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1 **The impact of C-Tactile Low threshold mechanoreceptors on affective touch and social**
2 **interactions in mice.**

3

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12

13 **Abstract**

14 **Affective touch is necessary for proper neurodevelopment and sociability. However, it**
15 **is still unclear how the neurons innervating the skin detect affective and social**
16 **behaviours. To clarify this matter, we targeted a specific population of somatosensory**
17 **neurons in mice, named C-low threshold mechanoreceptors (C-LTMRs), that appears**
18 **particularly well suited physiologically and anatomically to perceive affective and social**
19 **touch but whose contribution to these processes has not yet been resolved. Our**
20 **observations revealed that C-LTMRs functional deficiency from birth induced social**
21 **isolation and reduced tactile interactions in adults. Conversely, transient increase in C-**
22 **LTMRs excitability in adults using chemogenetics was rewarding, temporally promoted**
23 **touch seeking behaviours and thus had pro-social effects on group dynamics. This**
24 **work provides the first empirical evidence that specific peripheral inputs alone can drive**
25 **complex social behaviour, demonstrating the existence of a specialised neuronal circuit**
26 **originating from the skin wired to promote interaction with other individuals.**

27

28 **Introduction**

29 The rewarding value that emerges from touch is essential for decision making and motivation,
30 especially in social animals, and dysregulation of this process leads to debilitating psychiatric
31 or neurologic conditions, including autism, anxiety or depression ¹⁻³. Nonetheless, the neural
32 mechanisms underlying emotional tactile sensory processing relationship with social behaviour
33 are at the early stage of their understanding.

34 The skin is innervated by an array of functionally distinct populations of receptors contributing
35 to touch, which can be distinguished by their response properties, activation threshold,
36 conduction velocity, and the type of end organ that they innervate ⁴.

37 One class of these receptors, the C-Tactile fibers (CT), are particularly well responsive to tactile
38 stimulation categorized as “pleasant” and “affective”, by human subjects ^{5,6}. These neurons
39 are unmyelinated low-threshold mechanoreceptors (LTMRs) and respond to non-noxious
40 touch with a predilection for slow-moving and low-force, stroking stimuli, such as gentle
41 brushing ⁶⁻⁸. Activation of CTs in humans provides poor conscious spatial and qualitative
42 information to the subjects, who nonetheless still carry a positive feeling related to the gentle
43 brushing of the skin ⁹. Furthermore, the positively valent tactile information conveyed by CTs
44 make them particularly well suited to link tactile information to social bonding ^{10,11}.

45 Evidence for pleasant touch contribution to sociability and related disorders was also found in
46 laboratory animal models. For example, playful and pleasant touch is rewarding and
47 contributes greatly to social development in adolescent rats ¹². In mice, two recent studies
48 linked the disruption of LTMR functions with alterations of social behaviour typical of autism
49 spectrum disorder (ASD) ^{13,14}. Specifically, these studies used genetically modified mice
50 recapitulating mutations found in human patients within the *Mecp2*, *Shank3B*, and *Fmr1* genes.
51 Peripherally restricted conditional Knock-Out (KO) of these genes greatly altered Low-
52 threshold-mechanoreceptors (LTMRs) function which induced multiple phenotypes associated
53 with ASD, especially social deficits that are usually observed in constitutive KOs of these
54 genes. However, LTMR sensory neurons are known to be a highly heterogeneous population,
55 leaving open the question regarding the contribution of CT and pleasant touch to these social
56 deficits.

57 We and others genetically identified a population of primary sensory neurons in mice, named
58 C-Low-threshold-mechanoreceptors (C-LTMRs), with similar functional properties than CTs.
59 These studies characterized genes specific to C-LTMRs in rodents such as TAF4, but also a
60 set of genes allowing to differentiate C-LTMRs from other sensory neurons, such as tyrosine
61 hydroxylase (TH), VGlut3, TAF4 or Ca_v3.2, that may be used to gain genetic access to these
62 neurons ¹⁵⁻²⁰. These studies unveiled the contribution of C-LTMRs, and these genes, to pain
63 chronification in the context of neuropathic or inflammatory pathological pain. However, none
64 of these studies considered the role of C-LTMRs in touch sensation, within naturalistic
65 conditions and especially their role in social behaviours.

66 In the present study, we explored the role of C-LTMRs in social interactions. Using two mouse
67 transgenic models to decrease or facilitate C-LTMRs excitability, and a new tracking system

68 to automatically annotate social behaviour in groups of 4 animals, we clarified the specific
69 function of C-LTMRs in rodent inter-individual relations.

70 **Results**

71 **Social behaviours are impaired in Cav3.2^{Nav1.8}cKO**

72 First, we aimed at investigating the consequence of C-LTMRs hypofunction on social
73 behaviour. For that purpose, we used a genetic mouse model where the expression of the low
74 threshold calcium channel Ca_v3.2 is conditionally knocked out in C-LTMRs, by crossing Ca_v3.2
75 ^{GFP-flox}KI with Na_v1.8^{cre} mice as we previously described ¹⁶. In this mouse model
76 (Cav3.2^{Nav1.8}cKO), C-LTMR impaired function starts just before birth. Indeed, removing Ca_v3.2
77 expression from C-LTMRs increases the firing threshold of action potentials, reduces the firing
78 frequency and lowers the conduction velocity of these fibres transforming their mechanical
79 sensitivity into High Threshold Mechanoreceptors (HTMR) phenotype ¹⁶.

80 To evaluate the consequences of C-LTMRs deficiency on social preference behaviour, we
81 used the three-chamber paradigm (ie. Crawley test). Interestingly, when compared to control
82 Ca_v3.2^{GFP-flox}KI littermates, male Cav3.2^{Nav1.8}cKO mice spent less time interacting with an
83 unfamiliar mouse than with an unanimated object, which is summarized by the reduction in the
84 preference index (**Figure 1a, Supplementary Fig. 1a**). To further investigate the precise
85 quality of the social and tactile interactions that appears to be impaired in this model we used
86 a novel paradigm where mice could interact freely with each other. The live mouse tracker
87 (LMT), based on a machine learning analysis framework, was designed by de Chaumont and
88 colleagues for that specific purpose ²¹. This system allows the tracking and automatic
89 annotation of mice behaviours and social interactions in their environment for multiple days ²¹.
90 Using this system, we analysed the behaviour of 5 groups of 4 male mice (each group
91 composed of 2 controls Ca_v3.2^{GFP-flox}KI, and 2 C-LTMRs-impaired Cav3.2^{Nav1.8}cKO, all
92 littermates) for three consecutive nights (**Figure 1b**). For every time frame, the LMT detects
93 head, tail, ears, eyes and nose position, producing a geometrical mask for each mouse. These
94 data permit the computation of different behavioural events based on mice geometries
95 movements and localization compared to other mice. Overall, we detected 28 events that were
96 separated into seven categories: body configuration, isolated behaviour, position in contact,
97 type of contact, social configuration, social approach and social escape. These events were
98 analysed separately for both their number (**Figure 1c**) and their duration (**Figure 1d**) for each
99 individual. To compare C-LTMR-impaired mice and their control cagemates with the same
100 baseline and to reduce inter-experiment variability, the value of each behavioural trait for one
101 Cav3.2^{Nav1.8}cKO mouse was compared with the mean level of this trait from the two control
102 cagemates (called LMT index thereafter). An LMT index value above one indicates that the

103 Cav3.2^{Nav1.8}cKO mice perform more occurrences of a specific trait compared to controls
104 whereas a value below one means that the mutant mice do less of that trait than controls.

105 The LMT-index indicates that Cav3.2^{Nav1.8}cKO mice spent more time isolated than controls
106 (time *stop alone*: +18.2%±7), **Figure 1d**), without any noticeable differences in locomotor
107 activity (**Supplementary Fig. 1b**) or exploratory behaviour (stretch attending posture: (*SAP*)
108 **Figure 1e&d**). In social events, C-LTMR impaired mice showed a small, but statistically
109 significant, decrease in time spent engaged in all type of contacts (-15.7%±3.2 in average per
110 interaction bout, **Supplementary Fig. 1c**). Moreover, the duration of reciprocal (here
111 associated with nose-to-nose contacts and side-by-side contacts) and unilateral social
112 interactions (associated in these experiments with giving ano-genital contacts) were reduced,
113 even if the number of events is not statistically different (**Figure 1e&1f**). However passive
114 social interaction (associated in our experiment with receiving ano-genital contacts) were not
115 altered (**Supplementary Fig. 1d**). Cav3.2^{Nav1.8}cKO mice also displayed shorter social
116 approaches leading to a contact (*social approach*: -10.9%±1.9 and *make contact*: -7.3%±2.8,
117 **Figure 1d**) and spent less time in groups of three mice (-9.27%±1.9) (**Figure 1d**). However,
118 no difference was observed in the social escape behaviours category. Taking together, the
119 results from the 3-chamber test and the LMT revealed a deficit in sociability of Cav3.2^{Nav1.8}cKO
120 mice compared to their control littermates.

121 **A new viral strategy to specifically target C-LTMRs in mice**

122 Next, we deepened our investigations on C-LTMR role in sociability by designing a
123 chemogenetic strategy to selectively excite C-LTMRs remotely in adult mice independently of
124 Cav3.2 protein function and, importantly, without any postnatal functional perturbation of C-
125 LTMR. Our strategy to target C-LTMRs in adult mice, illustrated in **Figure 2a and b**, consists
126 on expressing a gene of interest under the control of the mini-Cav3.2 promoter in a Cre-
127 dependent manner. Indeed, C-LTMRs can be defined by the expression of both the sodium
128 channel Nav1.8 and the calcium channel Cav3.2^{16,22}. We previously engineered adeno
129 associated viruses (AAV) with a mini-Cav3.2 sequence and validated its faithful expression in
130 Cav3.2 positive neurons within the dorsal horn of the spinal cord²³. Here we used AAV-PHP-
131 S serotype that has a high tropism for peripheral sensory neurons²⁴, which we delivered into
132 Nav1.8^{Cre} heterozygote mice. This intersectional strategy combining the expression pattern of
133 Cav3.2 and Nav1.8 aims at restricting the expression of the viral payload into C-LTMRs. To
134 achieve C-LTMRs chemogenetic stimulation with this strategy, we inserted within the pAAV
135 vector the HA tagged-hM3Dq excitatory DREADD cassette.

136 HEK293T cells were transfected with the pAAV vector (pAAV-pCav3.2-FLEEx-HA-hM3Dq) to
137 validate its Cre-dependency and functionality. As the Cav3.2 promoter activity is highly

138 enhanced by the EGR1 transcription factor ²⁵, we co-expressed the murine EGR1 cDNA and
139 added or not the Cre recombinase (Cre-GFP fusion). We analysed the DREADD functionality
140 using Calcium fluorimetry in Fura2 loaded Cre-GFP positive cells. In a representative field, out
141 of 102 cells recorded, 28 were GFP positive and of those 17 responded to the hM3Dq
142 pharmacological actuator Clozapine-N-oxide (CNO) (**Supplementary Fig. 2a & b**). No
143 responses to CNO were observed in non-GFP cells.

144 We then packaged viral particles with this validated construct using the AAV-PHP-S capsid.
145 Although AAV PHP-S can be delivered systematically to access sensory neurons, we locally
146 injected the virus intrathecally with minimal invasiveness and a sole access to DRGs and no
147 other sensory neurons such as those of the vagal ganglia (**Figure 2b**)²⁴. Consistent with an
148 efficient C-LTMR targeting strategy, intrathecal injection of this viral construct leads to the
149 expression of the excitatory DREADD receptor HA-hM3Dq in C-LTMRs labelled by the tyrosine
150 hydroxylase (TH), 8 weeks post injection (**Figure 2c**). Overall, 71.2%±2.4 of HA-hM3Dq cells
151 were also positive to TH (N=5 mice). Inversely, HA-hM3Dq is present in 25.9%±2.6 of TH
152 positive dorsal root ganglia (DRG, T7 to L6) neurons (**Figure 2c**). In addition, no observation
153 was made of any specific HA immunostaining in the dorsal horn of the spinal cord
154 (**Supplementary Fig. 2c**)

155 To further confirm that hM3Dq was selectively expressed in C-LTMRs accordingly to the
156 defined strategy and kept its pro-excitatory nature, we evaluated the effect of CNO on cultured
157 DRG neurons from animal injected intrathecally with the rAAV_{PHPs}-pCa_v3.2-FLEX-HA-hM3Dq
158 cells were loaded with the Fura2 radiometric calcium indicator, and labelled with red-dye
159 conjugated IB4 to access the pharmacological and functional properties of large population of
160 neurons at the same time. Application of CNO (30µM) induced intracellular calcium increase
161 in neurons also responding to the TRPA1 agonist, allyl isothiocyanate (AITC, 200µM), and to
162 the P2Y1R agonist, MRS2365 (200nM) (**Figure 2d and 2e**). Of all CNO responsive neurons,
163 the large majority responded to AITC and MRS (15), 3 also responded to Capsaicin on top of
164 AITC and MRS, and only one was not responding to anything else. Among the MRS
165 responders, 56.2% were also responding to CNO while among AITC responders only 26.4%
166 were CNO responders. In mouse DRGs, TRPA1 is weakly expressed in C-LTMRs and P2Y1R
167 receptor is only expressed in C-LTMRs and TrkB positive Aδ-LTMRs ^{18,20}. As TRPA1 is not
168 express in TrkB neurons, responses to both MRS and AITC can only be observed in C-LTMRs.
169 Moreover, none of the CNO responsive neurons were labelled by IB4 (**Figure 2f**), in agreement
170 with the lack of reactivity of the lectin in mouse TH positive C-LTMR neurons ²⁶. Taken together,
171 our morphological and functional data provide supporting evidence toward a specific
172 expression of HA-hM3Dq in a large population of C-LTMRs (10.7% of all DRG neurons

173 recorded, N= 3 mice). Accordingly, we used this experimental approach in vivo to investigate
174 the impact of C-LTMRs stimulation in social behaviours.

