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# A hypothalamic novelty signal modulates hippocampal memory

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**The ability to recognize information incongruous with previous experience is critical for survival, thus novelty signals in the mammalian brain have evolved to enhance attention, perception and memory<sup>1-3</sup>. Although the importance of regions such as the ventral tegmental area<sup>4-6</sup> and locus coeruleus<sup>6,7</sup> in broadly signaling novelty has been well established, these diffuse monoaminergic transmitters have yet to be shown to convey specific information regarding the type of stimuli that drive them<sup>6</sup>. Whether distinct types of novelty, such as contextual and social novelty, are differently processed and routed in the brain remain unclear. Here we identify a novelty hub in the hypothalamus – the supramammillary nucleus (SuM)<sup>8</sup>. Unique about this region is that it not only responds broadly to novel stimuli, but segregates and selectively routes different types of information to discrete cortical targets, the dentate gyrus (DG) and CA2 fields of the hippocampus, for the modulation of mnemonic processing. Taking advantage of a novel SuM-Cre transgenic mouse, we found that DG-projecting SuM neurons are activated by contextual novelty while the SuM-CA2 circuit is preferentially activated by novel social encounters. Circuit-based manipulation demonstrated that divergent novelty channeling in these projections significantly modifies hippocampal-based contextual or social memory. This content-**

32 **specific routing of novelty signals represents a previously unknown mechanism that**  
33 **enables the hypothalamus to flexibly modulate select components of cognition.**

34 The hypothalamus supports evolutionary conserved functions, including the regulation of  
35 homeostatic states and innate behaviors<sup>9</sup>. However, given both the conservation of a behavioral  
36 novelty response across species and the hypothalamic connections with the hippocampus, this  
37 region may play a role in detecting novelty and influencing higher cognitive processing. Thus,  
38 we conducted a systematic examination of the expression of c-Fos, a marker of active neurons, in  
39 the hypothalamus of mice exposed to two omnipresent types of novelty, contextual and social  
40 (Fig. 1a). While c-Fos expression increased in multiple hypothalamic regions, the SuM, a small  
41 nucleus with direct projections to hippocampus<sup>8</sup>, exhibited a remarkably high response to both  
42 contextual<sup>10,11</sup> and social novelty (Fig. 1b and Extended Data Fig. 1), with similar proportions of  
43 neurons activated in both conditions (Fig. 1c, novel context:  $117.9 \pm 4.1$  c-Fos+ cells/mm<sup>2</sup>; novel  
44 animal:  $129.9 \pm 5.4$ ; familiar context (control):  $24.0 \pm 2.5$ ). To physiologically characterize the  
45 novelty response of SuM neurons, we performed *in vivo* single-unit recordings in behaving mice  
46 (Fig. 1d, e and Extended Data Fig. 2a–c). Each session consisted of exposure to a familiar  
47 context, a novel context and finally a novel animal (Fig. 1d). A total of 141 well-isolated units  
48 were recorded, comprised of 119 putative excitatory neurons (pEN, 84.4%) and 22 putative fast-  
49 spiking interneurons (pIN, 15.6%). The average firing rate of all pEN units was significantly  
50 higher during exposure to a novel context (2.5 Hz) or a novel social encounter (2.6 Hz)  
51 compared to familiar contexts (2.1 Hz; Fig. 1f, g), whereas pIN units showed no significant  
52 novelty modulation (Extended Data Fig. 2d–h). Subsets of pEN units significantly increased their  
53 activity in response to contextual novelty (21.8%, 26 of 119 units) and social novelty (21.0%, 25  
54 of 119 units), outweighing the subsets that showed decreased firing rates (8.4%, 10 of 119 units  
55 for contextual; 0.8%, 1 of 119 units for social; Fig. 1h, i). Notably, a large portion of SuM  
56 neurons (69.2%, 27 of 39 units) were specifically excited by one type of novelty but inhibited or  
57 non-responsive to the other (Fig. 1f, j). Both the context and social driven populations displayed  
58 a monotonic decrease in firing rates over time (Fig. 1k), suggesting uniform habituation of the  
59 novelty response.

60 The SuM and related circuits have been difficult to study due to their small size and diverse cell  
61 types<sup>8</sup>. Therefore, we developed a novel transgenic mouse line, Csf2rb2-Cre, to gain precise

62 genetic access to the SuM (SuM-Cre mouse; Fig. 2 and Extended Data Fig. 3). The pattern of  
63 Cre expression in this line, confirmed by crossing with a  $\beta$ -gal Cre reporter line<sup>12</sup> (Extended Data  
64 Fig. 3c, d), recapitulated the endogenous *Csf2rb2* pattern<sup>13</sup>, with robust expression in neurons of  
65 the SuM. Injection of Cre-dependent adeno-associated virus (AAV) vector expressing eYFP into  
66 the SuM resulted in a SuM-restricted signal (Fig. 2a, b) and strong terminal labeling in the  
67 pyramidal layer of CA2 and the granule cell layer of the DG in the hippocampus<sup>8</sup> (Fig. 2a, c and  
68 Extended Data Fig. 4). We further used tissue clearing techniques (ScaleS)<sup>14</sup> to image SuM  
69 projections in the whole brain and observed axons extending through the fornix superior and the  
70 fimbria to a wide dorsal-ventral range of DG and CA2 (Extended Data Fig. 5).

