth day of the 5cdRI test (n=8 mice per group, two-sided
1019 Mann–Whitney U test, $P = 0.5054$ between YFP and LTP groups, two-tailed unpaired *t*-test, $P = 0.0052$
1020 between YFP and LTD groups).

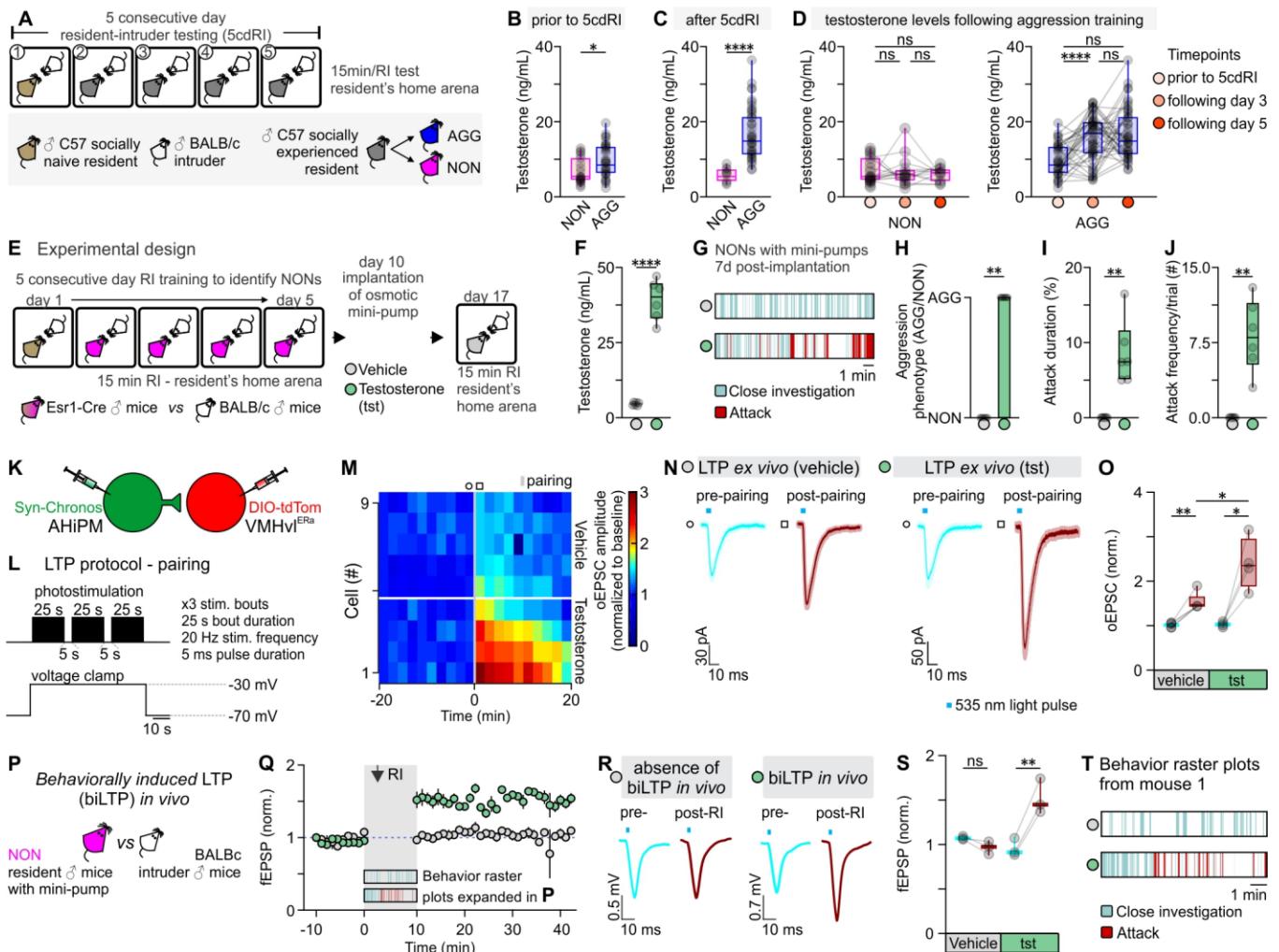
1021 (L) Representative behavior raster plots of YFP, LTP and LTD mouse behavior in novel arena towards a
1022 novel CD1 conspecific.

1023 (M) Quantification of aggression levels on the sixth day against a CD1 male (n=8 mice per group, two-
1024 tailed unpaired *t*-test, $P = 0.0387$ [observed power=0.999, Cohen's D=0.8980, difference between
1025 means=9.816±3.864%, 95% CI=0.6784 to 18.95] between YFP [lower 95% CI=0.8293, higher 95%
1026 CI=5.768] and LTP [lower 95% CI=5.190, higher 95% CI=21.04] groups, two-sided Mann–Whitney U test,
1027 $P = 0.0295$ [observed power=0.907, Cohen's D=0.7357, difference between means=2.161±1.069%, 95%
1028 CI=-4.616 to 0.2938] between YFP [lower 95% CI=0.1860, higher 95% CI=5.052] and LTD groups [lower
1029 95% CI=-0.2284, higher 95% CI =1.144]).

1030 (N) Quantification of attack latency on the sixth day against a CD1 male (n=8 mice per group, two-tailed
1031 unpaired *t*-test, $P = 0.0328$ [observed power=0.985, Cohen's D=1.0431, difference between
1032 means=227.00±82.23 sec, 95% CI =25.81 to 428.2] between YFP [lower 95% CI=67.01, higher 95%
1033 CI=477.9] and LTP groups [lower 95% CI=4.196, higher 95% CI=86.63], two-sided Mann–Whitney U test,
1034 $P > 0.9999$ between YFP and LTD groups).

1035 (O) Quantification of close investigation on the sixth day against a CD1 male (n=8 mice per group, two-
1036 tailed unpaired *t*-test, $P = 0.6973$ between YFP and LTP groups, $P = 0.6158$ between YFP and LTD
1037 groups).

1038 ns; not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$. In box-and-whisker plots, center
1039 lines indicate medians, box edges represent the interquartile range, and whiskers extend to the minimal
1040 and maximal values.