175 **Effect of exogenous C-LTMR activation on somatosensory perception**

176 First, we assessed the consequences of C-LTMRs exogenous activation on somatosensory
177 perception. Eight weeks after intrathecal injections of rAAV_{PHPS}-pCa_v3.2-FLEX-HA-hM3Dq
178 (named further: C-LMTRs^{hM3Dq}) or rAAV_{PHPS}-CAG-mCherry (Control) in Na_v1.8^{cre} mice, CNO
179 was administrated intraperitoneally (1mg/kg, IP) to all animals. 30 to 45 minutes after, a
180 decrease in reflex paw withdrawal frequency to low force von Frey stimulation (0.07g) was
181 observed, but not for higher forces (0.6g and 2g) or brushing in C-LMTRs^{hM3Dq} mice when
182 compared to control mice (**Supplementary Fig. 3a**). To note, CNO injection did not trigger any
183 alteration of motor activity or spontaneous nocifensive behaviours, such as paw shaking,
184 guarding, grooming, licking or jumping compared to the control group.

185 As C-LTMRs have been implicated into temperature perception^{15,16,27}, we probed thermal
186 sensitivity of control and C-LMTRs^{hM3Dq} mice in the thermal gradient test. 30 minutes after CNO
187 injection, mice were placed into a 1.5m corridor with the floor at one extremity cooled down to
188 5°C and the other heated up to 50°C, creating a gradient of temperature between the two
189 extremities. Once placed in the corridor, mice were allowed to explore the thermal gradient to
190 reveal their thermotaxis behaviour. The exploration was tracked during 90 minutes and the
191 animal's position was annotated according to the temperature zones they visited (**Figure 3a**).
192 When compared to control animals, CNO treated C-LMTRs^{hM3Dq} mice settled more quickly at
193 the comfort temperature of 30°C (**Figure 3c and 3d**) and spend more time overall at this
194 temperature (**Figure 3b and 3f**), without affecting locomotor (**Supplementary Fig. 3b and 3c**)
195 nor the preferred temperature that was similar between the two groups (**Supplementary Fig.**
196 **3d**). This interesting result suggests that exogenous activation of C-LTMRs can reinforce
197 motivational behaviour towards a pleasurable somatosensory stimulation.

198 **Positive valent information is associated with C-LTMRs stimulation**

199 As activation of C-LTMRs may results in positive feeling, next we investigated whether C-
200 LTMRs activation could be rewarding on its own by using the conditioned place preference
201 paradigm (CPP). Following one day of habituation to the CPP arena, control and C-LMTRs^{hM3Dq}
202 mice were conditioned to receive saline injection (CNO vehicle) in the compartment they
203 preferred during habituation and CNO (1mg/kg in saline) in the other compartment, for 3
204 consecutive days (**Figure 3g**). On the last day, mice were free to explore the entire arena and
205 their position was video tracked. While control animals did not develop a preference for the
206 side in which they either received saline or CNO injections, C-LMTRs^{hM3Dq} mice showed a
207 marked preference for the compartment associated with CNO injections (32%±1.4 increase,

208 **Figure 3h and 3i).** Overall, these two experiments suggest that activation of C-LTMRs is
209 rewarding and can increase the rewarding value of other sensory modalities.

210 **C-LTMR stimulation induces touch seeking and pro-social behaviours**

211 Taking into consideration the intrinsic emotional value conveyed by C-LTMRs activation, we
212 finally investigated whether C-LTMR activation can affect social behaviours and social group
213 organization. The LMT system was again used to analyse the behaviour of 5 groups of 4 mice
214 independently during 3 nights. Each group was composed of 2 Na_v1.8^{cre} mice injected with
215 rAAV_{PHPs}-CAG-mCherry (Control) and 2 mice injected with rAAV_{PHPs}-pCa_v3.2-FLEX-HA-
216 hM3Dq (C-LTMRs^{hM3Dq}), all male littermates. The first 24 hours were used as a habituation
217 phase, and just before the second dark cycle, we injected either a saline (CNO vehicle) or
218 CNO solution (IP, 1mg/kg in saline) to all animals (Control and C-LTMRs^{hM3Dq}). At the
219 beginning of the third dark cycle, groups that received a saline solution for the second dark
220 cycle received CNO and *vice versa* (**Figure 4a**). As for Cav3.2^{Nav1.8}cKO mice, we calculated
221 the LMT-index by taking the value of each trait for one C-LMTRs^{hM3Dq} mouse and compared it
222 with the mean level of that trait for the two control cagemates (**Figure 4 f to i**). The LMT-index
223 was calculated from cumulated value for both saline and CNO injection during the first half of
224 the dark cycle, at 30 minutes, 60 minutes, 90 minutes, 150 minutes (2.5 hours), 210 minutes
225 (3.5 hours), 270 minutes (4.5 hours), 330 minutes (5.5 hours), and 390 minutes (6.5 hours)
226 post injection. In addition, the LMT-index was also calculated at 1200 minutes during the
227 following light cycle (20 hours) post injection. To highlight the behaviours that were
228 exacerbated or inhibited by activation of the C-LTMRs following CNO injection, we then
229 computed another ratio for each behaviour by subtracting the LMT-index^{CNO} to LMT-index^{saline}.
230 The p-value of these ratios was then calculated for each different time points and represented
231 as a two-colour gradient heatmap (**Figure 4b, c d and e**).

232 The index ratios indicate that, overall, CNO but not saline injection, significantly reduced the
233 number of isolated events while it increased inter-individual events in C-LMTRs^{hM3Dq} mice and
234 not in controls 1 hour post-injection (*Move alone*: -122%±2.1, 60 minutes post CNO injection;
235 *stop alone*: -57%±1.2, 60 minutes post CNO injection; *move in contact*: +24%±0.7, 60 minutes
236 post CNO injection; *stop in contact*: +30%±0.7, 60 minutes post CNO injection; **Figure 4b, c,**
237 **d, e, h and i; Supplementary Fig. 4 d and e**). It is noteworthy that a single injection of CNO
238 was sufficient to significantly decrease the number of events corresponding to isolated
239 movement up to 6.5 hours following the injection (**Figure 4b and i**).

240 C-LMTRs^{hM3Dq} mice also appeared to be engaging more and, for longer periods, in all the
241 different types of contact post CNO injection. We also observed that some behavioural traits
242 were transiently altered, lasting for up to 30 to 90 minutes, while other were more durable (up

243 to 6.5 hours post injection, a time point largely exciding the CNO clearance ²⁸). Specifically,
244 behavioural traits associated with the time spent on social exploration were significantly
245 increased immediately after CNO injection and lasted up to 6 hours (duration of contact *nose-*
246 *anogenital*: +20.4%±0.7 at 60 minutes post CNO, number of *group of three*: +22% at 60
247 minutes post CNO; **Figure 4d, e and g**), whereas other behaviours were only significantly
248 increased for the first 60 to 90 minutes post CNO injection (duration and number of *contact*
249 *nose-nose*: +25%±0.4 and +20%±0.3 respectively 60 minutes post CNO injection, contact
250 *side-by-side*: +22.2%±0.8 90 minutes post CNO injection, and contact *side-by-side opposite*:
251 +26%±0.9 90 minutes post CNO injection; **Figure 4d, e and f**; **Supplementary Fig. 4a, b and**
252 **c**).

253 In conclusion activation of C-LTMRs transiently increases all kind of social interaction between
254 animals to the expense of isolated behaviour for up to 90 minutes post CNO injections,
255 including behaviour related to skin to skin contacts and social exploration. The impact of such
256 behavioural alteration appears to impact group dynamics for longer periods, especially groups
257 of 3 mice. Remarkably, some behavioural traits were still significantly increased up to 20 hours
258 after CNO injection, such as the duration of nose-to-nose interaction, social approach and
259 stops in contact (**Figure 4e**) as well as the number of social approach and social escape
260 (**Figure 4d**). In contrast, all behavioural traits in C-LMTRs^{hM3Dq} mice came back to control mice
261 level after 20 hours post injection.

262 Next, the relationship between each mouse and the group dynamics was analysed. First, we
263 focused on group of two mice and, in our condition, one mouse of a given genotype had a
264 probability of 1/3 to interact with a mouse from the same genotype, and 2/3 to interact with a
265 mouse from the other genotype (**Figure 5a**). Remarkably, after CNO but not saline injection,
266 C-LMTRs^{hM3Dq} mice interacted more with each other than expected by chance level
267 (+11.7%±0.2 compared to saline), while Control mice interacted less with each other (-
268 9.8%±0.2 compared to saline) (**Figure 5b and c**). In addition, Control mice had a higher
269 probability of contacting C-LMTRs^{hM3Dq} mice than expected (+9.7%±0.3 compared to saline).
270 This effect was visible for 30- and 90-minutes post CNO injection, depending on the couple
271 formed. These observations are only valid when all types of contacts are analysed as a whole
272 but not for individual behaviours related to social exploration where only C-LTMRs^{hM3Dq} mice
273 had a higher probability of interacting with each other (**Supplementary Fig. 5**). The mean
274 duration of the time spent in a group of 2 was however similar. Next, we focused our
275 investigation of the dynamic of groups of three mice. While looking at the combination of mouse
276 making or breaking groups-of-three, we did not observe any differences after CNO injection
277 compared to saline (**Figure 5d and f**), and this held true at all time point. However, it appears
278 that the groups of three mice formed by a C-LMTRs^{hM3Dq} mouse lasted longer than those

279 created by a Control mouse, especially if the C-LMTRs^{hm3Dq} mouse joined two other animals
280 to create a mix group (one C-LMTRs^{hm3Dq} mouse and two Controls) (**Figure 5e₂**). This effect
281 was particularly striking 1 hour after CNO injection, but decreased after 90 minutes and was
282 completely abolished after 390 minutes (**Figure 5e₃**).

283 **Discussion.**

284 Understanding how touch shapes social interactions, while keeping a level of ethological
285 validity is particularly challenging. In this study we overcame this challenge by combining a
286 unique genetic strategy and new tracking technologies to characterize the contribution of C-
287 LTMRs to affective and social touch in mice. By reducing, just before birth, the activity of this
288 specific population of primary sensory neurons defined genetically and physiologically, we
289 observed in adults a reduction of contacts with other mice, leading to an increase in isolated
290 behaviours. Conversely, exacerbation of C-LTMR excitability with a chemogenetic approach
291 in adults, led to an increase in social interaction, reduced isolated behaviour, and changed
292 groups social dynamics. We present evidence that C-LTMRs may be one of the main
293 contributors to social development and a potential target of treatment for neurodevelopmental
294 disorders.