71 To ask if these pathways are anatomically segregated, we simultaneously injected Cre-dependent  
72 retrograde AAVs expressing eYFP and mCherry into the DG and CA2 of single mice (Fig. 2d, e  
73 and Extended Data Fig. 6a). The majority of the identified SuM neurons were found to project  
74 solely either to the DG ( $37.3 \pm 2.2\%$ ,  $N = 6$  mice,  $n = 2,700$  neurons) or CA2 ( $39.4 \pm 3.8\%$ ,  $N = 6$   
75 mice,  $n = 2,700$  neurons) with less than a quarter ( $23.3 \pm 2.7\%$ ,  $N = 6$  mice,  $n = 2,700$  neurons)  
76 dually infected (Fig. 2e). The high viral titer and proximity of the CA2 and DG injection sites  
77 raised the possibility that the dually infected population could be overestimated; thus we injected  
78 small volumes of Alexa Fluor 488 and Alexa Fluor 594 cholera toxin subunit B (CTB) into the  
79 DG and CA2, respectively, and found a very small fraction of doubly labeled neurons<sup>15</sup> ( $2.6 \pm$   
80  $0.5\%$ ,  $N = 3$  mice,  $n = 708$  neurons, Extended Data Fig. 6b). Further, single injections of  
81 retrograde AAV into CA2 or DG revealed robust fibers only in the injection region, with no  
82 collaterals or synaptic transmission detected in the other (Extended Data Fig. 7a–d), confirming  
83 that these pathways are largely segregated.

84 We next asked if novelty-driven SuM neurons project to hippocampus and whether novelty  
85 information with distinct features, e.g. contextual and social, is differentially routed. We bred the  
86 SuM-Cre mice with the Tet-Tag mouse line<sup>16</sup>, carrying the c-Fos-tTA transgene to permit long-  
87 term tagging of c-Fos activity in the SuM (Fig. 3a–d). These mice allowed dual-color labeling of  
88 temporally segregated active SuM ensembles, visualized by c-Fos-triggered viral eYFP  
89 expression (green) and anti-c-Fos immunohistochemistry (red), respectively. Consistent with our  
90 electrophysiological results, sequential presentation of two different types of novelty revealed  
91 significant segregation of active ensembles (Fig. 3c, d). For simultaneous circuit tracing and c-

92 Fos labeling, we injected retrograde AAVs expressing mCherry either into the DG or CA2 and  
93 AAV-TRE-DIO-eYFP into the SuM of the double-transgenic mice (Fig. 3e–j). Significantly  
94 higher c-Fos activity was observed in the DG-projecting population compared to the CA2-  
95 projecting population in mice exposed to contextual novelty (Fig. 3j, DG-projecting:  $39.2 \pm 1.8\%$   
96 versus CA2-projecting:  $16.3 \pm 1.1\%$ ). In contrast, in mice exposed to a novel social stimulus, c-  
97 Fos expression was significantly elevated in the CA2-projecting neurons compared to the DG-  
98 projecting ones (Fig. 3j, CA2-projecting:  $32.8 \pm 2.2\%$  versus DG-projecting:  $12.4 \pm 1.1\%$ ).  
99 Similar results were observed when anti-c-Fos immunohistochemistry was used to assay activity  
100 in SuM-Cre mice injected with retrograde AAVs either into the DG or CA2 for circuit labeling  
101 (Extended Data Fig. 8a–f).

102 To validate this novelty-specific projection bias, we conducted *in vivo* single unit recordings in  
103 the SuM (Fig. 3k–t). Single-unit activity was recorded in the SuM during low-frequency laser  
104 stimulation to induce antidromic spikes in mice with ChR2 expression in the SuM and optic  
105 fibers above DG and CA2 (Fig. 3l–q and Extended Data Fig. 8g–l), permitting projection-  
106 specific optogenetic identification of SuM neurons. The majority of the DG-projecting neurons  
107 (10 of 12 units) increased their firing rate in a novel context (Fig. 3r, example cells 1 and 2) and  
108 the average firing rate change across the population was higher with novel contextual exposure  
109 than during novel social exposure (Fig. 3s, contextual: 0.39 versus social: 0.13). In contrast, most  
110 CA2-projecting neurons (15 of 17 units) exhibited a larger firing rate increase in response to  
111 social novelty than to contextual novelty (Fig. 3r, example cells 3 and 4; Fig. 3t, social: 0.43  
112 versus contextual: 0.18).

113 The novelty-selective activation of the SuM-DG and SuM-CA2 circuits led us to assess whether  
114 manipulation of either circuit impacts hippocampal memory in a feature (contextual or social)-  
115 dependent way. We employed optogenetics to dissect the two circuits by delivering light to axon  
116 terminals in either the DG or CA2 (Fig. 4; Extended Data Fig. 7). Contextual behavior was  
117 assessed using a one-day habituation protocol in a novel high-walled enclosure (Fig. 4a and  
118 Extended Data Fig. 9). Following three habituation sessions (A1–A3), across which all groups  
119 decreased exploration (Fig. 4b–e) indicating familiarization to the environment<sup>17</sup>, light was  
120 delivered to SuM terminals in the hippocampus (Session A4 in Fig. 4a). Only mice in which the  
121 SuM-DG pathway was activated altered their behavior, increasing exploration compared to the

122 controls (Fig. 4b, relative travel distance:  $58.1 \pm 3.4\%$  versus  $39.6 \pm 5.6\%$ ), consistent with an  
123 increased perception of contextual novelty. We then altered the now familiar context by  
124 displacing the local objects and changing the visual cues on the walls (Session B1 in Fig. 4a,  
125 Extended Data Fig. 9a). Control mice reacted to this contextual novelty by increasing their  
126 exploration during the session, however this response was absent in mice in which the SuM  
127 terminals in the DG were inhibited (Fig. 4c, relative traveling distance: DG/eNpHR:  $38.1 \pm 4.6\%$   
128 versus DG/EYFP:  $71.6 \pm 3.8\%$ ), suggesting a deficiency in contextual novelty recognition. A  
129 final session (B2) in the altered context in the absence of light confirmed that once relieved from  
130 SuM-DG inhibition the mice could recognize the contextual change (Fig. 4c). Identical sets of  
131 experiments were performed with ChR2- or eNpHR-transfected mice in the absence of light  
132 delivery and no changes were found in behavior (Extended Data Fig. 9f–i). These results  
133 demonstrate that the novelty signal routed by the SuM to DG projection can modulate behavior  
134 based on hippocampal contextual memory.