1041

1042 **Fig. 6. Testosterone administration leads to the expression of hypothalamic LTP and aggression**
1043 **in previously non-aggressive males.**

1044 (A) Schematic of the experimental design used to identify aggressive (AGG) and non-aggressive (NON)
1045 males, from which tails blood samples were collected for quantification of serum testosterone levels.

1046 (B) Serum testosterone levels in NON vs AGG mice prior to any aggression experience (n=24-36 samples
1047 per group, two-sided Mann-Whitney U test, $P = 0.0203$ between NON and AGG groups). Mice were
1048 assigned as NON or AGG, according to whether they expressed aggression on the 1st day of the 5cdRI
1049 test.

1050 (C) Serum testosterone levels in NON vs AGG mice after completion of the 5cdRI test (n=14-46 samples
1051 per group, two-tailed unpaired *t*-test, $P < 0.0001$ between NON and AGG groups). Mice that did not
1052 express any aggression/attack behavior throughout the 5cdRI test they were assigned to the NON group.
1053 All other mice they were included in the AGG group.

1054 (D) Left – Quantification of serum testosterone levels in NON mice throughout the 5cdRI test (n=14-24
1055 samples per group, Kruskal-Wallis one-way ANOVA with Dunn's post hoc test, $P > 0.9999$ between *prior*
1056 to 5cdRI and *following day 3*, $P > 0.9999$ between *prior to 5cdRI* and *following day 5*, and $P > 0.9999$
1057 between *following day 3* and *following day 5* groups). Right – Quantification of serum testosterone levels
1058 in AGG mice throughout the 5cdRI test (n=36-46 samples per group, one-way ANOVA with Tukey's test,
1059 $P < 0.0001$ between *prior to 5cdRI* and *following day 3*, $P < 0.0001$ between *prior to 5cdRI* and *following*
1060 *day 5*, and $P = 0.7060$ between *following day 3* and *following day 5* groups).

1061 (E) Schematic of the experimental design used to identify NON mice, and perform subcutaneous
1062 testosterone mini-pump implantation.

1063 (F) Serum testosterone levels in control vs. testosterone-treated mice (n=6 mice per group, two-tailed
1064 unpaired *t*-test, $P < 0.0001$ between vehicle and testosterone).

1065 (G) Representative behavior raster plots of vehicle vs testosterone-treated mice.

1066 (H) Quantification of the number of mice that switched aggression phenotype following vehicle vs
1067 testosterone administration (n=0/6 in the vehicle-treated group vs n=6/6 in the testosterone treated group,
1068 two-sided Mann-Whitney U test, $P = 0.0022$ between vehicle and testosterone).

1069 (I) Quantification of attack duration (n=6 mice per group, two-sided Mann-Whitney U test, $P = 0.0022$
1070 between vehicle and testosterone).

1071 (J) Quantification of attack frequency (# attacks/trial; n=6 mice per group, two-sided Mann-Whitney U
1072 test, $P = 0.0022$ between vehicle and testosterone).

1073 (K) Schematic of the experimental design used to study the induction and modulation of LTP by
1074 testosterone in the AHiPM→VMHvl synapse in brain slices from NON mice. Note that slices were taken
1075 from animals that received T injections, but no behavioral training or other social experience.

1076 (L) Schematic of the LTP induction protocol, utilizing simultaneous photostimulation of the AHiPM
1077 terminals in VMHvl through the opsin Chronos and depolarization of the VMHvl^{Esr1} neuron through voltage
1078 clamp at -30 mV.

1079 (M) Heat map illustrating the magnitude of LTP induction in VMHvl^{Esr1} neurons from vehicle- vs
1080 testosterone-treated mice.

1081 (N) Average current immediately prior to and following the induction of LTP in vehicle vs testosterone
1082 conditions (light color envelope is the standard error).

1083 (O) Quantification of the optically evoked excitatory post-synaptic current (oEPSC), prior to and following
1084 the induction of LTP in vehicle vs testosterone conditions (pre-[lower 95% CI= 0.9508, higher 95%
1085 CI=1.084] vs post-[lower 95% CI=1.274, higher 95% CI=1.786] pairing in vehicle conditions, n=5 cells
1086 from 3 mice, two-tailed paired *t*-test, *P* = 0.0038 [observed power=0.992, Cohen's D=2.704, difference
1087 between means=0.5124±0.0847, 95% CI=0.2771 to 0.7478], pre-[lower 95% CI=0.9349, higher 95%
1088 CI=1.120] vs post-[lower 95% CI=1.456, higher 95% CI=3.334] pairing in testosterone conditions, n=4
1089 cells from 3 mice, two-tailed paired *t*-test, *P* = 0.0209 [observed power=0.889, Cohen's D=2.232,
1090 difference between means=1.368±0.3063, 95% CI=0.3926 to 2.342], post-pairing in vehicle [lower 95%
1091 CI=1.274, higher 95% CI=1.786] vs testosterone [lower 95% CI=1.456, higher 95% CI=3.334] conditions,
1092 n=4-5 cells from 6 mice, two-tailed unpaired *t*-test, *P* = 0.0174 [observed power=0.932, Cohen's
1093 D=0.6388, difference between means=0.8652±0.2974, 95% CI=0.2044 to 1.526]).

1094 (P) Schematic of the experimental design used to trigger and record behaviorally induced LTP *in vivo* in
1095 NONs.

1096 (Q) Field EPSP amplitude (fEPSP) over time, prior to and following social behavior in the resident intruder
1097 assay, in vehicle- vs testosterone-treated NON mice (average fEPSP from n=3 mice per group).

1098 (R) Average fEPSP amplitude immediately prior to and following the expression of social behavior in the
1099 resident intruder assay, in vehicle- vs testosterone-treated NON mice.