295 *C-LTMRs, Ca_v3.2 and sociability*

296 Even if the C-LTMR hypofunction and the C-LTMRs remote activation share a similar genetic
297 strategy, the behavioural phenotypes observed in adults may result from totally different
298 mechanisms. C-LTMRs inhibition using Ca_v3.2 cKO^{Nav1.8} mice is a conditional knock-out where
299 pro-excitatory calcium channel Ca_v3.2 expression is removed in C-LTMRs as soon as Nav1.8
300 promoter and the Cre recombinase start to be active, around E16-E17^{29,30}. Thus, this “late”
301 DRG Cav3.2 conditional KO spares any in utero development presumably dependent of Cav3.2,
302 while it starts to abolish Cav3.2 function in neuronal excitability at perinatal stage. We and
303 others documented the impact of Ca_v3.2 in helping LTMR neurons to fire in burst by lowering
304 action potential threshold and by generating an after depolarisation potential^{16,31–33}.
305 Accordingly, C-LTMR remained mechanosensitive after Ca_v3.2 conditional knock out, but
306 responded to higher threshold stimuli as the result of dampened excitability¹⁶. Touch
307 perception defects from birth may have dramatic consequences on nurturing touch which can
308 lead to impaired somatosensory development, increased stereotyped behaviours and deficits
309 in social behaviour and cognitive abilities^{34–36}. Because of the affective nature of the
310 information carried by CTs, and potentially their rodent equivalent, C-LTMRs, it has been
311 hypothesized that these neurons play a critical role in this process. Interestingly,
312 Ca_v3.2^{Nav1.8}cKO mice share some phenotypes with two mouse models of ASD which have
313 been phenotyped with the LMT (Shank 2 and Shank 3 KO)²¹. Specifically, Shank 2 KO and

314 $Ca_v3.2^{Nav1.8}cKO$ mice share common phenotypes with regards to isolated behaviours and the
315 contact reduction. On the other hand, C-LTMRs $Ca_v3.2$ cKO mice do not show any deficit in
316 exploratory behaviours **Figure1 c and d and Supplementary Fig. 1**)²¹. The fact that no
317 difference was observed in either social escape behaviours or in passive social interaction in
318 these mice while unilateral and reciprocal interactions were decreased also suggests that
319 $Ca_v3.2^{Nav1.8}cKO$ mice do not seek to avoid inter-individual tactile stimuli, but rather do not
320 actively seek it. Nonetheless, if these mice do not show signs of tactile avoidance, based on
321 the parameters automatically extracted by the LMT and on the Crawley test results where only
322 the time of interaction is affected, this could be due to the experimental paradigm. Indeed, it
323 may be difficult for a mouse to avoid 3 others in the LMT arena (50*50cm).

324 It is also interesting to note that $Ca_v3.2^{Nav1.8}cKO$ mice have an excessive rearing behaviour
325 when isolated (**Figure1 c and d**), which can be considered a manifestation of anxiety³⁷.
326 Rearing behaviour's relationship with anxiety level is a matter of debate, this phenotype,
327 associated with decreased sociability in the LMT and in the Crawley test is strikingly similar to
328 those observed in the *Mecp2* and *Shank3* peripherally restricted KO¹⁴. These observations
329 strongly suggest that C-LTMRs are a key component of social interactions and may play a
330 critical role in neurodevelopmental disorder such as ASD, as suggested from human
331 observation. Indeed, individuals with ASD have altered tactile sensitivities and autism-
332 associated behavioural deficits and neural responses to C-LTMR-triggered affective touch
333 stimuli are inversely correlated³⁸. Such observations suggest that people with greater numbers
334 of autism-relevant traits have impaired processing of affective touch. Because most young
335 children with ASD are averse to touch, caregivers often provide less nurturing touch, and this
336 lack of tactile input may have a profound impact on subsequent behaviour and development
337³⁹. The asocial phenotypes observed in this study in adult C-LTMRs $Ca_v3.2^{Nav1.8}cKO$ could then
338 be the reflect of long-term consequences of altered bottom up effect of touch on shaping the
339 developing social brain during early life. Finally, the asocial traits revealed here may have
340 translational relevance in clinic. Indeed, they nicely parallel the presence of congenital
341 missense mutations in distinct ASD patients within the *Cacna1H* gene leading to *Cav3.2*
342 functional defects⁴⁰. While in this case, mutations have body wide consequences, we can
343 speculate a substantial contribution of tactile sensory deficits to explain the genotype-
344 phenotype relations. Consequently, a perspective of developing peripherally restricted *Cav3.2*
345 selective T-type calcium channel activators could represent a therapeutic opportunity to correct
346 early ASD defects. Conversely, caution should be warranted with the use of T-type calcium
347 channels inhibitors specifically with the risk of hitting the critical period of social brain
348 development in child's early life.

349 *C-LTMRs and pleasant touch.*

350 Using a model of chemogenetic activation of C-LTMRs we demonstrated that this population
351 of neurons is sufficient to create a pleasant experience. Not only does C-LTMR stimulation
352 induce a rewarding experience in a CCP paradigm with no specific context, but it also
353 reinforces thermotaxis centred around 30°C, consistent with the idea that the functioning of
354 these neurons is tuned to the temperature of a skin-stroking caress (~30°C) as in humans ⁴¹.
355 Consistently, we previously documented that Cav3.2^{Nav1.8}cKO mice has the exact opposite
356 phenotype with a weakened thermotaxis ¹⁶. Furthermore, the temperature at which the LMT
357 experiments are performed is 24°C, which is cool for mice. Therefore, the CNO reinforcement
358 of thermotaxia evidenced in the gradient paradigm, is likely to contribute to the animal seeking
359 for group formation within the LMT where the warm body temperature of each other mouse in
360 duo, trio, or even tetrad further amplify the C-LTMR activation during skin to skin contacts. This
361 data reinforces our confidence that the population we defined as C-LTMRs by using different
362 gene expression such as TFAFA4, TH and the duo Ca_v3.2-Na_v1.8 is the correlate of the sensory
363 fibres supporting affective touch in humans.

364 It is interesting to note that the mini Ca_v3.2 promoter used in our viral constructs may not be
365 as efficient as those usually used in similar strategy such as CAG, hSyn, or Ef1A. By contrast,
366 our approach likely results in hM3Dq expression levels just sufficient to potentiate C-LTMRs
367 excitability rather than over-excite them, thus explaining the subtle behaviour changes
368 observed. In vitro calcium imaging in cultured DRG from DREADD expressing mice confirms
369 that most of the neurons functionally responding to DREADD agonist CNO had a unique C-
370 LTMR chemo response pattern consistent with their transcriptional profile regarding the
371 expression of channels and receptors coupled to cytosolic calcium variations. This includes
372 responses to agonists of TRPA1 agonist as reported before ^{15,16}, as well as to the purinergic
373 metabotropic receptor P2RY1 that is consistently reported to be highly expressed in C-LTMRs
374 across species from rodents to non-human primates ^{18,20,42,43}. In addition, the CNO responsive
375 neurons were all negative to live staining with fluorescent IB4, as expected from previous
376 studies ¹⁵.

377 To our knowledge, only one study presently published has been able to demonstrate that a
378 population of primary sensory neurons, the population expressing MRGPRB4, is able to drive
379 a motivational behaviour in mice upon DREADD stimulation approach ⁴⁴. Unfortunately,
380 whether or not this neuronal population can also drive specific inter-individual behaviours has
381 not been investigated.

382 Whether the genetically defined C-LTMR population MRGPRB4 or TH/VGluT3/TFAFA4 (or both)
383 is the murine equivalent of C-tactile fibres in humans remains controversial. MRGPRB4-
384 expressing population is strikingly different from the TH-expressing C-LMTRs population.

385 Despite both belonging to a class of mechanoreceptive C-fibres, MRGPRB4 is expressed in a
386 population not as well defined functionally and genetically across development than TH-
387 expressing neurons^{45,46}. For example MRGPRB4-expressing neurons also express TRPV1
388 and respond to capsaicin⁴⁴ similarly to numerous C-nociceptors, whereas TH-expressing
389 neurons do not²⁰. Interestingly humans C-Tactile fibres do not seem to be sensitized by
390 capsaicin, suggesting that they do not express TRPV1³. In addition, T-type channel blocker
391 TTA-A2 applied in human glabrous skin completely abolished sensibility to innocuous tactile
392 stimuli supporting expression of T-Type calcium channel in C-Tactile afferences, similar to
393 mouse TH-expressing C-LTMRs, but not MRGPRD4 fibres^{16,47}.

394 *C-LTMRs as a motivational drive toward social interaction.*

395 Activation of C-LTMRs greatly increase the number of contacts between adult animals. In
396 accordance with C-LTMRs known innervation of the hairy skin, we observed a large increase
397 of what we considered skin-to-skin contact (contact *side by side* and *side by side opposite*,
398 cumulative duration and number of events **Figure 5 d-e**)¹⁷. More surprisingly, we also
399 witnessed an increase in nose-to-nose contacts and even more nose-to-anogenital contacts,
400 involving skin areas that are not supposedly innervated by C-LTMRs, although recent work
401 suggest that C-LTMRs innervates more skin areas than expected in humans, such as glabrous
402 skin⁴⁸. Such an observation may be due to a priming or reinforcing influence of C-LTMRs
403 activation on inter individual interaction to induce more complex social contacts such as social
404 investigation (nose to nose and nose to anogenital contacts). For example, C-LTMRs
405 stimulation via side to side contact, may act as an appeasing signal to engage communication
406 through face to face sniffing or may be the starter for soliciting play behaviour such as pounce
407 and crossover (not annotated by the LMT)^{49,50}. This may also explain the surprising results
408 concerning the changes observed in dyadic and triadic interactions dynamics where C-LTMRs
409 activation in one mouse reinforce social interaction with the other C-LTMR-stimulated mouse
410 as well as with control mice where C-LTMRs are not potentiated. The LMT is a close arena
411 where all four mice are free to interact with each other. Consequently, a change in occurrence
412 of one given social behavioural trait in any mouse logically impact the rest of the mice, including
413 control mice. Thus, explaining the observed significative alteration of interaction probability of
414 Control mice with C-LTMRs. In addition, C-LTMR-stimulated mice may act as social stress
415 buffers, appeasing social tension in the whole group, thus increasing seeking for inter-
416 individual contact in all animals, including in Controls. However, our results suggest that even
417 if Control mice behaviour appeared to be impacted by the CNO treatment of all four mice, the
418 effect is more robust and potent in C-LTMR-stimulated mouse, suggesting that a C-
419 LMTRs^{hM3Dq} mouse will have a preference toward another C-LMTRs^{hM3Dq} mouse over control

420 mice after CNO treatment. Thus, reciprocal stimulation of C-LTMRs may be more reinforcing
421 than unilateral stimulation.

422 In conclusion, while clinical studies documented that affective information conveyed through
423 the skin have powerful impact on social behaviour, the direct causality of C-Tactile/C-LTMRs
424 primary afferents in this bottom up social brain regulation remained to be rationally
425 demonstrated. Our study, combining mouse genetics and in-depth ethological analysis,
426 provides a first demonstration that in healthy naïve adults, enhancing skin C-LTMRs activity
427 for a few tens of minutes is sufficient to induce immediate and lasting prosocial effects, while
428 conversely impairment of C-LTMRs functions from birth to adulthood negatively impact
429 sociability with behavioural traits resembling those found in genetic mouse models of Autistic
430 Spectrum Disorders. We hope that the type of preclinical approaches developed here to
431 modulate C-LTMRs with a level a selectivity that has not been yet achieved, will prefigure
432 future investigations aimed at better understanding the pathophysiology of CNS circuits of
433 social behaviours driven by affective touch.

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449 **Authors contribution:** AF conceptualized performed and analysed most of the experiments.
450 EB and PF helped to conceptualize the experiments and designed the AAV vectors. DH and
451 FJ helped with the LMT experiments. EB performed the calcium imaging on dissociated DRG.
452 MM performed and analysed the three-chamber social interaction test. AF wrote the
453 manuscript with the help of all the authors.

454

455 **Methods**

456 **Study approval**

457 All animal procedures complied with the welfare guidelines of the European Community and
458 were approved by the local ethic comity, the Hérault department Veterinary Direction, France
459 and the French ministry for higher education, research and innovation (Agreement Number
460 :2017100915448101).

461 **Animals:**

462 Cav3.2 KI^{GFP-flox}, Cav3.2^{Nav1.8}cKO (Cav3.2 KI^{GFP-flox} x Nav1.8^{cre}) and Nav1.8^{cre} mouse lines
463 were bred and housed in a Specific Pathogen Free (SPF) animal facility at the Institute of
464 Functional genomics under approved laboratory conditions (12 hours day/night cycle, 22–24 °C,
465 50 ± 5% humidity, food and water *ad libitum*). All behavioural tests were conducted in the
466 same animal facility with the SPF sanitary status.