135 Next we employed a direct interaction test to explore the impact of SuM inputs on social  
136 memory (Fig. 4f–k). Subject mice were first exposed to a novel mouse without light stimulation  
137 and interaction time was scored. One hour later mice were either re-exposed to the same mouse  
138 or exposed to a second novel mouse, with light delivered to the DG or CA2. In CA2-targeted  
139 mice we found that photostimulation caused a deficit in the expression of social memory, as  
140 these mice exhibited high levels of social interaction, resulting in a significantly higher  
141 habituation index compared to the controls (Fig. 4h, CA2/ChR2:  $0.116 \pm 0.081$  versus  
142 CA2/eYFP:  $-0.373 \pm 0.103$ ). Optogenetic manipulations targeting the DG, regardless of  
143 stimulation or inhibition, showed no effect on social interaction (Fig. 4g, j). These data suggest  
144 that the SuM to CA2 pathway can drive social novelty-linked exploratory behavior and routes  
145 social instead of contextual novelty to the hippocampus.

146 To understand how these projections influence the hippocampus we examined how the two  
147 discrete SuM-hippocampal circuits differ in neurotransmission (Extended Data Figs. 6c–h and  
148 10). Multiplex fluorescence *in situ* hybridization (RNAscope, Extended Data Fig. 6c–h) in mice  
149 injected with retrograde AAVs expressing eYFP into the DG or CA2 found that while Cre  
150 recombination occurs in both glutamatergic and GABAergic neurons, the majority of DG-  
151 projecting neurons are positive for both VGLUT2 and VGAT<sup>15,18,19</sup>, whereas most CA2-

152 projecting neurons are only VGLUT2+. Consistent with this, whole cell voltage-clamp  
153 recordings in acute hippocampal slices (Extended Data Fig. 10) revealed DG-projecting SuM  
154 neurons simultaneously release both glutamate and GABA (Extended Data Fig. 10f–j)<sup>15,18,19</sup>,  
155 while SuM-CA2 transmission is entirely glutamatergic (Extended Data Fig. 10k–o). While both  
156 inputs induced modest excitatory post-synaptic currents (EPSCs) in their target hippocampal  
157 principal neurons, they shared a robust feed-forward inhibitory component, evidenced by the  
158 large inhibitory post-synaptic currents (IPSCs) seen in DG granule cells (GCs) or CA2 pyramidal  
159 neurons (PNs) upon SuM axon stimulation. This regulation of the local excitation/inhibition (E/I)  
160 balance in the hippocampus has been speculated to contribute to the fine tuning of neuronal  
161 excitability and the dynamic range of neuroplasticity during mnemonic processing<sup>19-21</sup>. Thus,  
162 SuM transmission may provide an alternative and highly flexible mode of novelty signaling for  
163 shaping hippocampal encoding, in complement to the modulatory effects of monoaminergic  
164 innervation.

165 To better understand how the wide anatomical connectivity<sup>8</sup> of the SuM underlies this signaling  
166 we used projection-specific tracing to profile upstream inputs to the DG- and CA2-innervating  
167 SuM neurons (Extended Data Fig. 11). Both populations receive extensive inputs from  
168 subcortical regions, and although there were no significant statistical differences, inputs to the  
169 DG-projecting population were skewed towards brain regions in the reward and motor systems,  
170 while the CA2 projectors received proportionally more inputs from neurons in socially engaged  
171 regions, particularly the paraventricular hypothalamic nucleus<sup>22</sup> and medial preoptic area<sup>23</sup>  
172 (Extended Data Fig. 11g, h). This projection-specific connectivity may underlie the ability of the  
173 SuM to segregate contextual and novelty signals. In contrast to these diverse inputs, the  
174 ascending outputs of the hippocampal projecting SuM neurons are fairly restricted to the  
175 hippocampus (Extended Data Figs. 4 and 5). Therefore, the SuM may integrate internal states  
176 with cognitive input, extracting the contextual and social salience of stimuli and routing this  
177 signal for the modulation of mnemonic processing.

178 The observed selectivity of the SuM-DG and SuM-CA2 circuits supports the idea of specialized  
179 information processing along the longitudinal axis of the hippocampus<sup>24</sup>. The DG<sup>25-27</sup> can  
180 rapidly detect and encode contextual changes, while the loss of synaptic plasticity in DG GCs  
181 impairs context discrimination and novelty recognition<sup>28,29</sup> but not social memory<sup>30</sup>. Therefore,

182 the SuM input to DG GCs, via regulation of local E/I balance, may serve as a key facilitator of  
183 plasticity at the perforant pathway when new contextual information is encoded<sup>19</sup>. In contrast,  
184 interventions targeting pyramidal cells in CA2 have no impact on contextual learning, but rather  
185 result in profound changes in social memory<sup>31-36</sup>. We found that activation of the SuM input in  
186 CA2, which engages strong feed-forward inhibition (Extended Data Figs. 10), could induce a  
187 social novelty response (Fig. 4h), however inhibition could not impair an endogenous response  
188 (Fig. 4k), suggesting redundancy in this circuitry. We speculate that the SuM inputs to dorsal  
189 CA2 may shift local E/I balance and modulate downstream information flow in the hippocampus  
190 that favors the encoding of new social information in its ventral subregions.

191 Our work provides mechanistic evidence for a hypothalamic content-specific novelty signal that  
192 can contribute to shaping the engagement of distinct hippocampal subregions during encoding of  
193 different types of memory. SuM modulation is probably not the sole source of such distinct  
194 specificity; however, our findings demonstrate that within this small hypothalamic nucleus,  
195 distinct novelty features can be detected, segregated and transmitted. How differences in input  
196 circuitry permit novelty segregation in the SuM and how the signaling of segregated novelty  
197 modifies hippocampal activity on the physiological level during behavior await further  
198 investigation.