1100 (S) Quantification of fEPSP amplitude, prior to and following the induction of LTP in vehicle vs
1101 testosterone conditions (pre-[lower 95% CI=1.027, higher 95% CI=1.123] vs post-[lower 95% CI=0.7907,
1102 higher 95% CI=1.143] pairing in vehicle conditions, n=3 mice, two-tailed paired *t*-test, *P* = 0.1020
1103 [observed power=0.999, Cohen's D=1.6667, difference between means=0.1081±0.0374, 95% CI=
1104 0.2692 to 0.05303], pre-[lower 95% CI=0.7055, higher 95% CI=1.209] vs post-[lower 95% CI=1.027,
1105 higher 95% CI=2.012] pairing in testosterone conditions, n=3 mice, two-tailed paired *t*-test, *P* = 0.0098
1106 [observed power=0.786, Cohen's D=5.7787, difference between means=0.5625±0.0562, 95% CI=0.3207
1107 to 0.8043]).

1108 (T) Representative behavior raster plot of the same mouse treated with vehicle and 8 days after with
1109 testosterone and used for *in vivo* electrophysiology experiments.

1110 ns; not significant, **P* < 0.05, ***P* < 0.01, *****P* < 0.0001. In box-and-whisker plots, center lines indicate
1111 medians, box edges represent the interquartile range, and whiskers extend to the minimal and maximal
1112 values. In bar graphs, data are expressed as mean ± s.e.m.

1113

1114 Supplementary Information for

1115 **Experience-dependent plasticity in an innate social behavior is mediated by**
1116 **hypothalamic LTP**

1117 *Stefanos Stagkourakis^{1*}, Giada Spigolon², Grace Liu¹, David J. Anderson^{1,3*}*

1118

1119 ¹ Division of Biology and Biological Engineering 156-29, Tianqiao and Chrissy Chen Institute for
1120 Neuroscience, California Institute of Technology, Pasadena, California 91125, USA

1121 ² Biological Imaging Facility, California Institute of Technology, Pasadena, California 91125, USA

1122 ³ Howard Hughes Medical Institute, California Institute of Technology, 1200 East California Blvd,
1123 Pasadena, California 91125, USA

1124 ***Corresponding author information:** Stefanos Stagkourakis, David J. Anderson

1125 **Email:** stefanos.stagkourakis@caltech.edu, wuwei@caltech.edu

1126 **Supplementary Information Text**

1127 **Extended materials and methods**

1128 **Animals.** All mice were housed in ventilated micro-isolator cages in a temperature-controlled environment (median
1129 temperature 23 °C), under a reversed 12h dark-light cycle, and had *ad libitum* access to food and water. Mouse
1130 cages were changed weekly on a fixed day on which experiments were not performed.

1131 **Brain slice electrophysiology.** Acute mouse brain slices were prepared. Slices were cut on a vibratome (Leica
1132 VT1000S) to 300 µm thickness and continuously perfused with oxygenated aCSF containing (in millimolar): NaCl
1133 (127), KCl (2.0), NaH₂PO₄ (1.2), NaHCO₃ (26), MgCl₂ (1.3), CaCl₂ (2.4), and D-glucose (10). See also Table S1.
1134 Whole-cell current- and voltage-clamp recordings were performed with micropipettes filled with intracellular solution
1135 containing (in millimolar), K-gluconate (140), KCl (10), HEPES (10), EGTA (10), and Na₂ATP (2) or Cesium
1136 methanesulfonate (140), KCl (10), HEPES (10), EGTA (10), and Na₂ATP (2) (pH 7.3 with KOH). Recordings were
1137 performed using a Multiclamp 700B amplifier, a DigiData 1440 digitizer, and pClamp 11 software (Molecular
1138 Devices). Slow and fast capacitative components were semi-automatically compensated. Access resistance was
1139 monitored throughout the experiments, and neurons in which the series resistance exceeded 15 MΩ or changed
1140 ≥20% were excluded from the statistics. The liquid junction potential was 9.7 mV and not compensated. The
1141 recorded current was sampled at 20 kHz. Baseline recordings of EPSCs, IPSCs and optogenetically-evoked
1142 synaptic currents were performed in normal aCSF conditions and in the absence of GABA and NMDA receptor
1143 blockers. Spontaneous excitatory currents were sampled at the reversal of Cl⁻ ($V_{HOLD}=-70$ mV), and spontaneous
1144 inhibitory currents were sampled at the reversal of fast excitatory neurotransmission ($V_{HOLD}=0$ mV). All recordings
1145 were performed at near-physiological temperature (33±1°C). Reagents used in slice electrophysiology experiments;
1146 Neurobiotin™ tracer (Vector laboratories) was used in combination with Streptavidin conjugated to Alexa Fluor 647.
1147 MATLAB and OriginPro9 were used for electrophysiological data analysis. CNQX (10 µM), D-AP5 (25 µM), TTX
1148 (500 nM), and 4-AP (100 mM) were bath applied to block excitatory transmission and to test if optogenetically
1149 evoked responses are monosynaptic (137). All drugs were pre-applied for 5 min in the slice chamber prior to data
1150 acquisition.

1151 **Brain slice Ca²⁺ imaging.** The spontaneous activity of mouse VMHvl^{Esr1} neurons was monitored by imaging
1152 fluorescence changes of the jGCaMP7s biosensor, using a CCD camera (Evolve® 512, Photometrics), mounted on

1153 an Olympus BX51WI microscope. Recordings were 5 min in duration. As a subpopulation of VMHvl^{Esr1} neurons
1154 expresses T-type Ca²⁺ channels (*unpublished data*), the Ca²⁺ transients reported in Fig. 1 likely reflect both action
1155 potentials and subthreshold synaptic potentials. A 60x water-dipping objective was used to focus on VMHvl. Ca²⁺
1156 imaging analysis was performed using the MIN1PIPE one-photon based calcium imaging signal extraction pipeline
1157 (138), in combination with custom-written MATLAB routines.

1158 **Cell filling and reconstruction.** Mouse *Esr1*⁺ VMHvl neurons were recorded in whole-cell mode with intracellular
1159 pipette solution as above, with the addition of 0.2% neurobiotin. After recording, slices were placed in fixative (4%
1160 paraformaldehyde/0.16% picric acid), washed in PBS and incubated at 4°C for 72h in a solution containing
1161 streptavidin conjugated to Alexa Fluor 647. After extensive washing, slices were mounted with 2.5% DABCO in
1162 glycerol. VMHvl^{Esr1} neuron identity of all filled cells was confirmed with colocalization studies of viral-induced
1163 tdTomato expression.