467 **Plasmids and Viruses**

468 The excitatory DREADD hM3Dq with a N-terminal HA tag epitope was cloned into a pAAV viral
469 vector under the control of a 1.5kb minimal Cav3.2 promoter²³ and between double-floxed
470 inverse Orientation (also called FLEX) sequences, allowing to switch the DREADD open reading
471 frame in the correct orientation upon Cre mediated recombination. The pAAV-promCav3.2-
472 DIO-ChR2-ires-YFP-WPRE²³ served as template. The ChR2-ires-YFP was replaced by the
473 HA-hM3Dq sequence, coming from pAAV-hDlx-GqDREADD-dTomato (gift from Gordon
474 Fishell, Addgene plasmid # 83897), using HIFI DNA assembly method (New England Biolab,
475 Evry - France). A 3xHA version of the DREADD was further created by inserting a KpnI-NheI
476 cassette obtained by gene synthesis (Proteogenix, Strasbourg - France). All constructs were
477 verified by DNA sequencing. Before viral production, the functionality of the constructs was
478 demonstrated by Fura2 calcium imaging following transient transfection in HEK cells together
479 with expression vectors expressing the Cre recombinase (pCAG-Cre-ires-GFP, a gift from
480 Jérôme Dujardin), and the Egr1 transcription factor (pcDNA3-Egr1, a gift from Eileen Adamson,
481 Addgene plasmid #11729) known to stimulate the Cav3.2 promoter²⁵.

482 AAV-PHPs viruses were custom made either by the Plateforme de Vectorologie de Montpellier
483 (PVM, Montpellier, France) or by the Canadian Neurophotonics Platform Viral Vector Core
484 Facility (RRID:SCR_016477) (Quebec - Canada) using the ad hoc capsid plasmid (pUCmini-
485 iCAP-PHP.S, a gift from Viviana Gradinaru; Addgene plasmid # 103006;²⁴). Control rAAV
486 PHPs CAG mCherry was bought from Addgene (viral prep # 59462-PHP.S)

487 **Primary sensory neuron culture and calcium imaging**

488 Lumbar/thoracic DRGs (L4 to T10) were prepared and cultured on Laminin coated μ -Dish
489 chambers (Ibidi, Germany) as described previously (Francois et al., 2013) from AAV injected
490 mice (18 weeks) Recording were made within 24h of culture. Prior to recording, neurons were
491 incubated with 5 μ M fura-2AM in Tyrode's solution for 1 hour at 37°C, then washed and kept
492 for an additional 20 minutes in Tyrode with no Fura2 for desesterification. Fluorescence
493 measurements were sampled at 1Hz with an inverted microscope (Olympus IX70) equipped
494 with an Evolve Photometrics EMCCD camera (Roper Scientific, France). Fura-2 was excited
495 at 340nm and 380nm and ratios of emitted fluorescence at 510nm were acquired using
496 Metafluor software (Universal Imaging). Drugs were applied with a gravity driven perfusion (1-
497 2ml/min) Pharmacological agonists of hM3Dq (CNO 30 μ M, Tocris, France), P2YR1 (MRS2365
498 200nM, Tocris France), TRPV1 (Capsaicin 500nM), TRPA1 (allyl isothiocyanate 200 μ M), KCl
499 (40mM) were prepared into the Tyrode solution and applied sequentially to the neurons for a
500 few seconds. Data were analyzed offline using metafluor, excel, and graphpad. Similar
501 procedure for Fura2 loading and imaging was used for transiently transfected HEK cells for the
502 verification of pAAV functionality prior to custom virus production. Otherwise stated, all
503 chemicals were from Sigma Aldrich (L'isle d'Abeau Chesnes - France).

504 **Intrathecal injection and Stereotaxic surgeries.**

505 6 to 8week old mice were anesthetized by inhalation of a 2%isoflurane/1.5% oxygen mixture.
506 For intrathecal injection, 5 μ l of viral vector (1.10^{13}) was injected into the lumbar subarachnoid
507 space using a 20 μ l Hamilton syringe and 26g removable minimal dead volume needle.
508 Wounds were sutured and surgical sites infiltrated with 2% lidocaine in saline. Animals were
509 placed on a heating pad in an oxygenated chamber and monitored until fully recovered.

510 **Behaviour**

511 *Three chambers social interaction test:*

512 8 to 12 weeks old male mice were first habituated for half an hour to the experimental room in
513 their home cage. The three-chamber arena is composed of 3 compartment box, each
514 20x40x22(h)cm and separated by two sliding doors (5x8(h)cm) with one prison placed in the
515 upper right corner of the right compartment and another one on the lower left corner of the left
516 compartment. After room habituation, each animal was placed individually in the arena, starting
517 from the middle compartment and was let free to explore for 10 minutes with the two prisons
518 empty. Then, the mouse was locked in the middle compartment and one mouse, stranger to
519 the tested mouse (C57B6J/n bred and housed in the IGF facility), was place in one of the two
520 prison and an inanimate object (made from Lego blocks, roughly shaped like a mouse) was
521 placed on the other prison. The tested mouse was then set free to explore all compartments

522 again for 10 minutes. The all experiment was video recorded and animal position was tracked
523 using Ethovision XT13 (Nodlus). The preference index was calculated with the following
524 formula (Time in stranger side - Time in object side)/exploration time. A mouse was considered
525 interacting automatically by Ethovision when the tested animal was facing the middle of the
526 prison inside a 1cm perimeter around the prisons. The side where the stranger mouse and the
527 inanimate object were placed was alternated between each tested mouse to avoid bias.
528 Experiments were performed in the morning (from 7 to 10 a.m.) under 180 lumens. The
529 experimenter was blinded to the animal conditions.

530 Live Mouse Tracker:

531 The LMT setup was built following the instruction of De Chaumont et al.,²¹. We added to the
532 original design two water dispensers to the arena on two sides.

533 Before going into the LMT each group of 4 mice were housed together for at least 2 weeks
534 (following RFID chip implantation under anaesthesia). LMT recordings were performed with 10
535 to 16 weeks old $Ca_v3.2^{GFP-floxKI}$ or $Cav3.2^{GFP-floxKI} \times Nav1.8^{cre}$ mice, and with 14 to 18 weeks
536 old $Nav1.8^{cre}$ mice (injected with rAAV_{PHP5}-CAG-mCherry or rAAV_{PHP5}-Ca_v3.2-FLEX-HA-
537 hM3Dq). During recording, the animals were kept under the same condition as in the housing
538 facility (12hour Daylight, 500 lumens, food and water *ad libidum*) and the experimenter came
539 once per day to perform injection if necessary or to check the water and food level.

540 We used Python scripts provided to analyse all the data acquired by the system.
541 (www.livemousetracker.com; <https://github.com/fdechaumont/lmt-analysis>). We only
542 performed analyse during the activity phase (night cycle) as during the day mice nest together
543 which impaired the tracking and provide unreliable annotation.

544 von Frey

545 8 weeks after intrathecal injections, mice were first habituated for half an hour to the
546 experimental room in their home cage. Each mouse was then placed individually into small
547 arena (8*8cm) over a von Frey mesh for 45 minutes for habituation. Then, animals were
548 injected peritoneally with 1mg/kg CNO diluted in sterile saline (prepared fresh each time). 30
549 to 45 minutes after the injections, von Frey filaments (0.07g, 0.6g, 2g) and the brush was
550 applied 5 time on each hind paw and withdrawal was then scored. Experiments were
551 performed in the morning (from 8 to 11 a.m.) under 500 lumens. The experimenter was blinded
552 to the animal conditions.

553 Gradient

554 8 weeks after intrathecal injections, mice were first habituated for half an hour to the
555 experimental room in their home cage and then injected with 1mg/kg CNO diluted in sterile
556 saline (prepared fresh each time). 30 minutes later, mice were placed into the bioseb thermal
557 gradient (2 corridors of 1m50 with one extremity cooled down to 5°C and the other to 50°C,
558 creating a thermal gradient spited into 20 thermal zone). Mice were free to explore for 90
559 minutes during which animal's positions were tracked and annotated accordingly to the
560 temperature zones. For each run, a C-LTMRs^{hM3Dq} mouse and a control mouse were tested
561 simultaneously. However, the groups were labelled as such as he experimenter was still blind
562 to the animal conditions. Mice corridor distribution was alternated between each run to avoid
563 any corridor biased. Experiments were performed in the morning (from 8 to 12 a.m.) under 180
564 lumens.

565 Conditioned place preference (CPP)

566 8 weeks after intrathecal injections, mice were subjected to the conditioned place preference
567 protocol. This protocol consisted into 6 experimental days: the first day consisted into an
568 exploration and habituation phase of 30 minutes per mouse where mice were free to explore
569 the CPP arena (compartments: 25cm*20cm, corridor: 5cm*20cm . From the second to the fifth
570 day, animals were conditioned to associate a saline injection in the morning to their preferred
571 compartment (defined during the first day), and a CNO injection (1mg/kg) to the other
572 compartment in the afternoon. During this conditioning, animals received the injection and
573 stayed in their homecage for 30 minutes and then were placed into the designated
574 compartment for 30 minutes, to allow the CNO to reach its molecular target. To avoid that the
575 CNO was still active between two conditioning, we choose to stretch as much as possible the
576 period of time between the CNO injection from one given day and the next day saline injection.
577 On the sixth and last day animals were free to explore the all arena again for 30 minutes. The
578 position of the animal was detected by a network of infrared beams. The CPP rack allowed us
579 to perform 4 experiments at a time (Imetronic place preference setup), thus each run was
580 balanced to test 2 C-LTMRs^{hM3Dq} and 2 control mice, however the groups were labelled as
581 such as the experimenter was blinded to the animal conditions. Experiments were performed
582 in the morning (from 8 to 12 a.m.) under 180 lumens.

583 **Immunohistology**

584 Tissue collection and processing. Animals were transcardially perfused with phosphate-
585 buffered saline (PBS) followed by 10% formaldehyde in PBS. Brain and spinal cord were
586 dissected, post-fixed in 10% formaldehyde for 24 hours, and cryoprotected in 30% sucrose in
587 PBS. DRGs were cryoprotected directly after formaldehyde perfusion. Tissues were then
588 frozen in Optimum Cutting Temperature (OCT, Tissue Tek) and sectioned using a cryostat

589 (Leica). Spinal cord and brain were sectioned at 40 μ m and stored in PBS + 0.05% azide at
590 4°C. For DRGs, tissues were sectioned at 18 μ m, collected on Superfrost Plus slides (Fisher
591 Scientific), and stored at -80°C.

592 Immunofluorescence. Tissues were incubated for 1 hour and blocked in a solution consisting
593 of 0.1 M PBS with 0.3% Triton X-100 (Sigma) plus 5% normal donkey serum. Primary and
594 secondary antibodies were diluted in 0.1 M PBS with 0.3% Triton X-100 plus 1% normal
595 donkey serum. Sections were then incubated overnight at 4°C in primary antibody solution,
596 washed in 0.1 M PBS with 0.3% Triton X-100 for 40 min, incubated for 2 hrs in secondary
597 antibody at room temperature (RT), and washed again in 0.1 M PB for 40 min. Sections were
598 then mounted using Dako fluorescence mounting medium. Images were acquired with a Leica
599 SP8 confocal microscope.

600 Primary antibodies: anti-TH: Millipore (sheep; 1:500), anti-HAtag: Covenant (mouse, 1:1000);
601 Anti-Vglut3 : Synaptic system (Rabbit, 1:500). To identify IB4-binding cells, Fluorophore-
602 conjugated IB4 (VectorLab, 1:500) was used in place of primary and secondary antibodies.
603 Secondary antibodies: Alexa Fluor®-conjugated secondary antibodies were acquired from
604 Invitrogen and Jackson Immunoresearch Labs.

605 **Statistics**

606 Statistics were performed with Graphpad Prism 8. Data are represented as mean \pm s.e.m. For
607 bar graphs, each individual data points were superimposed under mean mean \pm s.e.m.
608 Statistical tests used to compare values are indicated in each figure legends.