199

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313 **Author contributions**

314 S.C. conceived the study and mainly conducted experiments and analyzed data under guidance  
315 and supervision of T.J.M. S.C., L.H., Y.T., A.J.H. and T.J.M performed tracing and histology.  
316 S.C., L.H., M.E.W. and A.Z.W. performed behavioral experiments. S.C. performed in vivo  
317 electrophysiology and analyzed data; R.B. and L.H. contributed to data collection; D. P. and  
318 S.J.M contributed to data analysis. T.J.M. and A.J.H. generated the mouse lines. A.J.H. produced  
319 all AAVs. V.R., L.T., V.C. and R.A.P. performed in vitro electrophysiology,

320 immunohistochemistry and analysis. K.N., H. Hama and A.M. performed *Scale*; H. Hioki  
321 provided AAV vectors for *Scale*. All figures were prepared by S.C. with inputs from all authors.  
322 S.C. and T.J.M. wrote the manuscript. All authors discussed the manuscript.

323 **Data availability**

324 Data supporting the findings of this study are available from the corresponding authors upon  
325 reasonable request.

326 **Code availability**

327 Custom code used in this study is available from the corresponding authors upon reasonable  
328 request.

329 **Methods**

330 **Animals.** All mouse lines were maintained on a C57BL/6J background, bred in-house and raised  
331 in a temperature- and humidity-controlled room with a 12 h light/dark cycle (lights on from  
332 08:00 A.M. to 8:00 P.M.). All experimental protocols were approved by the RIKEN Institutional  
333 Animal Care and Use Committee, animal protocol approval W2019-2-045 (2) and recombinant  
334 DNA approval Sho2018-013(9), Sho2019-054(1), Sho2018-014(1).

335 **Generation of the SuM-Cre (*Csf2rb2*-Cre) mouse line.** The bacterial artificial chromosome  
336 (BAC) RP23-233L7 (BACPAC Resources Center) containing the colony stimulating factor 2  
337 receptor, beta2 (*csf2rb2*) was modified using the Quick & Easy BAC Modification Kit (Gene  
338 Bridges GmbH) to insert a Cre-FRT-Amp-FRT cassette at the start site of *csf2rb2* translation.  
339 The ampicillin (Amp) marker was deleted utilizing Flp-706 recombination and the Cre-modified  
340 BAC's were confirmed by PCR analysis. The confirmed Cre-modified RP23-233L7 BAC was  
341 subsequently purified using the QIAGEN Large Construct Kit (QIAGEN). To prepare the  
342 modified BAC for microinjection, 50 µg of Cre-modified RP23-233L7 BAC was linearized  
343 using AscI (New England Biolabs (NEB)) and then buffer exchanged using the Sepharose CL-  
344 4B matrix (Pharmacia/Pfizer) into an injection buffer composed of 10 mM Tris-HCl, pH 7.4, 0.1  
345 mM EDTA, and 100 mM NaCl. The purified BAC was injected into C57BL/6 fertilized  
346 pronuclei and fertilized blastocysts were implanted into pseudopregnant females. From the  
347 microinjections, four *Csf2rb2*-Cre B6 founders (lines 252, 267, 271, 272) were generated. The  
348 252 *Csf2rb2*-Cre line was determined to express Cre selectively in the supramammillary area of  
349 the hypothalamus using immunohistochemistry with the anti-Cre recombinase antibody  
350 (Millipore, MAB 3120). The 252 line was maintained in a pure C57BL/6 background for all the  
351 experiments herein described and male mice between the ages of 2 and 5 months were used for  
352 all experiments.

353 **Generation of the SuM-Cre/ *Rosa-NLSLacZ* mouse line.** The 252 *Csf2rb2*-Cre line was bred  
354 with the *Rosa-NLSLacZ*<sup>12</sup> line. The double transgenic 252 *Csf2rb2*-Cre x *Rosa-NLSLacZ* mouse  
355 line was confirmed with PCR genotyping for both the Cre and β-Gal genes and designated as  
356 R252. The two lines used to generate the double transgenic line were maintained in a pure  
357 C57BL/6 background for all the experiments herein described.

358 **Generation of the SuM-Cre/c-Fos-tTA mouse line.** The 252 *Csf2rb2-Cre* line was bred with  
359 the *Tg(Fos-tTA, Fos-EGFP\*)IMmay* line (JAX 018306)<sup>16</sup>. The double transgenic 252 *Csf2rb2-*  
360 *Cre* x *Tg(Fos-tTA, Fos-EGFP\*)IMmay* mouse line was confirmed with PCR genotyping for  
361 both the Cre and EGFP genes and designated as I252. The two lines used to generate the double  
362 transgenic line were maintained in a pure C57BL/6 background for all the experiments herein  
363 described and male I252 mice between the ages of 2 and 5 months were used for all experiments.

364 **Adeno-associated viruses (AAV) vector constructions.** The  
365 pAAV.EF1a.DIO.hChR2(H134R).EYFP and pAAV.EF1a.DIO.eNpHR3.0.EYFP recombinant  
366 AAV vectors were obtained from the Deisseroth Laboratory (Stanford University). The  
367 pAAV.SynTetOff.DIO.EGFP and pAAV.SynTetOff.DIO.mCherry recombinant AAV vectors  
368 were obtained from the Hioki Laboratory (Kyoto University).

369 The recombinant AAV vectors pAAV.EF1a.DIO.EYFP and pAAV.EF1a.DIO.mCherry were  
370 generated at our laboratory. For the construction of pAAV.EF1a.DIO.EYFP, the EYFP fragment  
371 was generated using the high fidelity Phusion DNA Polymerase (NEB). At the 5' end of the  
372 DNA fragment, the *NheI* restriction enzyme site is followed by the Kozak sequence and 5' ORF  
373 of the EYFP from the pAAV.DIO.hChR2(H134R).EYFP plasmid (Stanford University). The 3'  
374 end of the EYFP ORF contains the *AscI* restriction enzyme site. The PCR-generated cassette was  
375 digested with *NheI* (NEB) and *AscI* (NEB). The vector backbone for this construct was the  
376 pAAV.EF1a.hChR2(H134R).EYFP plasmid digested with *NheI* and *AscI* and treated with calf  
377 intestine phosphate (NEB). The approximately 5.7 kB DNA fragment containing the vector  
378 backbone was ligated to the digested EYFP ORF DNA fragment with T4 DNA ligase buffer  
379 (NEB) and transformed into One Shot Stbl3 Chemically Competent Cells (Invitrogen) and  
380 colonies were screened by restriction digest analysis. The putative positive colonies were  
381 submitted for DNA sequencing and one clone with the correct DNA sequence was designated as  
382 pAAV.EF1a.DIO.EYFP.