1164 **Ex vivo optogenetics.** Photostimulation during slice whole-cell recordings was performed via a 3.4 watt 535 nm
1165 LED mounted on the microscope fluorescence light source and delivered through the 60X objective's lens.
1166 Photostimulation was controlled via the analog outputs of a DigiData 1440A, enabling control over the duration and
1167 intensity. The photostimulation diameter through the objective lens was ~310 µm with illumination intensity typically
1168 scaled to 0.35 mW/mm².

1169 **In vivo optogenetics.** Subjects were coupled via a ferrule patch cord to a ferrule on the head of the mouse using
1170 a zirconia split sleeve (Doric Lenses). Ferrules and fiber-optic patch cords were purchased from Thorlabs and Doric
1171 Lenses, respectively. The optical fiber was connected to THORLABS fiber-coupled LED (M530F2, 9.6 mW) via a
1172 fiber-optic rotary joint (FRJ_1x1_FC-FC, Doric Lenses) to avoid twisting of the cable caused by the animal's
1173 movement. Prior to a testing session, following the coupling of the patch cords with the optic fiber ferrules, *Esr1*^{Cre/+}
1174 animals were given 10 min to acclimate in their home cage in the absence of an intruder. The frequency and duration
1175 of photostimulation were controlled using the programmable train generator Pulse Pal (139). Light power was
1176 controlled by dialing an analog knob on the LED driver (T-Cube™ LED Driver with Trigger Mode, Thorlabs,
1177 LEDD1B). Light power was measured from the tip of the ferrule in the patch cord at different laser output settings,
1178 using an optical power energy meter and a photodiode power sensor (Thorlabs, PM100D, and S130VC). Light
1179 power was dialed at 0.5 mW at the fiber tip. Upon identification of the fiber placement coordinates in brain tissue

1180 slides, irradiance (light intensity) was calculated using the brain tissue light transmission calculator based on
1181 (<http://www.stanford.edu/group/dlab/cgi-bin/graph/chart.php>) using laser power measured at the tip and the
1182 distance from the tip to the target brain region measured by histology. Animals showing no detectable viral
1183 expression in the target region and/or ectopic fiber placement were excluded from the analysis.

1184 ***In vivo electrophysiology.*** *In vivo* electrophysiology recordings were performed in freely moving mice, using
1185 chronic silicon probe implants. All extracellular recordings were conducted in the left VMHvl, and all mice included
1186 in the present study were validated using the following criteria: identification of the lipophilic dye (DiD) tract targeting
1187 VMHvl, phototagging of VMHvl^{Esr1} neurons, and photostimulation-evoked low-latency attack against a conspecific
1188 through optrode mediated VMHvl^{Esr1} neuron photoactivation. Recordings were performed using an optrode based
1189 on the A1x32-Poly2-10mm-50s-177 NeuroNexus probe and a 100 μ m optic fiber placed along the probe's shank
1190 terminating 50 μ m above the probe's first recording sites. Photostimulation was delivered using fiber-coupled
1191 Thorlabs LEDs (M530F2, 9.6 mW for LTP/LTD studies, and M617F2, 13.2 mW for phototagging), and light power
1192 was dialed at 0.33 mW at the optrode's fiber tip. The probe was implanted 200 μ m above the intended recording
1193 site, and using the NeuroNexus OH32LP oDrive was lowered over a period of four days to the target coordinates
1194 (lowering by 50 μ m/day). Only channels that showed photo-responses in the local field potential were used for LFP
1195 analysis. Recordings were performed using the Open Ephys acquisition board with a sampling rate of 30 kHz, the
1196 Open Ephys I/O board, and the Open Ephys GUI (140). The LTP signal was obtained by applying low pass-filtering
1197 with a cut-off at 100 Hz on the raw voltage traces.

1198 **Immunohistochemistry.** Mice were anesthetized with ketamine (KetaVed, VEDCO) and xylazine (AnaSed, NDC
1199 59399-110-20), then transcardially perfused with 20 mL of ice-cold fixative. Whole brains were dissected, immersed
1200 in ice-cold fixative for 90 min then stored in 0.1M PBS (pH 7.4) containing 20% sucrose, 0.02% bacitracin and
1201 0.01% sodium azide for three days, before freezing with dry ice. Coronal sections were cut at a thickness of 14 μ m
1202 on a cryostat (Microm, Walldorf) and thaw-mounted onto gelatine-coated glass slides. For GFP staining, brain
1203 sections were incubated overnight at 4°C using a chicken anti-GFP antibody (Aves Labs, Inc., GFP-1010) at 1:500
1204 dilution. For tdTomato staining brain sections were incubated overnight at 4°C using a rabbit anti-DsRed antibody
1205 (Takara, 632392) at 1:500 dilution. Primary antibody incubation was followed by Alexa-488-conjugated goat anti-
1206 chicken secondary antisera (1:500; Invitrogen), and/or Alexa-568-conjugated donkey anti-rabbit secondary antisera

1207 (1:500; Invitrogen). DAPI solution (1mg/mL) was used at 1:10000 dilution. For further details on reagents, see also
1208 Table S1.

1209 **Confocal microscopy.** Brain slices were imaged by confocal microscopy (Zeiss, LSM 800). Brain areas were
1210 determined according to their anatomy using Paxinos and Franklin Brain Atlas (141).

1211 For cell reconstructions, each entire neurobiotin-filled neuron was acquired at 63X (NA = 1.4), 1 μ m step size using
1212 a Zeiss LSM880 confocal microscope. Imaris 9.3 (Bitplane) was used to visualize the topology of the dendritic tree
1213 and the centrifugal branch ordering method was chosen to sort dendrites, assigning order 1 to the root. 2nd order
1214 dendrites were then selected for further imaging acquisition to perform spine quantification. 70-90 μ m-long dendritic
1215 segments were acquired at 63X (NA = 1.43), 0.1 μ m step size and 0.06x0.06 pixel-size using Airy-scan detector at
1216 the LSM880. Two segments were acquired for dendrites longer than 200 μ m.