609

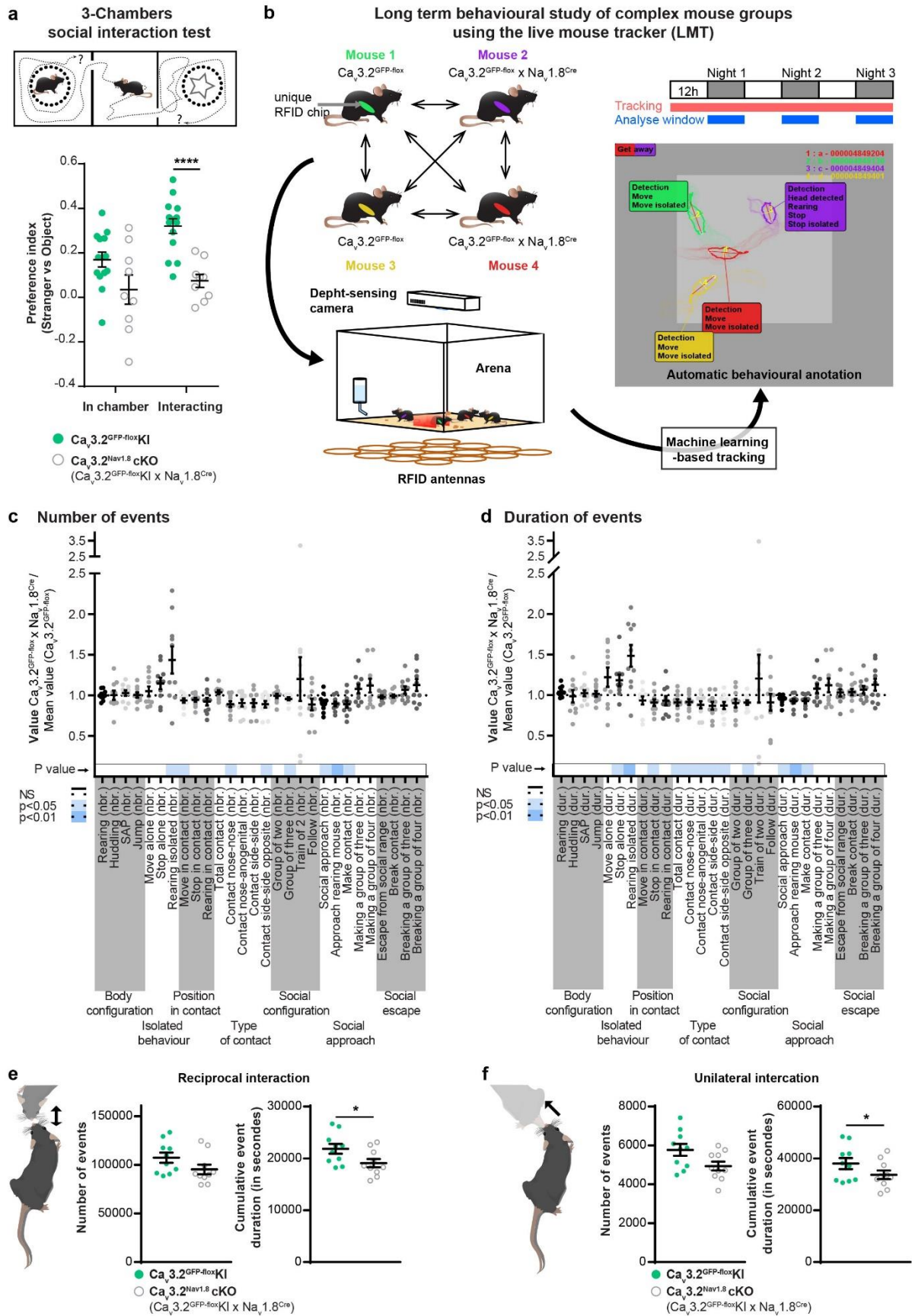
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677

678 **Figure 1: C-LTMRs impairment via $Ca_v3.2$ deletion reduces social behaviour.**

679 a. In a three-chamber social interaction test, control $Ca_v3.2^{GFP-flox}KI$ littermates have a
680 significantly higher preference index toward a stranger mouse than $Cav3.2^{Nav1.8}cKO$. Unpaired
681 t-Test $p < 0.001$, $n_{Cav3.2^{GFP-flox}KI} = 14$ (light green circles), $n_{Cav3.2^{Nav1.8}cKO} = 9$ (open grey
682 circles).

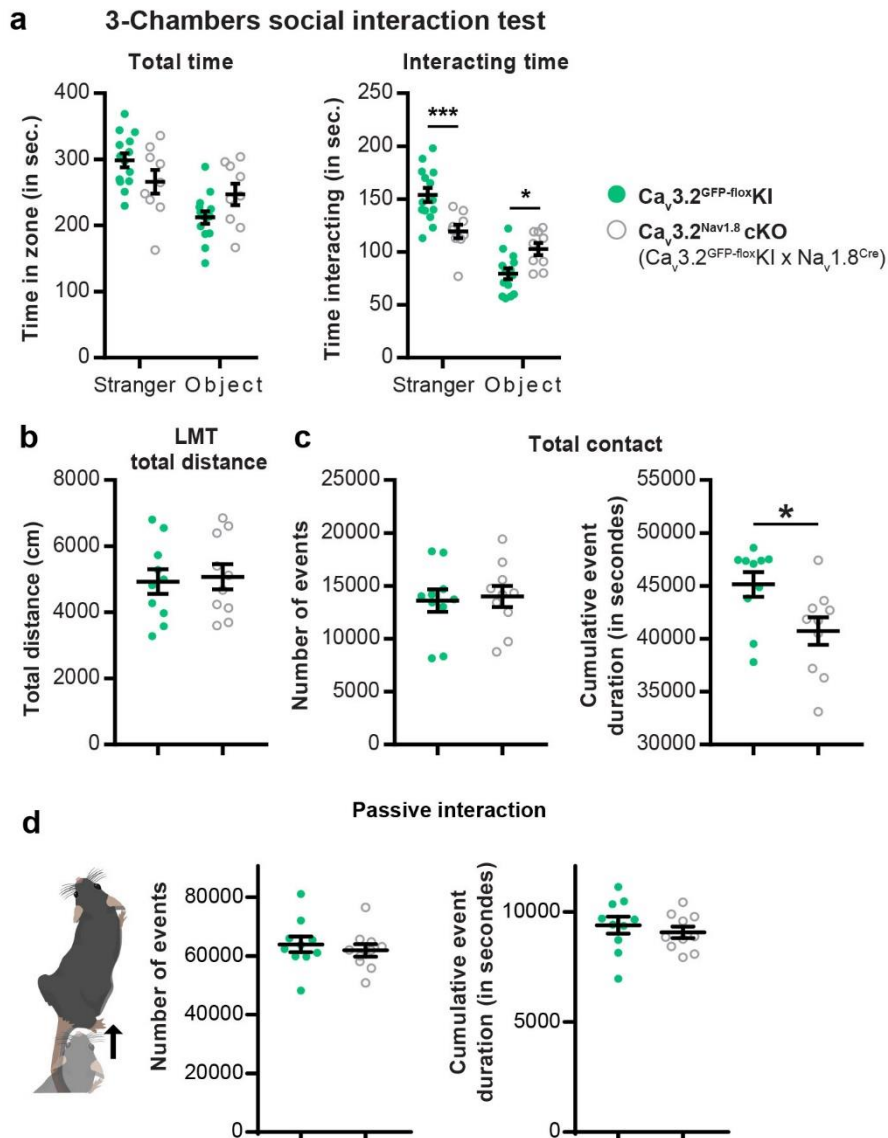
683 b. Live mouse tracker configuration description. Each animal is implanted with a RFID chip for
684 authentication and tracking. The system includes 16 RFID antennas that cover the 50 by 50
685 cm arena which allow, with the deep sensing camera and the machine learning algorithm, to
686 track and automatically annotated the behaviour of 4 mice. Each group of mice includes 2
687 $Ca_v3.2^{GFP-flox}KI$ and 2 $Cav3.2^{Nav1.8}cKO$. the experiment was performed over 3 days and each
688 behaviour was analysed during the nocturnal activity phases.

689 c. LMT index for 28 behavioural traits. Sum of all the number of events for the three nights.

690 d. LMT index for 28 behavioural traits. Sum of the duration of events for the three nights.

691 For c. and d.: The index for each trait was compared to one using one-sample two-sided
692 Student's t-tests (corrected for multiple testing, because 28 tests were conducted for each
693 strain). The p values were color-coded in shades of blue depending of the p value as indicated
694 on the left. $n = 10$

695 e. f. Raw values extracted from the LMT for three consecutive nights for $Ca_v3.2^{GFP-flox}KI$ (light
696 green circles; $n = 10$) and $Cav3.2^{Nav1.8}cKO$ (open grey circles; $n = 10$). e. number of events and
697 cumulative duration of reciprocal social interaction (* $p = 0.0372$). f. number of events and
698 cumulative duration of unilateral social interaction (* $p = 0.0429$). unpaired t-test



699

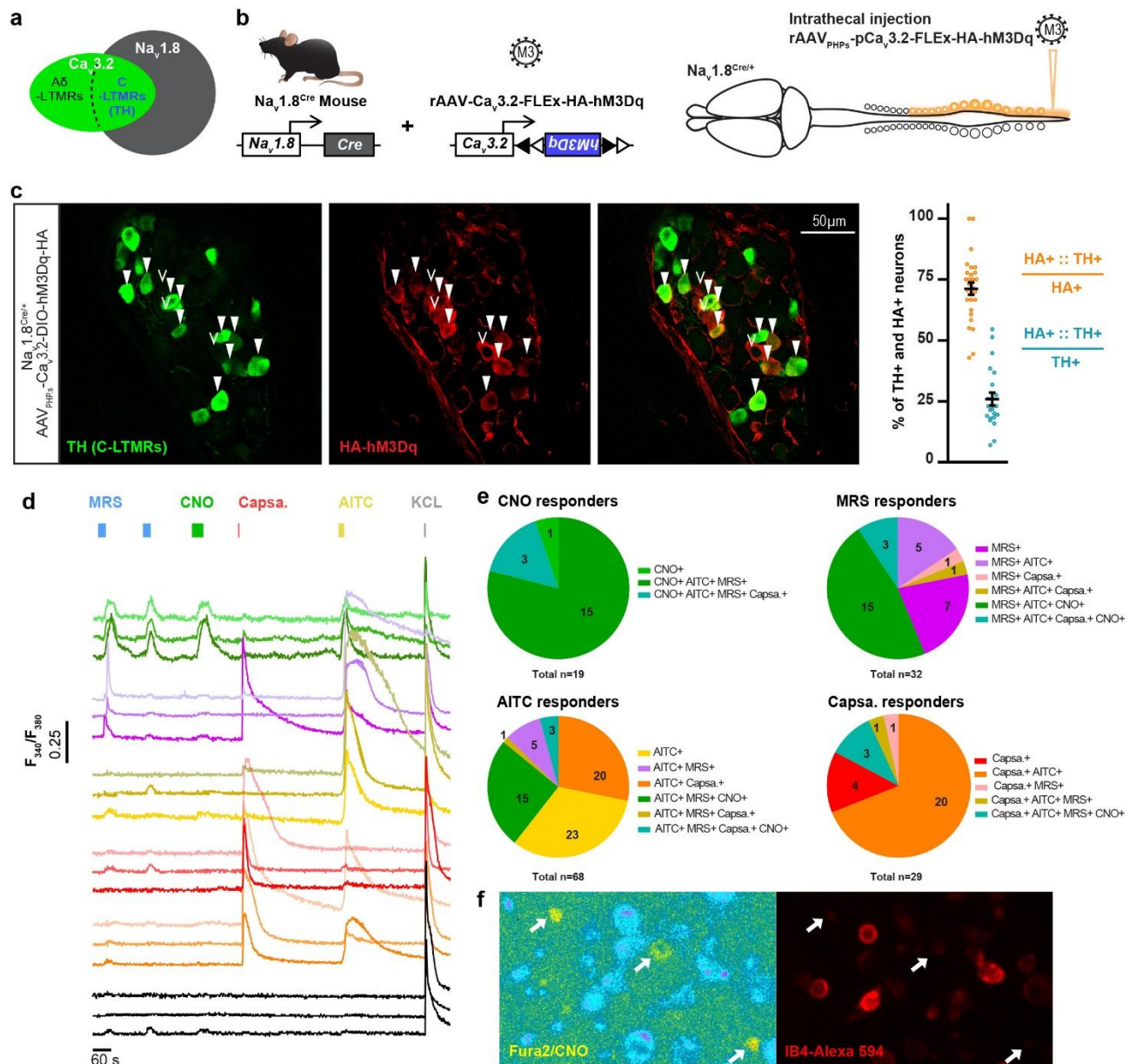
700 **Figure Supplementary 1: C-LTMRs hypo excitability via $Ca_v3.2$ deletion reduces social**
701 **behaviour.**

702 a. In a three-chamber social interaction test, control $Ca_v3.2^{GFP-floxKI}$ littermates spent more time
703 interacting with a stranger mouse and less time with an inanimate object than $Ca_v3.2^{Nav1.8cKO}$.
704 2 way ANOVA with Bonferroni post hoc test, *** $p=0.0007$; * $p=0.262$, $n_{Ca_v3.2^{GFP-floxKI}} = 14$
705 (light green circles), $n_{Ca_v3.2^{Nav1.8cKO}} = 9$ (open grey circles).