383 For the construction of pAAV.EF1a.DIO.mCherry, the mCherry fragment was generated using  
384 the high fidelity Phusion DNA Polymerase (NEB). At the 5' end of the DNA fragment, the *NheI*  
385 restriction enzyme site is followed by the Kozak sequence and 5' ORF of the mCherry from the  
386 pAAV.DIO.hChR2(H134R).mCherry plasmid (Stanford University). The 3' end of the mCherry  
387 ORF contains the *AscI* restriction enzyme site. The PCR-generated cassette was digested with

388 NheI (NEB) and AscI (NEB). The vector backbone for this construct was the  
389 pAAV.EF1a.hChr2(H134R).EYFP plasmid digested with NheI and AscI and treated with calf  
390 intestine phosphate (NEB). The approximately 5.7 kB DNA fragment containing the vector  
391 backbone was ligated to the digested EYFP ORF DNA fragment with T4 DNA ligase buffer  
392 (NEB) and transformed into One Shot Stbl3 Chemically Competent Cells (Invitrogen) and  
393 colonies were screened by restriction digest analysis. The putative positive colonies were  
394 submitted for DNA sequencing and one clone with the correct DNA sequence was designated as  
395 pAAV.EF1a.DIO.EYFP.

396 Subsequently, the recombinant AAV vector pAAV.TRE.DIO.EYFP was generated at our  
397 laboratory. For the construction of pAAV.TRE.DIO.EYFP, the TRE promoter fragment was  
398 generated by digestion of the pAAV.TRE.EYFP plasmid (previously described Chen, Weitemier,  
399 et al.) with MluI (NEB) and XbaI (NEB). The vector backbone for this construct was the  
400 pAAV.EF1a.DIO.EYFP plasmid digested with MluI and XbaI and treated with calf intestine  
401 phosphate (NEB). The approximately 4.8 kB DNA fragment containing the vector backbone was  
402 ligated to the digested TRE promoter DNA fragment with T4 DNA ligase buffer (NEB) and  
403 transformed into One Shot Stbl3 Chemically Competent Cells (Invitrogen) and colonies were  
404 screened by restriction digest analysis. The putative positive colonies were submitted for DNA  
405 sequencing and one clone with the correct DNA sequence was designated as  
406 pAAV.TRE.DIO.EYFP.

407 For the rabies virus experiments, the recombinant AAV vector pAAV.EF1a.DIO.TVA950 was  
408 generated at our laboratory. For the construction of pAAV.EF1a.DIO.TVA950, the EF1a  
409 promoter fragment was generated by digestion of the pAAV.EF1a.DIO.mCherry with MluI  
410 (NEB) and XbaI (NEB). The vector backbone for this construct was the  
411 pAAV.UbiquitinC.DIO.TVA950 (Johansen Laboratory, RIKEN Center for Brain Science)  
412 plasmid digested with MluI and XbaI and treated with calf intestine phosphate (NEB). The  
413 approximately 4.8 kB DNA fragment containing the vector backbone was ligated to the digested  
414 EF1a promoter DNA fragment with T4 DNA ligase buffer (NEB) and transformed into One Shot  
415 Stbl3 Chemically Competent Cells (Invitrogen) and colonies were screened by restriction digest  
416 analysis. The putative positive colonies were submitted for DNA sequencing and one clone with  
417 the correct DNA sequence was designated as pAAV.EF1a.DIO.TVA950.

418 Finally, the recombinant AAV vector pAAV.EF1a.DIO.H2B.GFP.2A.oG was generated at our  
419 laboratory. For the construction of pAAV.EF1a.DIO.TVA950, the EF1a promoter fragment was  
420 generated by digestion of the pAAV.EF1a.DIO.mCherry with MluI (NEB) and XbaI (NEB). The  
421 vector backbone for this construct was the pAAV.UbiquitinC.DIO.H2B.GFP.2A.oG (Johansen  
422 Laboratory, RIKEN Center for Brain Science) plasmid digested with MluI and XbaI and treated  
423 with calf intestine phosphate (NEB). The approximately 7.2 kB DNA fragment containing the  
424 vector backbone was ligated to the digested EF1a promoter DNA fragment with T4 DNA ligase  
425 buffer (NEB) and transformed into One Shot Stbl3 Chemically Competent Cells (Invitrogen) and  
426 colonies were screened by restriction digest analysis. The putative positive colonies were  
427 submitted for DNA sequencing and one clone with the correct DNA sequence was designated as  
428 pAAV.EF1a.DIO.H2B.GFP.2A.oG.