1217 For spine quantification, images of dendritic segments were rendered in Imaris using the *Blend* algorithm and the
1218 *Filament* module was used to reconstruct dendrites and spines. Specifically, the auto-path method was chosen and
1219 thinnest spine diameter (between 1.5 and 2 μ m), maximal distance from the dendrite (between 3 and 8 μ m) and
1220 fluorescence intensity threshold were defined in every single dendrite to detect spines. The statistics module in
1221 Imaris was used to extract spine density values. Three to six segments per neuron were quantified and values were
1222 averaged.

1223 **Tail-tip whole blood sampling.** Whole blood samples of 40-70 μ L were collected from the lateral tail vein of
1224 restrained mice (142). Only blood samples acquired within 2 min post-restraining were used for hormone
1225 measurements, and the subjects were then returned to their home cage. Briefly, the rodent's tail was immersed for
1226 30 sec in 40°C water to dilate the tail blood vessels. Immediately after, a 23G needle was used to puncture the
1227 lateral tail vein, and whole blood was collected. Bleeding was stopped via applying gentle pressure to the tail at the
1228 level of the puncture with surgical cleaning tissue, and 2% chlorhexidine antiseptic solution was used for tail
1229 disinfection at the end of the procedure. Blood samples were refrigerated at 4°C for 30 min and then centrifuged at
1230 4°C at 2000 RCF. Following centrifugation, serum was collected and was frozen at -80°C for a maximal period of 2
1231 months prior to performing ELISA measurements. All blood samples were acquired during the dark phase of the
1232 12h/12h light/dark cycle. For further details on reagents, see also Table S1.

1233 **Testosterone ELISA.** 96-well plates were used in a ready-to-use kit for testosterone ELISA (R&D systems –
1234 Catalog number KGE010). Linear regression was used to fit the optical densities for the standard curve vs the
1235 concentration. The standard curve range for corticosterone was 300 to 100000 pg/mL. Concentrations were
1236 calculated from the optical density at 450 nm of each sample. Appropriate sample dilutions were carried out to
1237 maintain detection in the linear part of the standard curve and typically involved 1 to 10 for mouse serum samples.
1238 Data acquired from the performed ELISAs are presented as absolute values. Differences between groups were
1239 identified by Student's *t*-test or ANOVA.

1240 **Viral vectors.** For *ex vivo* Ca²⁺ imaging studies of VMHvl neurons, *Esr1Cre/+* male mice were injected in VMHvl
1241 with 200 nL of AAV9-Syn-FLEX-jGCaMP7s-WPRE (addgene 104491-AAV9) 5.3×10^{12} genomic copies per mL.
1242 For *ex vivo* optogenetic studies, *Esr1Cre/+* male mice were injected in VMHvl with 200 nL of AAV9-FLEX-tdTomato
1243 (addgene 28306-AAV9) 4.2×10^{12} genomic copies per mL and in AHiPM with 100 nL of AAV5-Syn-Chronos-GFP
1244 (addgene 59170-AAV5) 3.7×10^{12} genomic copies per mL. For *in vivo* optogenetic and electrophysiology
1245 experiments, *Esr1Cre/+* male mice were injected in VMHvl with 100 nL of AAV5-Syn-FLEX-rc[ChrimsonR-
1246 tdTomato] (addgene 62723-AAV5) 4.1×10^{12} genomic copies per mL and in AHiPM with 100 nL of AAV5-Syn-
1247 Chronos-GFP (addgene 59170-AAV5) 3.7×10^{12} genomic copies per mL. Control groups were injected in VMHvl
1248 with 100 nL of AAV9-FLEX-tdTomato (addgene 28306-AAV9) 4.2×10^{12} genomic copies per mL and in AHiPM with
1249 100 nL of AAV5-CAG-GFP (37825-AAV5) 5.9×10^{12} genomic copies per mL. For further details on reagents, see
1250 also Table S1.

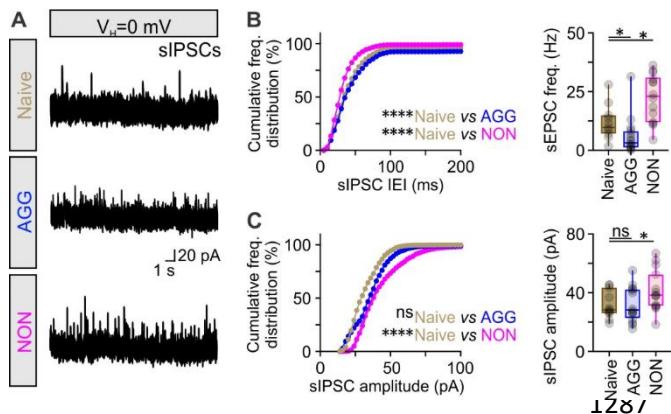
1251 **Stereotactic surgery and viral gene transfer.** Adult heterozygous *Esr1Cre/+* males were single-housed for at
1252 least five days before undergoing surgical procedures and were operated on at 16–20 weeks of age. Mice were
1253 anesthetized using isoflurane (5% induction, 1–2% maintenance, in 95% oxygen) and placed in a stereotaxic frame
1254 (David Kopf Instruments). Body temperature was maintained using a heating pad. An incision was made to expose
1255 the skull for stereotaxic alignment using the inferior cerebral vein and the Bregma as vertical references. We based
1256 the coordinates for the craniotomy and stereotaxic injection of VMHvl on an anatomical magnetic resonance atlas
1257 of the mouse brain (AP: -4.68 mm; ML: ± 0.78 mm; DV: -5.80 mm), as previously described (47). Virus suspension
1258 was injected using a pulled-glass capillary at a slow rate of 8–10 nL/min, 100 nl per injection site (Nanoject II,

1259 Drummond Scientific; Micro4 controller, World Precision Instruments). The glass capillary was withdrawn 10 min
1260 after the cessation of injection.

1261 **Osmotic mini-pumps.** Testosterone was dissolved at 30 mg/ml in sesame oil and was administered for 2 weeks
1262 at a rate of 0.75 mg/hour via subcutaneous osmotic mini-pumps (Alzet, model 1002) (143-145). For further details
1263 on reagents, see also Table S1.