706 b. Sum of the total distance travelled by each mouse during the three nights for $Ca_v3.2^{GFP-floxKI}$
707 $Ca_v3.2^{GFP-floxKI}$ KI (light green circles; $n = 10$) and $Ca_v3.2^{Nav1.8cKO}$ (open grey circles; $n=10$).

708 c. raw values extracted from the LMT for three consecutive nights. Left: number of total contact,
709 right, cumulative event duration of contacts (* $p=0.0220$).

710 d. number of events and cumulative duration of passive social interaction. Wilcoxon matched-
711 unpaired t-test



712

713 **Figure 2: Viral expression of DREADD receptors HA-hM3Dq in C-LTMRs.**

714 a. Diagrams representing primary sensory neurons expressing Na_v1.8 and Ca_v3.2 and the
715 population defined by the expression of both ion channels.

716 b. Strategy to express HA-hM3Dq in C-LTMRs using intrathecal injection of AAV_{PHPs} serotypes
717 in Nav1.8^{Cre} mice.

718 c. Left: representative images of an Immunofluorescence of Tyrosine Hydroxylase (TH, C-
719 LTMRs marker, green) and HA tag (HA-hM3Dq, red) in thoracic dorsal root ganglia (T13) of a
720 Nav1.8^{Cre} mice injected with the AAV_{PHPs}-pCa_v3.2-FLEX-HA-hM3Dq. Filled arrowheads
721 indicate examples of neurons considered positive for HA and TH. Empty arrowheads indicate
722 examples of neurons considered positive for HA only. Right: Bar graph of the percentage of

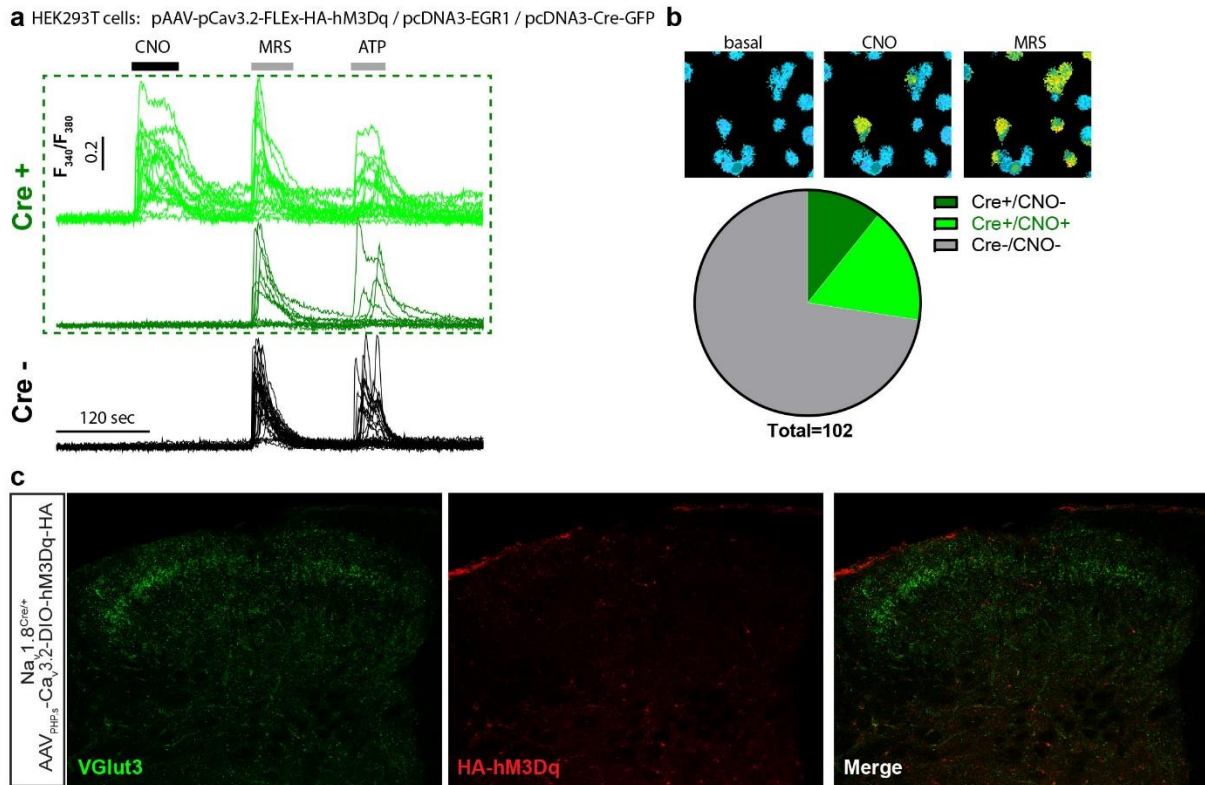
723 neurons positive for HA and TH immunostaining over the all HA positive population (Orange
724 dots) or over the all TH positive population (Tile dots) in thoracic and lumbar ganglions. N=3
725 mice; Thoracic: T7 to T13; Lumbar: L1 to L6.

726 d. Representative individual calcium influx in cultured DRG neurons using Fura2 following bath
727 perfusion of MRS2365 (200nM, blue) CNO (30 μ M, green), Capsaicin (Capsa., 500nM, red)
728 AITC (200 μ M, yellow) or KCL (40mM, Grey).

729 e. Pie charts of the number of neurons responding to the different chemical compounds. Based
730 on the amplitude of the calcium influx responses, neurons were classified as CNO responder
731 (Top Left), MRS responder (Top right), AITC responder (bottom left) and Capsaicin
732 responders). A total of 168 neurons were recorded from 3 animals.

733 f. representative images of ratiometric calcium imaging on cultured DRG from a Na_v1.8Cre
734 mice injected with the AAV_{PHPs}-pCa_v3.2-FLEX-HA-hM3Dq. Left panel: ratiometric Fura2 after
735 CNO bath perfusion, right panel: live IB4-Alexa 594 staining of the same field of view. Arrows
736 indicates CNO-responsive DRG neurons.

737



738

739 **Figure Supplementary 2: validation of the viral construct used to target C-LTMRs**

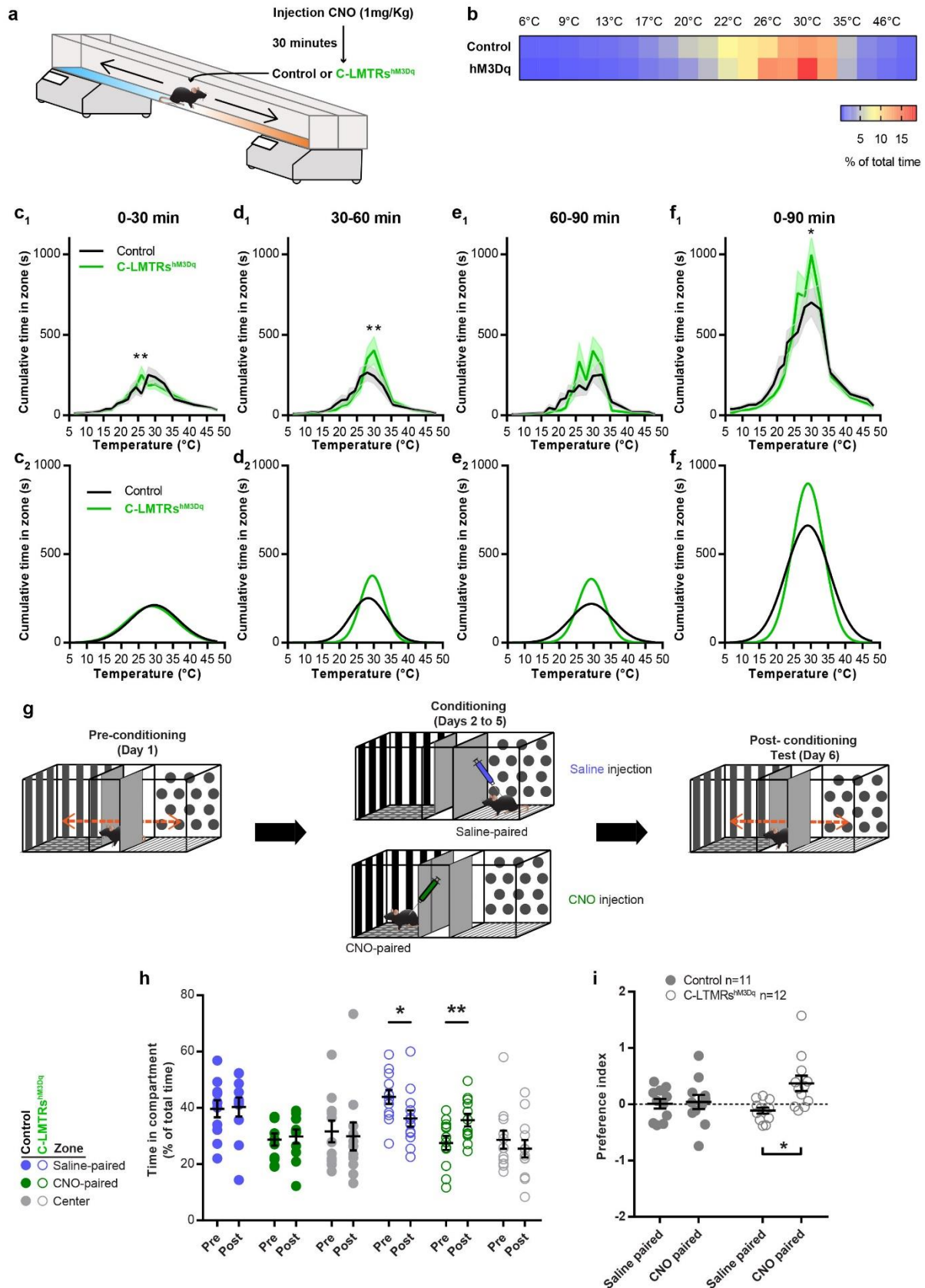
740 a. Representative individual Fura2 calcium influx in cultured tsA201 cells transfected with
741 pAAV-pCav3.2-FLEEx-HA-hM3Dq, pcDNA3-EGR1, and pcDNA3-Cre-GFP, following bath
742 perfusion of CNO (30 μ M), MRS2365 (200nM) to stimulate endogenous P2Y1 receptors or ATP
743 (30 μ M) to stimulate all endogenous P2Y receptors. Green traces: cells expressing the Cre
744 recombinase (visualized with the GFP). Black traces: cells that do not express the Cre
745 recombinase.

746 b. Top panels, representative images of ratiometric calcium imaging on tsA201 cells
747 transfected with pAAV-Cav3.2-FLEEx-HA-hM3Dq, pcDNA3-EGR1, and pcDNA3-Cre-GFP
748 following bath perfusion of CNO to stimulate hM3Dq (30 μ M, middle), and MRS2365 (200nM,
749 right). Bottom panel: Pie chart of the number of cells responding to CNO perfusion. 102 cells
750 recorded

751 c. Representative images of VGlut3 (Green) and HA (Red) immunostaining in the caudal part
752 of the lumbar spinal cord.