429 **AAV and Rabies Virus Preparation.** For adeno-associated virus production, we used the AAV  
430 Helper Free System (Agilent Technologies). The adeno-associated virus vectors,  
431 pAAV.DIO.EYFP, pAAV.TRE.DIO.EYFP, pAAV.DIO.hChr2(H134R).EYFP, and  
432 pAAV.DIO.eNpHR3.0.EYFP were individually co-transfected with pAAV-DJ/8 (Cell Biolabs),  
433 which supplies AAV2 replication proteins and AAV-DJ/8 capsid proteins, and pHelper (Agilent  
434 Technologies) which supplies the necessary adenovirus gene products required for the AAV  
435 production into the 293FT cell line (Invitrogen) utilizing the 293fectin transfection reagent  
436 (Invitrogen). The adeno-associated virus vectors, pAAV.DIO.EYFP, AAV.DIO.mCherry,  
437 pAAV.SynTetOff.DIO.EGFP, pAAV.SynTetOff.DIO.mCherry, pAAV.EF1a.DIO.TVA950 and  
438 pAAV.EF1a.DIO.H2B.GFP.2A.oG were individually co-transfected with retroAAV  
439 (Addgene), which supplies AAV2 replication proteins and retrograde AAV capsid proteins, and  
440 pHelper (Agilent Technologies) which supplies the necessary adenovirus gene products required  
441 for the AAV production into the 293FT cell line (Invitrogen) utilizing the 293fectin transfection  
442 reagent (Invitrogen). For the AAV.DJ/8 serotype AAV's, the supernatant was collected after 72  
443 hours and centrifuged at 3,000 rpm for 30 minutes and then filtered through a 0.45 $\mu$ M filtration  
444 unit (Millipore). For the retrograde serotype AAV's, the supernatant and cells were collected  
445 after 72 hours and subjected to 3 cycles of freeze-thaw at  $-80^{\circ}\text{C}$  and  $37^{\circ}\text{C}$ . The whole cell lysate  
446 and supernatant were then centrifuged at 3,000 rpm for 30 minutes and then filtered through a  
447 0.45 $\mu$ M filtration unit (Millipore). Subsequent purification of the DJ/8 serotype AAV and  
448 retrograde serotype AAV was carried out by ultracentrifugation (87,000 g,  $4^{\circ}\text{C}$ , 2 h) with a 20%

449 sucrose cushion. After ultracentrifugation, the supernatant was removed and the pellet was  
450 resuspended in phosphate-buffered saline (PBS), aliquoted and stored at  $-80^{\circ}\text{C}$  for long term  
451 storage. The AAV stocks were titered using a custom ordered AAV stock purchased from  
452 Virovek (Hayward, CA) as the reference standard. AAV titer quantification was performed using  
453 qPCR with the StepOne Plus Real Time PCR System (Applied Biosystems), FastStart Universal  
454 SYBR Green Master (Roche, Basel), and QPCR primers for an approximately 100 bp fragment  
455 of the woodchuck hepatitis virus posttranscriptional response element (WPRE) found in all our  
456 adeno-associated virus vectors. The titers for the five AAV stocks ranged between  $10^{12}$  to  $10^{13}$   
457 viral genome (vg)/mL for the DJ/8 serotype AAV's and between  $10^{11}$  to  $10^{12}$  viral genome  
458 (vg)/mL for the retrograde serotype AAV's. For the pseudotyped SAD rabies virus, the  
459 EnvA.RV.mCherry was generously provided by the Johansen Lab (RIKEN Center for Brain  
460 Science). The titer of the EnvA.RV.mCherry rabies virus was approximately  $10^9$  infectious units  
461 (I.U.)/mL.

462 **Stereotaxic injection and optic fiber implants.** All surgeries were performed in a stereotaxic  
463 frame (Narishige). Mice were anaesthetized using 500 mg/kg Avertin. AAV's were injected  
464 using a 10  $\mu\text{L}$  Hamilton microsyringe (701LT, Hamilton) with a beveled 33 gauge needle  
465 (NF33BL, World Precision Instruments (WPI)). A microsyringe pump (UMP3, WPI) and its  
466 controller (Micro4, WPI) were used to control the speed of the injection. The needle was slowly  
467 lowered to the target site and remained in place for 5 min before the beginning of the injection.  
468 AAVs were typically injected at a speed of 100 nL/min. The needle was removed 10 min after  
469 infusion was complete. Injections were targeted to the SuM ( $-2.7$  mm AP,  $+0.4$  mm ML,  
470  $-5.0$  mm DV), bilaterally to the DG ( $-1.9$  mm AP,  $\pm 1.2$  mm ML,  $-2.1$  mm DV) and CA2  
471 ( $-1.9$  mm AP,  $\pm 2.2$  mm ML,  $-1.8$  mm DV).

472 The same procedure was used for CTB injections. For the dual fluorescence tracing  
473 visualization, injections of Alexa Fluor 488 CTB (1 mg/mL, 100 nL, Thermo Fisher) were  
474 targeted bilaterally to the DG while the Alexa Fluor 594 CTB (1 mg/mL, 100 nL Thermo Fisher)  
475 injections were targeted bilaterally to CA2.

476 For retrograde tracing of upstream inputs to the DG and CA2 projecting SuM neurons,  
477 retrograde AAV.EF1a.DIO.TVA950 and retrograde AAV.EF1a.DIO.H2B.GFP.2A.oG,  
478 injections were targeted bilaterally to the DG or CA2. Twenty-one days post-AAV injections,

479 the EnvA.RV.mCherry SAD rabies virus was targeted to the SuM and performed as described  
480 for the stereotaxic AAV injections above.

481 Optic fibers (200  $\mu$ m core diameter) were bilaterally lowered above the target site (DG: -1.9 mm  
482 AP, +/- 1.2 mm ML, -1.7 mm DV; CA2: -1.9 mm AP, +/- 2.2 mm ML, -1.6 mm DV). The  
483 implant was secured to the skull with two jewelry screws and black opaque dental cement, which  
484 also prevented light leakage from the skull.

485 **Immunohistochemistry and histology.** Mice were transcardially perfused with 4%  
486 paraformaldehyde (PFA) in 0.1 M PBS (Nacalai Tesque). Brains were post-fixed in 4% PFA and  
487 50  $\mu$ m thick vibratome sections were prepared on a Vibratome (Leica).

488 Anti- $\beta$ -galactosidase immunohistochemistry. After 3 x 10 min PBS washes, the sections were  
489 blocked in TNB (Tris-NaCl blocking buffer) with 3% normal goat serum for 1.5 h, and incubated  
490 in primary antibody (goat anti- $\beta$ -galactosidase, 1:500, Abcam) at 4°C for 20 h. After 3 x 10 min  
491 washes in TNT (Tris-NaCl-Tween-20), the sections were incubated with secondary antibody  
492 (goat anti-chicken IgY, Alexa Fluor 488, 1:200, Abcam) for 2 h at room temperature followed by  
493 3 x 10 min washes in TNT. Fluorescence images were acquired on the Keyence BZ-X810 All-in-  
494 One fluorescence microscope with either a 4x or 10x objective.