1264 **Social behavior assays.** The aggression phenotype of animals defined as aggressive (AGG), or non-aggressive
1265 (NON) in the present study was based on the expression of aggressive behavior in the five consecutive day resident-
1266 intruder test (5cdRI). Animals that did not express any aggressive behavior in the 5cdRI were identified as NONs,
1267 while all AGGs expressed aggression in a minimum of three out of the five trials, with the majority expressing attack
1268 behavior in all five days. As described in Fig. 1, the 5cdRI composed of a 15 min social interaction test per day in
1269 the resident's home arena, with socially naïve 4-5 month-old residents. Intruders were BALB/c males 2-3 months
1270 old and of lower weight/size. Three follow up tests were performed in the 5cdRI experimental design presented in
1271 Fig. 1, specifically, 2 weeks, 4 weeks and 12 weeks following the completion date of the 5cdRI assay. Note that
1272 only 15 out of a total of 106 aggressive mice, were used to quantify the effect of aggression training. This was based
1273 on the finding that following behavioral analysis of the first 15 mice used in the study, the power of the ANOVA test
1274 reached $P < 0.0001$. This suggested that including additional observations would not aid the power of the statistical
1275 test. In Fig. 5, following the 5cdRI, on day six a social interaction test was performed in a novel home-cage-sized
1276 arena. In addition to the C57 male, a male with a larger bodyweight/size CD-1 conspecific was introduced. The
1277 duration of this experiment was 15 min, following which both animals were returned to their home cage.

1278 **Statistics.** No statistical methods were used to predetermine sample sizes but our sample sizes are similar to those
1279 reported in previous publications (35, 44, 47). Data met the assumptions of the statistical tests used and were tested
1280 for normality and variance. Normality was determined by D'Agostino–Pearson normality test. All *t*-tests and one-
1281 way ANOVAs were performed using GraphPad Prism software (Graphpad Software Inc.). Statistical significance
1282 was set at $P < 0.05$.



1288 **Fig. S1. Presynaptic plasticity of inhibitory input in VMHvl^{Esr1} neurons of non-aggressive male**
1289 **mice.**

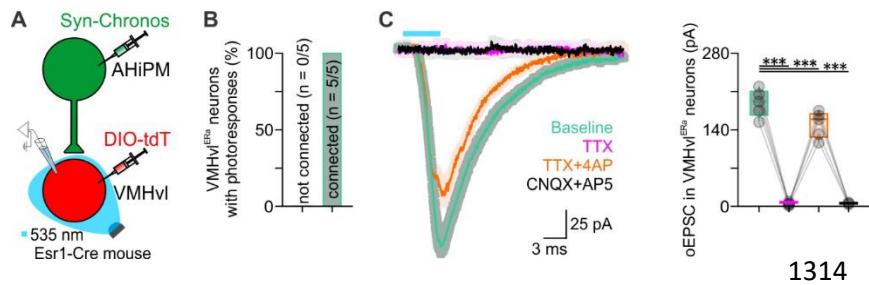
1290 (A) Representative recordings of spontaneous inhibitory post-synaptic currents (sIPSCs) from VMHvl^{Esr1}
1291 neurons, from socially naive, aggressive (AGG) and non-aggressive (NON) mice.

1292 (B) Left – cumulative frequency distribution plot of sIPSC inter-event interval (IEI) in voltage-clamp
1293 recordings collected from VMHvl^{Esr1} neurons from socially naive, AGG and NON mice (n=11-14 VMHvl^{Esr1}
1294 neuron recording per group, collected from 8-10 mice per group, Kolmogorov-Smirnov test, $P < 0.0001$
1295 between socially naive and AGG mice, $P < 0.0001$ between socially naive and NON mice). Right –
1296 comparison of sIPSC frequency in voltage-clamp recordings collected from VMHvl^{Esr1} neurons from
1297 socially naive, AGG and NON mice (n=11-14 VMHvl^{Esr1} neuron recording per group, collected from 8-10
1298 mice per group, Kruskal-Wallis one-way ANOVA with uncorrected Dunn's post hoc test, $P = 0.0425$
1299 between socially naive and AGG mice, $P = 0.0480$ between socially naive and NON mice).

1300 (C) Left – cumulative frequency distribution plot of sIPSC amplitude in voltage-clamp recordings
1301 collected from VMHvl^{Esr1} neurons from socially naive, AGG and NON mice (n=11-14 VMHvl^{Esr1} neuron
1302 recording per group, collected from 8-10 mice per group, Kolmogorov-Smirnov test, $P = 0.2780$ between
1303 socially naive and AGG mice, $P < 0.0001$ between socially naive and NON mice). Right – comparison of
1304 sIPSC amplitude in voltage-clamp recordings collected from VMHvl^{Esr1} neurons from socially naive, AGG

1305 and NON mice (n=11-14 VMHvl^{Esr1} neuron recording per group, collected from 8-10 mice per group,
1306 Kruskal-Wallis one-way ANOVA with uncorrected Dunn's post hoc test, $P = 0.8995$ between socially
1307 naive and AGG mice, $P = 0.0476$ between socially naive and NON mice).

1308 ns; not significant, * $P < 0.05$, **** $P < 0.0001$. In box-and-whisker plots, center lines indicate medians, box
1309 edges represent the interquartile range, and whiskers extend to the minimal and maximal values.