753

754

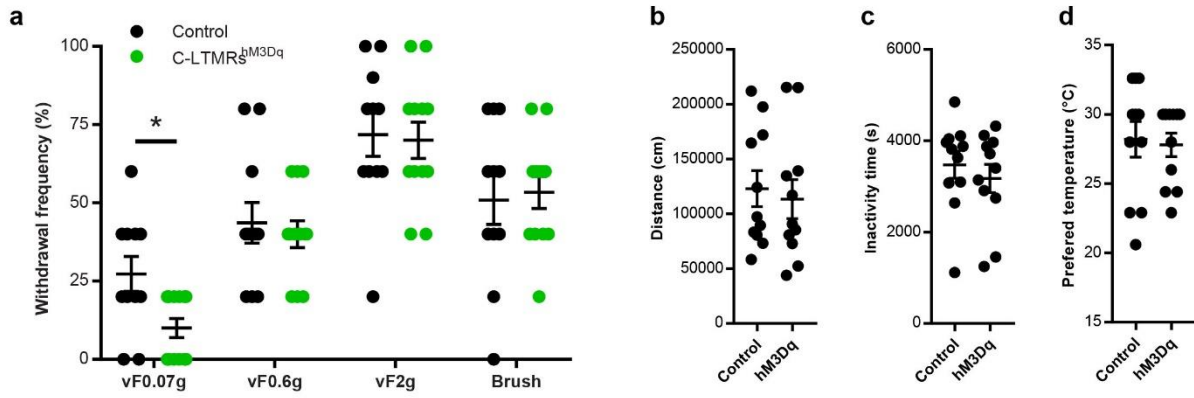


755

756 **Figure 3: C-LTMRs exogenous activation increases thermal preference, and induced**

757 **conditioned place preference.**

- 758 a. Thermal gradient protocol to assess thermotaxis of Control (Nav1.8^{Cre}; AAV_{PHPs}-CAG-
759 mCherry; in black) and C-LTMRs^{hM3Dq} (Nav1.8^{Cre}; AAV_{PHPs}-pCa_v3.2-FLEX-HA-hM3Dq; in
760 green) mice.
- 761 b. Heatmap of the zone occupancy (in % or total time) in the thermal gradient for 90 minutes.
- 762 c1. Cumulative time in the thermal gradient zones during the first 30 minutes. c2. Predictive
763 curves based on a Gaussian fit of the results in c1.
- 764 d1. Cumulative time in the thermal gradient zones between 30 and 60 minutes. d2. Predictive
765 curves based on a Gaussian fit of the results in d1.
- 766 e1. Cumulative time in the thermal gradient zones between 60 and 90 minutes. e2. Predictive
767 curves based on a Gaussian fit of the results in e1.
- 768 f1. Cumulative time in the thermal gradient zones for the all 90 minutes. f2. Predictive curves
769 based on a Gaussian fit of the results in f1.
- 770 (two-way ANOVA, Bonferroni post hoc test, **p = 0.0037; **p=0.0019; *p=0.0112; Control n
771 =11, C-LTMRs^{hM3Dq} n =12).
- 772 g. Conditioned placed preference protocol.
- 773 h. Time spent in compartments (in % of total time) before (pre-) and after (post-) 3 days of
774 conditioning. Wilcoxon matched-pairs signed rank test (**p = 0.0093; *p=0.0210; Control n
775 =11, C-LTMRs^{hM3Dq} n =12).
- 776 i. Conditioned placed preference index (time post - time pre/time pre). (two-way RM ANOVA,
777 Bonferroni post hoc test, *p = 0.0141)
- 778
- 779



780

781 **Figure Supplementary 3: Light touch perception is altered by C-LTMRs exogenous**
782 **activation.**

783 a. Withdrawal frequency to von Frey filament 0.07g, 0.6g and 2g of Control (Nav1.8^{Cre};
784 AAV_{PHPs}-CAG-mCherry; in black) and C-LTMRs^{hM3Dq} (Nav1.8^{Cre}; AAV_{PHPs}-pCa_v3.2-FLEX-HA-
785 hM3Dq; in green) mice. Mann-Whitney test; * p=0.0221; Control n =11, C-LTMRs^{hM3Dq} n =12)

786 b. Distance travelled during 90 minutes on the thermal gradient.

787 c. Cumulative time of inactivity during 90 minutes on the thermal gradient.

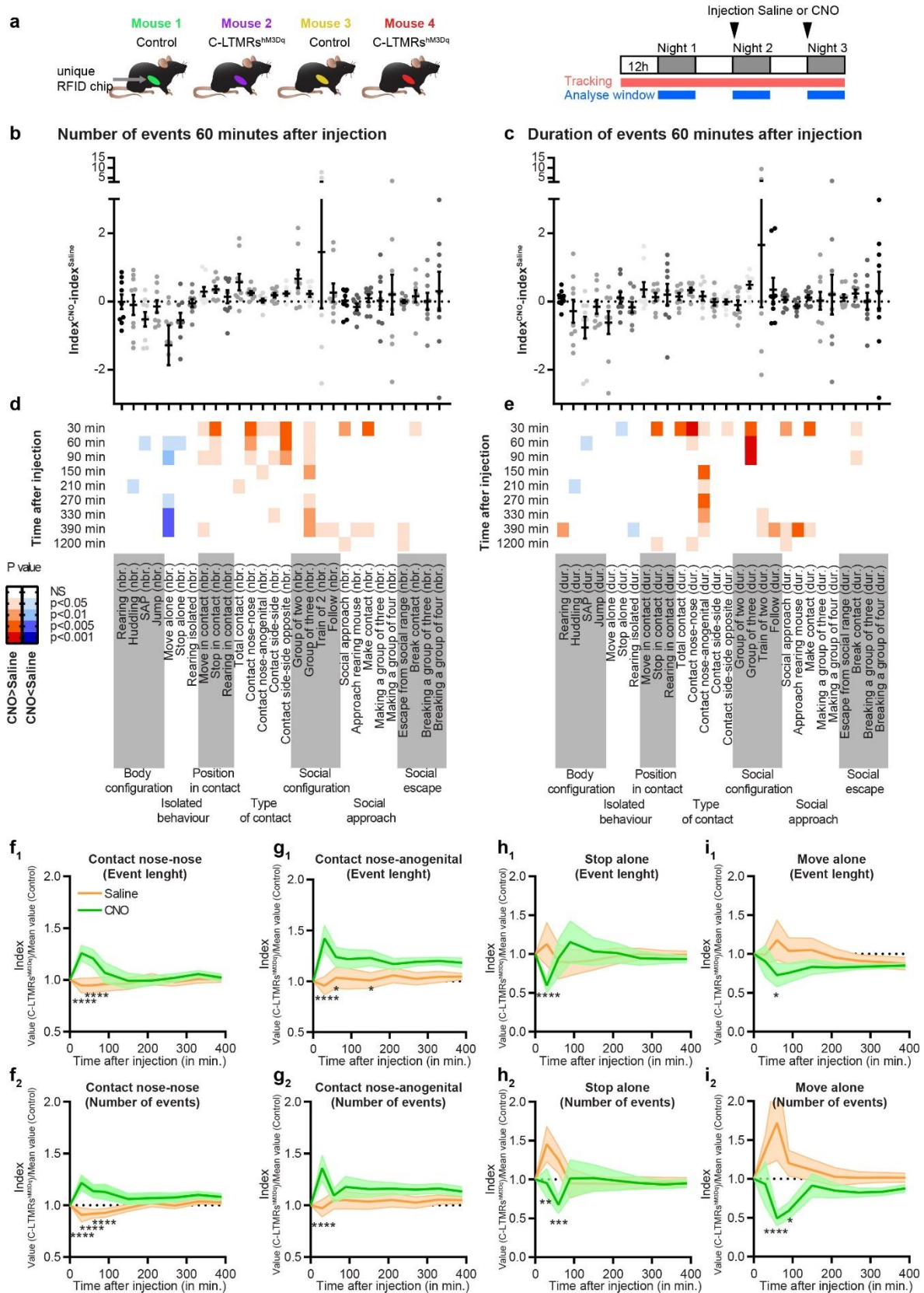
788 d. Average of the temperature where the animals spent the most of their time.

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794 **Figure 4: C-LTMRs exogenous activation transiently increases contact between animals**
 795 **and reduces isolated behaviour.**

796 a. Left: Cage composition of each group analysed in the LMT: 2 Na_v1.8^{cre} mice injected with
797 rAAV_{PHPs}-CAG-mCherry (Control) and 2 mice injected with rAAV_{PHPs}-Ca_v3.2-FLEX-HA-hM3Dq
798 (C-LMTRs^{hM3Dq}). Right: Animals were recorded for 3 consecutive days, and received on
799 injection of saline at the beginning of the second night cycle and one injection of CNO (1mg/kg)
800 at the beginning of the third, or vice versa.

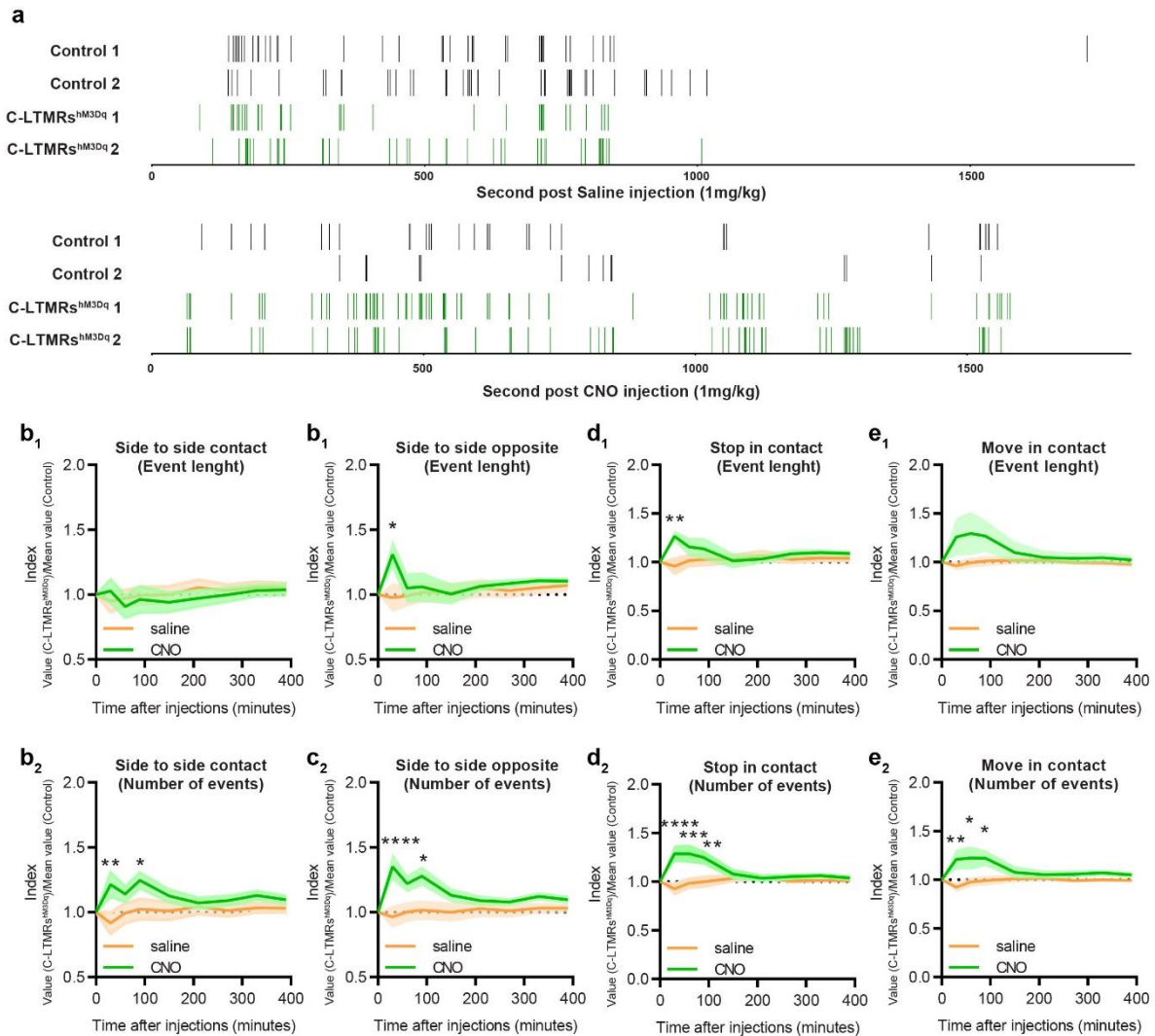
801 b. LMT index^{CNO}-LMT index^{saline} ratio of the number of events 60 minutes after injection for
802 each behavioural trait automatically annotated by the LMT.

803 c. LMT index^{CNO}-LMT index^{saline} ratio of the events duration 60 minutes after injection for each
804 behavioural trait automatically annotated by the LMT.

805 d. and e. P values obtained by comparing the index for each trait to zero, at 9 different time
806 points post injection (30 minutes, 60 minutes, 90 minutes, 150 minutes (2.5 hours), 210
807 minutes (3.5 hours), 270 minutes (4.5 hours), 330 minutes (5.5 hours), 390 minutes (6.5 hours)
808 and 1200 minutes (20 hours) using one-sample two-sided Student's t-tests (corrected for
809 multiple testing). The P values were color-coded as indicated on the left depending of the level
810 of significativity and the polarity of the mean used for comparison: negative (shade of blue) or
811 positive (shade of red). n = 10.