495 Anti-GFP immunohistochemistry. Anti-GFP immunohistochemistry was performed to label  
496 SuM cells infected by retrograde AAV.EF1a.DIO.H2B.GFP.2A.oG injected into DG or CA2.  
497 After 3 x 10 min PBS washes, the sections were blocked in TNB (Tris-NaCl blocking buffer)  
498 with 3% normal donkey serum for 1.5 h, and incubated in primary antibody (goat anti-GFP,  
499 1:200, Abcam) at 4°C for 20 h. After 3 x 15 min washes in TNT (Tris-NaCl-Tween-20), the  
500 sections were incubated with secondary antibody (donkey anti-goat IgG, 1:200, Abcam) for 1 h  
501 at room temperature followed by 3 x 15 min washes in TNT. The sections were then incubated  
502 with a working solution for the TSA Plus Fluorescein Amplification Reagent (PerkinElmer) for  
503 10 min at room temperature followed by 3 x 5 min washes in TNT. Fluorescence images were  
504 acquired on the Keyence BZ-X810 All-in-One fluorescence microscope with either a 4x or 10x  
505 objective.

506 Anti-PCP4 immunohistochemistry. After 3 x 10 min PBS rinses, the sections were blocked in  
507 PBST (PBS + 0.3% Triton X-100) with 3% normal donkey serum for 1.5 h, and incubated in

508 primary antibody (rabbit anti-PCP4, 1:1000, Santa Cruz Biotechnologies) at 4°C for 20 h. After 3  
509 x 10 min washes in PBS, the sections were incubated with secondary antibody (donkey anti-  
510 rabbit, Alexa Fluor 594, 1:500, Life Technologies) for 2 h at room temperature followed by 3 x  
511 10 min washes in PBS. Confocal fluorescence images were acquired on an Olympus IX81  
512 confocal laser scanning microscope with a 10x or 20x objective.

513 Anti-c-Fos immunohistochemistry and histology. After 3 x 10 min PBS rinses, the sections were  
514 blocked in PBST (PBS + 0.3% Triton X-100) with 3% normal donkey serum for 1.5 h, and  
515 incubated in primary antibody (rabbit anti-c-Fos, 1:1000, Synaptic Systems) at 4°C for 20 h.  
516 After 3 x 10 min washes in PBS, the sections were incubated with secondary antibody (donkey  
517 anti-rabbit, Alexa Fluor 594, 1:500, Life Technologies) for 2 h at room temperature followed by  
518 3 x 10 min washes in PBS.

519 Confocal fluorescence images were acquired on an Olympus IX81 confocal laser scanning  
520 microscope with a 20x objective. To calculate the c-Fos+ cell density in Fig. 1b, three 50 µm-  
521 thick sections at representative SuM coordinates (AP: -2.5, -2.8 and -3.0 mm, as shown in Fig.  
522 2b) were prepared from each animal and imaged. The total number of c-Fos+ cells in the SuM  
523 across the three sections was counted and divided by the total area of the SuM region to derive  
524 the c-Fos+ cell density. Data from four animals was obtained for each condition (exposure to a  
525 familiar context, a novel context or a novel animal) and plotted. To analyze the overlap between  
526 fluorescent markers (anti-c-Fos and EYFP, mCherry and EYFP), a z-stack function was used to  
527 montage 5 optical stacks (1 µm each, step size 4 µm). Filtered fluorescent images were digitally  
528 combined to produce composite images and the number of cells with overlapping signals was  
529 counted in the region of interest (ROI) with ImageJ. The overlap ratio in Fig. 3d was calculated  
530 as (number of cells labeled in both green and red) / (total number of cells labeled in green). The  
531 overlap ratio in Fig. 3j was calculated as (number of cells labeled in both green and red) /  
532 (number of cells labeled in red). The overlap ratio in Extended Data Fig. 8f was calculated as  
533 (number of cells labeled in both green and red) / (number of cells labeled in green). For statistics,  
534 slices from at least 3 animals were obtained, imaged and analyzed. All imaging and analyses  
535 were performed blind to the experimental conditions.

536 **RNAscope.** Mice were transcardially perfused with 4% PFA in 0.1 M PBS (Nacalai Tesque).  
537 Brains were post-fixed in 4% PFA and 50 µm-thick vibratome sections were prepared on a

538 Vibratome (Leica). RNAscope hybridization was performed using the RNAscope Multiplex  
539 Fluorescent Reagent Kit v2 Assay (Advanced Cell Diagnostics). After  $4 \times 10$  min TBS washes,  
540 free-floating slices were treated with pretreatment solutions of proprietary compositions  
541 (Advanced Cell Diagnostics). First, the slices were placed RNAscope Hydrogen Peroxide for 30  
542 minutes at 25°C, followed by 2 x 1 min 1x TBS washes, and 2 x 1 min 0.5x TBS washes, the  
543 slices were mounted on Superfrost Plus Slides (Fisher Scientific) were baked in a dry oven for 1  
544 h at 60°C. The mounted slices were then placed in Target Retrieval Reagent for 15 min at 100–  
545 104°C, followed by washes in ddH<sub>2</sub>O and then 100% ethanol. The slices were baked in a dry  
546 oven for 30 min at 60°C. The slices were then placed in RNAscope Protease Plus for 30 min at  
547 40°C followed by 2x washes in ddH<sub>2</sub>O. The slices were incubated with the following trio of  
548 RNAscope probes, Mm-Slc17a6 (vGluT2), Mm-Slc32a1-C3 (vGAT), and EYFP-C2 (Advanced  
549 Cell Diagnostics) for 2 h at 40°C, followed by amplifying hybridization processes, AMP1,  
550 AMP2, and AMP3. The RNAscope probes were developed in the following order: Mm-Slc17a6  
551 HRP-C1.Opal 570, Mm-Slc32a1-C3.Opal 690, and lastly EYFP-C2.Opal 520 (Ayoka  
552 Biosciences). The slices were counterstained with RNAscope 4',6-diamidino-2-phenylindole  
553 (DAPI) (Advanced Cell Diagnostics) for 1 min at 25°C followed by 1 – 2 drops of Prolong Gold  
554 Antifade Mountant (Thermo Fisher) before placing coverslip and sealing slides. Fluorescence  
555 images were acquired on the Keyence BZ-X810 All-in-One fluorescence microscope with either  
556 a 4x or 10x objective.