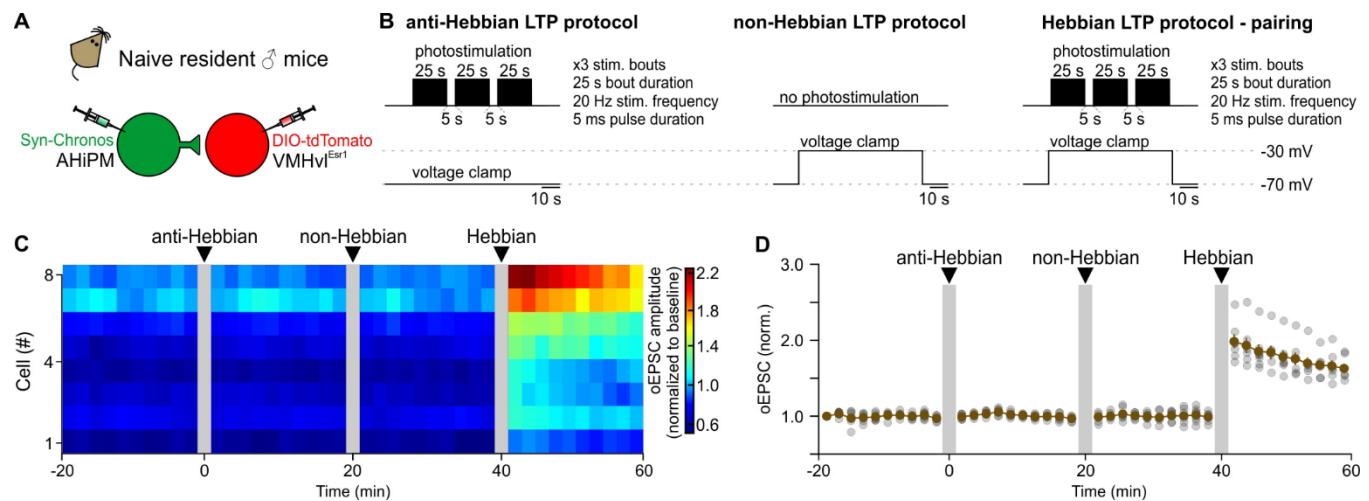
1315 **Fig. S2. Monosynaptic connectivity between AHiPM and VMHvl^{Esr1} neurons.**

1316 (A) Schematic illustration of the experimental design, transducing AHiPM neurons with Chronos and
1317 optically evoking postsynaptic responses in VMHvl^{Esr1} neurons *ex vivo*.

1318 (B) Quantification of VMHvl^{Esr1} neurons with optically-evoked EPSCs (oEPSCs).

1319 (C) Averaged amplitudes of oEPSCs evoked on baseline (green), TTX (magenta), TTX + 4AP (orange),
1320 and in CNQX and AP5 (black); n=5 brain slices, collected from n=5 mice, one-way ANOVA with Dunnett's
1321 post hoc test, $P = 0.0002$ between baseline and TTX conditions, $P = 0.0001$ between baseline and
1322 TTX+4AP conditions, $P = 0.0002$ between baseline and CNQX+AP5 conditions. Shaded region
1323 represents the standard error. The vertical scale bar defines current and the horizontal scale bar time.

1324 *** $P < 0.001$. In box-and-whisker plots, center lines indicate medians, box edges represent the
1325 interquartile range, and whiskers extend to the minimal and maximal values.



1326

1327 **Fig. S3. Characterization of LTP-inducing stimulation protocols at the AHiPM→VMHvl^{Esr1} synapse.**

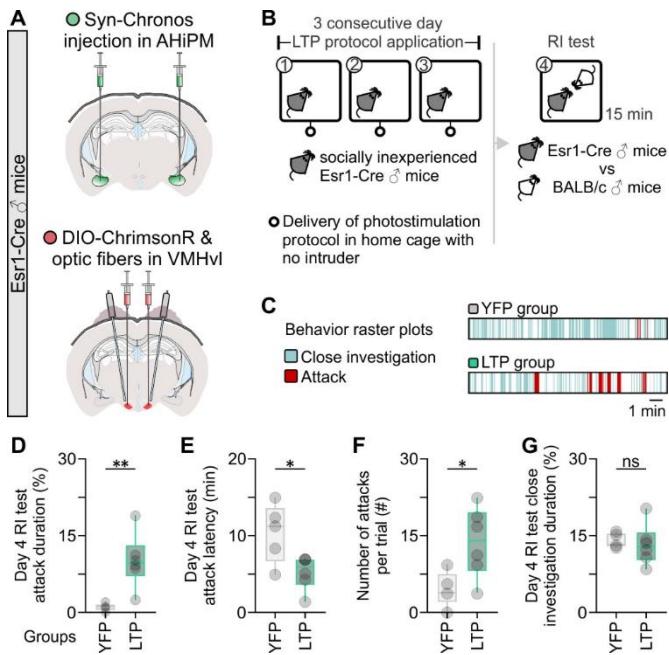
1328 (A) Schematic of the experimental design used to identify the appropriate stimulation protocol for LTP
1329 induction *ex vivo* in socially naïve mice.

1330 (B) Illustration of the experimental protocols tested to induce LTP in the AHiPM→VMHvl synapse.

1331 (C) Monitoring the optically induced EPSC (oEPSC) prior to, and following application of each of three
1332 stimulation protocols (n=8 cells, n=5 socially naïve mice).

1333 (D) Alternative quantification/illustration of optically induced EPSC (oEPSC) prior to, and following
1334 application of each of three stimulation protocols (n=8 cells, n=5 socially naïve mice – similar to panel C).

1335



1343

1344 **Fig. S4. Optogenetic induction of LTP at AHiPM→VMHvl^{Esr1} synapses in socially naïve mice leads**
1345 **to elevated aggression in the first resident-intruder test.**

1346 (A) Schematic indicative of the experimental design used to induced hypothalamic LTP in the
1347 AHiPM→VMHvl synapses, via Chronos-eYFP expression in AHiPM, and ChrimsonR expression in
1348 VMHvl^{Esr1} neurons.

1349 (B) Schematic of the behavior test design used to identify whether induction of LTP in the AHiPM→VMHvl
1350 synapses, influences the innate expression of aggression.

1351 (C) Representative behavior raster plots of a control (YFP) and opsin-expressing (LTP) mouse, in the
1352 resident-intruder test against a novel BALBc conspecific.

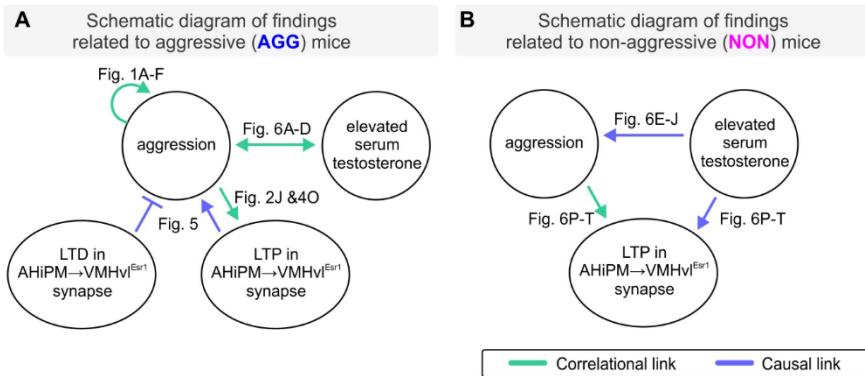
1353 (D) Quantification of attack duration (n=5-6 mice per group, two-tailed unpaired *t*-test, *P* = 0.0046 between
1354 YFP and LTP groups).