812 f to i. LMT index obtained at 30, 60, 90, 150, 210, 270, 330, and 390 minutes post saline
813 injection (orange) or post CNO injection (green) for the cumulative duration (f₁) and the
814 cumulative number (f₂) of contact nose to nose, the cumulative duration (g₁) and the cumulative
815 number (g₂) of contact nose anogenital and, the cumulative duration (h₁) and the cumulative
816 number (h₂) of events stop alone and their length, or the duration (i₁) and the number (i₂) of
817 event move alone. two-way RM ANOVA, Bonferroni post hoc test, *p < 0.05 ; ** p<0.01; ***
818 p<0.005; ****p<0.0001

819



820

821 **Figure Supplementary 4: C-LTMRs exogenous activation transiently increases social**
822 **exploratory contacts between animals.**

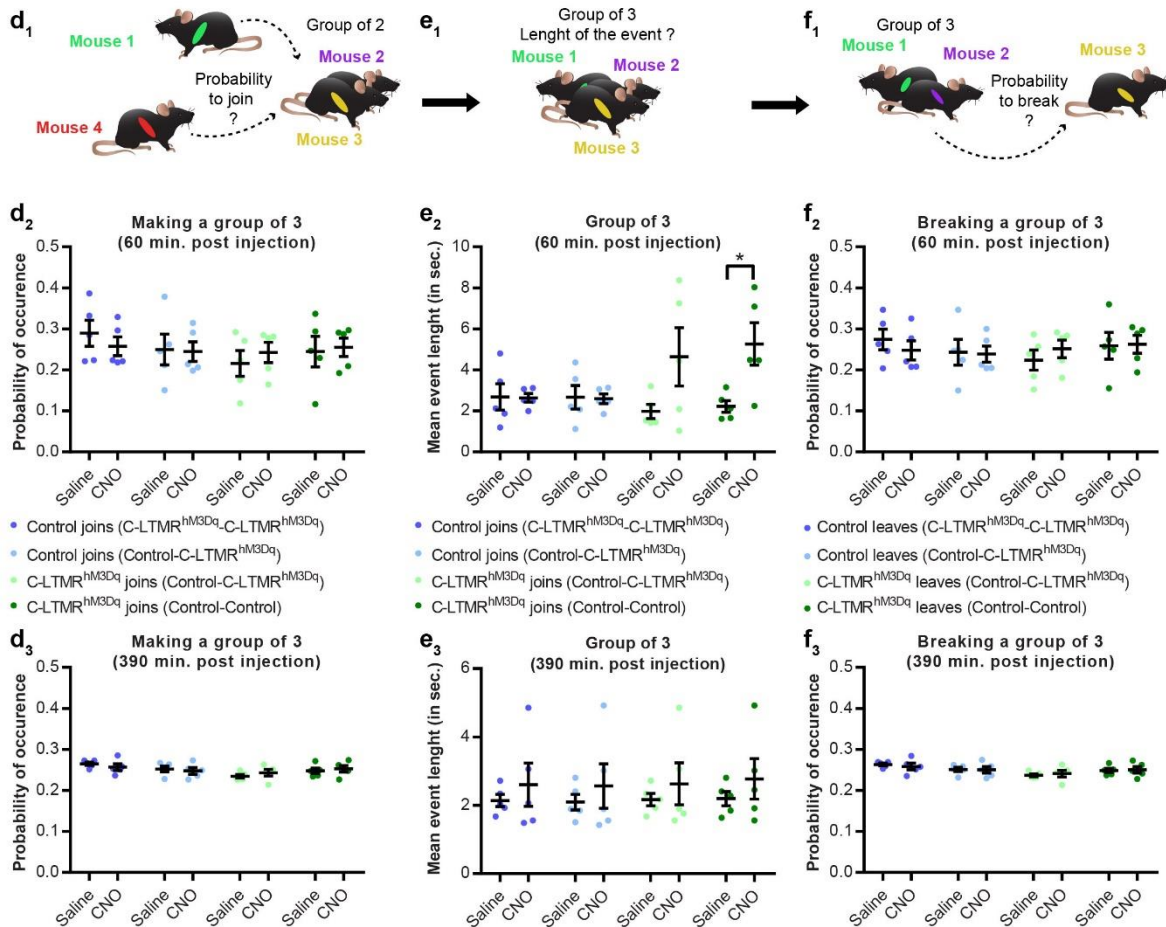
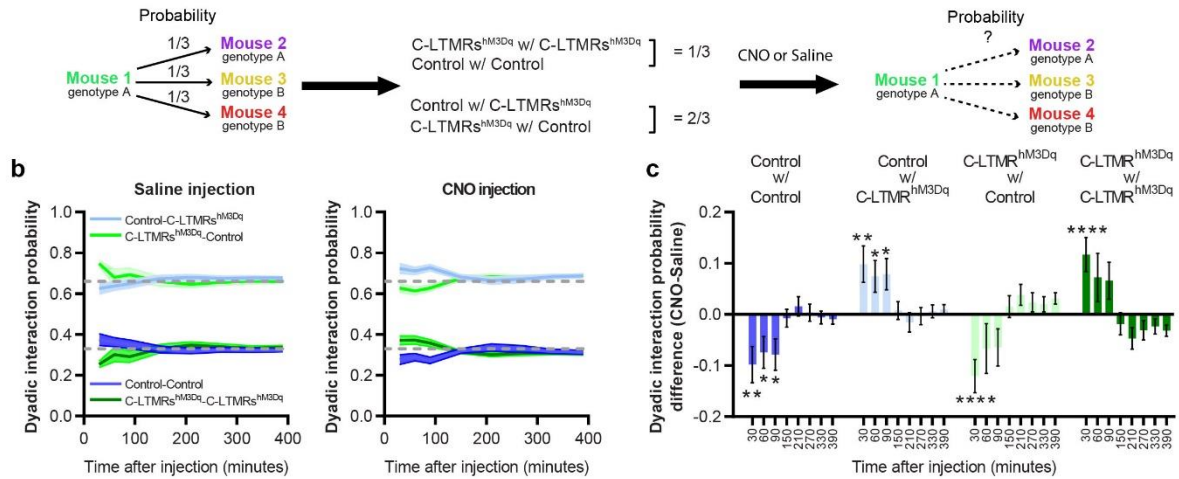
823 a. Example from the 4th group of nose-to-nose contacts events for the first 30 minutes post
824 saline (upper panel) or CNO (lower panel) injections.

825 b to e. LMT index obtained at 30, 60, 90, 150, 210, 270, 330, and 390 post saline injection
826 (orange) or post CNO injection (green) for the cumulative number of contact side to side (b2)
827 and their cumulative length (b1), the cumulative number of contact side to side opposite (c2)
828 and their cumulative length (c1), the cumulative number of events stop in contact (d2) and their
829 cumulative length (d1), or the cumulative number of event move in contact (e2) and their
830 cumulative length (e1). two-way RM ANOVA, Bonferroni post hoc test, *p < 0.05 ; ** p<0.01;
831 *** p<0.005; ****p<0.0001

832

833

a Theoretical composition of a group of 2 and probability of occurrence



834

835 **Figure 5: C-LTMRs exogenous activation shakes group dynamics and inter individual**
 836 **interaction probabilities.**

837 a. Theoretical composition of a group of two based on its probability of occurrence

838 b. Dyadic interaction probability for all 4 possibilities at 30, 60, 90, 150, 210, 270, 330, and 390

839 minutes after saline (left) and CNO (right) injection.

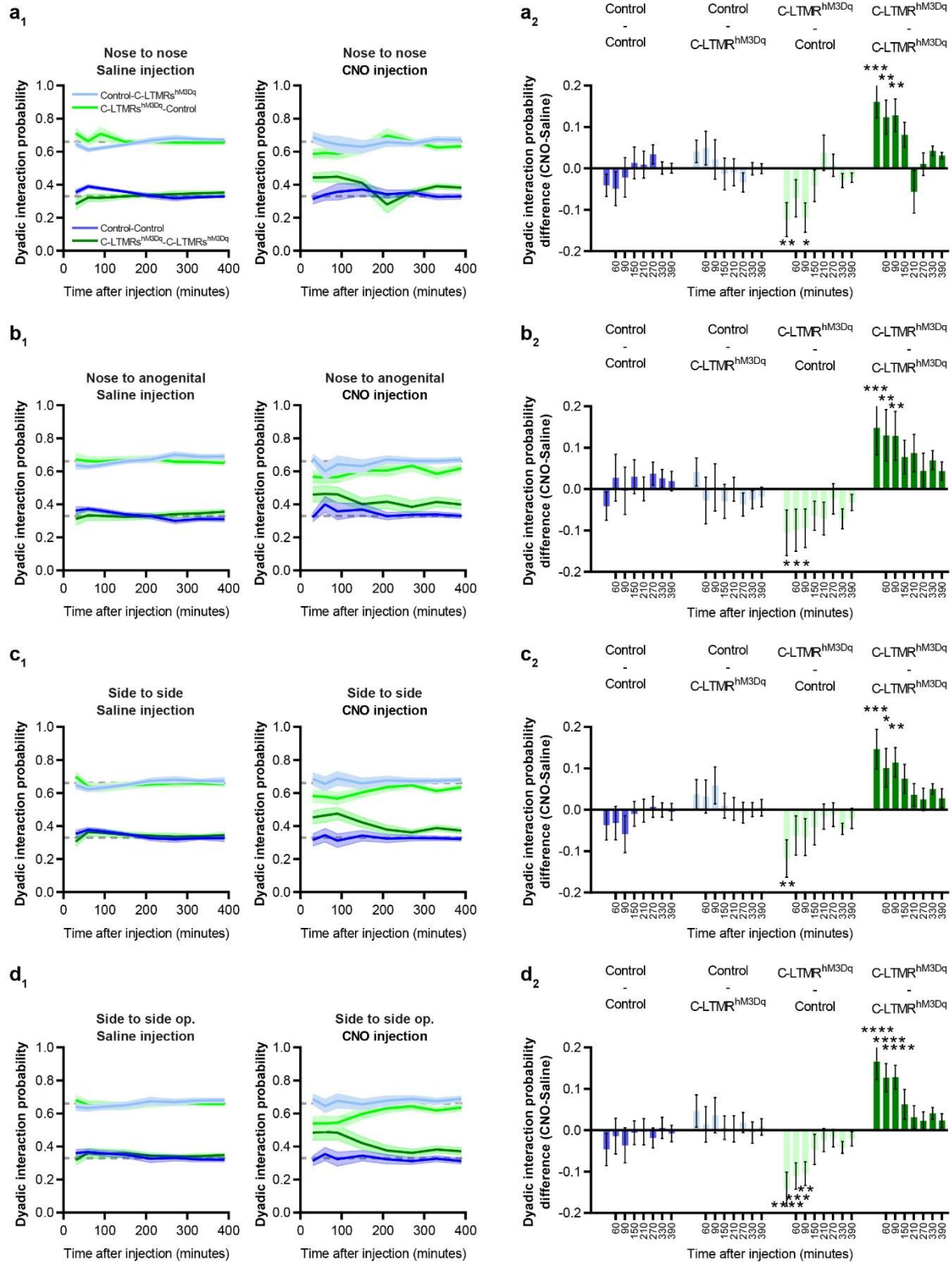
840 c. Dyadic interaction probability difference between CNO and saline injections at 30, 60, 90,
841 150, 210, 270, 330, and 390 minutes post injection. two-way RM ANOVA, Bonferroni post hoc
842 test, * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$

843 d. Probability of occurrence for a mouse of any given genotype to create a group of three of all
844 possible composition at 60 (d2) or 390 (d3) minutes post saline or CNO injection.

845 e. length of the group 3 event created by a given mouse depending of the total composition of
846 the group at 60 (e2) or 390 (e3) minutes post saline or CNO injection.

847 f. Probability of occurrence for a mouse of any given genotype to break a group of three of all
848 possible composition at 60 (f2) or 390 (f3) minutes post saline or CNO injection.

849 Paired t test $p = 0.0486$



850

851 **Figure Supplementary 5: C-LTMRs exogenous activation exacerbates interaction**
 852 **probabilities between C-LTMR^{hM3Dq} mice.**

853 a1 to d1. Specific dyadic interaction probability for all 4 possibilities at 30, 60, 90, 150, 210,
 854 270, 330, and 390 minutes after saline (left) and CNO (right) injection. A1 nose to nose dyadic

855 interaction; b1 nose to anogenital dyadic interaction; c1 side to side dyadic interaction; d1 side
856 to side opposite dyadic interaction.

857 a2 to d2. Specific dyadic interaction probability difference between CNO and saline injections
858 at 30, 60, 90, 150, 210, 270, 330, and 390 minutes post injection. a2 nose to nose dyadic
859 interaction; b2 nose to anogenital dyadic interaction; c2 side to side dyadic interaction; d2 side
860 to side opposite dyadic interaction.

861 two-way RM ANOVA, Bonferroni post hoc test, * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$

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