1355 (E) Quantification of attack latency (n=5-6 mice per group, two-tailed unpaired *t*-test, $P = 0.0214$ between
1356 YFP and LTP groups).

1357 (F) Quantification of number of attacks per trial (n=5-6 mice per group, two-tailed unpaired *t*-test, $P =$
1358 0.0235 between YFP and LTP groups).

1359 (G) Quantification of close investigation duration (n=5-6 mice per group, two-tailed unpaired *t*-test, $P =$
1360 0.7106 between YFP and LTP groups).

1361 * $P < 0.05$, ** $P < 0.01$. In box-and-whisker plots, center lines indicate medians, box edges represent the
1362 interquartile range, and whiskers extend to the minimal and maximal values.



1368

1369 **Fig. S5. Schematic summary.**

1370 (A) The schematic summarizes the findings from AGG mice, and the suggested links between
1371 aggression, serum testosterone and hypothalamic LTP.

1372 (B) Similar to panel (A), but summarizing results from experiments in NON mice - this schematic
1373 summarizes the identified links among aggression, serum testosterone and hypothalamic LTP. Our
1374 results do not distinguish whether the effect of elevated serum testosterone to increase LTP *in vivo* (Fig.
1375 6P-T) is direct, or rather indirect via an effect to increase aggressive behavior, which in turn enhances
1376 LTP. However, exogenous administration of T to NON mice (in the absence of any aggressive
1377 experience) enhances LTP amplitude and persistence as tested *ex vivo* (Fig. 6K-O).

1378

Materials and Methods

1379

Table S1. Reagents and resources.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit monoclonal anti-DsRed	Takara	632392
Anti-GFP rabbit serum	Invitrogen	A-6455
Chicken polyclonal anti-GFP	Aves Labs, Inc.	GFP-1010
Donkey anti-Mouse IgG- Alexa Fluor 488	ThermoFisher	A-21202
Donkey anti-Rabbit IgG- Alexa Fluor 488	ThermoFisher	A-21206
Donkey anti-Rabbit IgG- Alexa Fluor 568	ThermoFisher	A-10042
Donkey anti-Rabbit IgG- Alexa Fluor 647	ThermoFisher	A-31573
Goat anti-Chicken IgY- Alexa Fluor 488	ThermoFisher	A-11039
Biotinylated Goat Anti-Rabbit IgG Antibody	Vector Laboratories	BA-1000
Donkey anti-Rabbit IgG- Alexa Fluor 568	ThermoFisher	A-10042
Chemicals, Peptides, and Recombinant Proteins		
Picric acid	Sigma-Aldrich	P6744
4% paraformaldehyde (PFA) in PBS	Santa Cruz Biotech.	CAS30525-89-4
Streptavidin conjugated to Alexa Fluor 647	ThermoFisher	CS32357
Neurobiotin tracer	VectorLabs	SP-1120-50
Sodium chloride	Sigma-Aldrich	S9888
Sodium bicarbonate	Sigma-Aldrich	S6297
D-(+)-Glucose	Sigma-Aldrich	G7528
Sodium phosphate monobasic dihydrate	Sigma-Aldrich	71505
Potassium chloride	Sigma-Aldrich	P9333
Magnesium sulfate heptahydrate	Sigma-Aldrich	63138
Calcium chloride dihydrate	Sigma-Aldrich	C5080
4-Aminopyridine	Sigma-Aldrich	275875
CNQX disodium salt	TOCRIS	1045
D-AP5	TOCRIS	0106
Tetrodotoxin citrate	Alomone labs	T-550
DAPI solution (1mg/mL)	ThermoFisher	62248
OCT Cryomount	Histolab	45830
Normal donkey serum (NDS)	Sigma-Aldrich	D9663
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A2153
Triton X-100	Sigma-Aldrich	T8787
Sucrose	Sigma-Aldrich	S7903
DiD' solid	Invitrogen	D7757
Vectastain ABC kit	Vector Laboratories	PK-6100
3,3-Diaminobenzidine tetrahydrochloride hydrate (DAB)	Sigma-Aldrich	D5637
Testosterone	Sigma-Aldrich	T1500
Sesame oil	Sigma-Aldrich	S3547-250ML
Phosphate buffer saline (PBS)	Santa Cruz Biotech.	SC-24946

ELISA kit		
Testosterone Parameter Assay Kit	R&D systems	KGE010
Experimental Models: Organisms/Strains		
<i>Esr1</i> ^{Cre/4}	Lee et al., 2014	Own breeding
BALB/cAnNCr mouse line	Charles River	https://www.criver.com/
Crl:CD1(ICR)	Charles River	https://www.criver.com/
AAV mediated gene transfer		
AAV5-CAG-GFP	addgene	37825-AAV5
AAV9-FLEX-tdTomato	addgene	28306-AAV9
AAV5-Syn-Chronos-GFP	addgene	59170-AAV5
AAV5-Syn-FLEX-rc[ChrimsonR-tdTomato]	addgene	62723-AAV5
AAV9-Syn-FLEX-jGCaMP7s-WPRE	addgene	104491-AAV9
Software		
Clampfit 11	MOLECULAR DEVICES	https://www.moleculardevice.com/
MATLAB 2018	MathWorks	https://www.mathworks.com/
OriginPro 9	OriginLab	https://www.originlab.com/
ImageJ	NIH; Schneider et al., 2012	https://imagej.nih.gov/ij/
Prism 8	GraphPad	https://www.graphpad.com/scientific-software/prism/
Illustrator CC 2020	Adobe Systems	http://www.adobe.com
CorelDrawX8	CorelDRAW graphics suite	https://www.coreldraw.com/
Photoshop 2020	Adobe Systems	http://www.adobe.com

1380

1381