557 **ScaleS and AbScale.** For selective plane illumination microscopy of whole brains, adult mice  
558 were deeply anesthetized with pentobarbital (Somnopentyl) and transcardially perfused with 4%  
559 PFA/PBS(-). The whole brain was extracted and subjected to post-fixation in 4% PFA/PBS(-) at  
560 4°C for 24 hrs. Then, the brain samples were cleared by ScaleS as described previously<sup>23</sup>.  
561 Observation was performed with a ZEISS Lightsheet Z.1 equipped with a 5x objective lens  
562 (Plan-Neofluor, NA = 0.16, RI = 1.45) and a 5x illumination lens (LSFM, NA = 0.1). The EYFP  
563 fluorescence was observed using a 488-nm diode laser and a 504–545 emission filter. A cleared  
564 sample was glued to the tip of a syringe (1 ml) and was positioned in front of the objective in the  
565 mounting medium (ScaleS4). Images were acquired every 6.126  $\mu$ m along the z-axis.

566 For c-Fos expression visualized by AbScale, a 0.8-mm-thick coronal brain slice was prepared  
567 from a SuM-Cre mouse infected with the Cre-dependent EYFP AAV. The EYFP fluorescent

568 images of 4%(w/v) PFA-fixed brain slices were acquired by a fluorescence stereo-microscope  
569 (Leica MZ16F) equipped with a 1× objective lens (Leica PLANAPO, NA = 0.141) and a color  
570 CCD camera (Zeiss AxioCam 506 color). The filters of ET-GFP (470 ± 20 nm) and ET-GFP  
571 (525 ± 25 nm) were used for excitation and emission, respectively. Then, AbScale was  
572 performed as described previously<sup>14</sup>. The brain slices were reacted with a Cy5-conjugated rabbit  
573 anti-cFos polyclonal antibody (F7799, Sigma) (1.67 µg protein/mL). Multiple adjacent regions in  
574 the slice were imaged with an upright laser scanning confocal microscopy system (Olympus  
575 FV1200) equipped with a motorized xy stage module in addition to a motorized focus module (z-  
576 drive), and a 10× dipping objective lens (Olympus XLPLN10XSVMP, NA = 0.6, WD = 6 mm,  
577 RI = 1.33–1.52). The EYFP fluorescence was observed using 473 nm laser. The Cy5  
578 fluorescence was observed using 635 nm laser. The adjacent regions were overlapped by 10%  
579 for precise alignment. The z step size was 10 µm.

580 ***In vivo* electrophysiology.** We recorded extracellularly from SuM neurons using custom-built  
581 screw-driven microdrives. The drives contain four to six independently adjustable nichrome  
582 tetrodes (14 µm), gold-plated to an impedance of 200 to 250 kΩ. The relative position of each  
583 tetrode exiting the cannulas on the bottom of the recording drive was mapped prior to surgery.  
584 The microdrive was implanted with the tetrodes placed approximately 4.5 mm ventral from the  
585 skull surface. Two days post-surgery, tetrodes were slowly lowered over several days to the  
586 SuM. As the tetrode were implanted at depth, we could ensure they entered the brain smoothly  
587 and perpendicularly, thus maintaining their relative positions even at their tip. During the  
588 adjustment period the animal was habituated every day in a same bucket for 15 min (Familiar  
589 Context) to ensure at least 10 days of habituation before the recordings started.

590 Recordings commenced once the SuM was reached based on the presence of theta oscillations  
591 and high amplitude spiking. At the start of each day the tetrodes were finely adjusted to  
592 maximize cell yield. Recordings started from 15-min exposure to the Familiar Context, followed  
593 by 5-min exposure to a Novel Context, a second 15-min exposure to the Familiar Context and a  
594 5-min exposure to a Novel Animal.

595 Neural signals and time stamps for behavior were recorded using a DigiLynx recording system  
596 running Cheetah v 5.7.4 acquisition software (Neuralynx). Broadband signals from each wire  
597 filtered between 0.1 and 9,000 Hz were recorded continuously at 32 kHz. The location and head

598 direction of the animal were accurately tracked by the red and green diodes affixed above the  
599 animal's head.

600 After recordings were completed, mice underwent terminal anesthesia with Avertin and electrode  
601 positions were marked by electrolytic lesioning of brain tissue (30 mA current for 5 s through  
602 each tetrode). After transcardial perfusion with 4% PFA brains were removed and post-fixed for  
603 another 24 hr in 4% PFA. Coronal slices (50  $\mu$ m thick) were prepared and inspected by standard  
604 light microscopy to confirm electrode placement. We cross-referenced the pattern of lesions  
605 across multiple sections with the tetrode positions mapped before the implantation of the  
606 recording drive to ensure precise positional assignment of each tetrode.

607 **Circuit-specific optogenetic identification and recordings.** The microdrive was fabricated in  
608 the same way as described above except for the inclusion of an optic fiber with precisely  
609 designed geometry to allow its placement above the CA2 or DG (DG:  $-1.9$  mm AP,  $\pm 1.2$  mm  
610 ML,  $-1.7$  mm DV; CA2:  $-1.9$  mm AP,  $\pm 2.2$  mm ML,  $-1.6$  mm DV). Same procedures for  
611 adjustment and recording were used, except for an additional optogenetic identification session  
612 in the beginning of the recording. In this session, trains of 10 light pulses ( $473$ nm,  $10$  mW/mm<sup>2</sup>)  
613 were delivered to the CA2 or DG, each 20-ms long, at 10 Hz. Spike shape of the SuM neurons  
614 was simultaneously measured using a broadband signal ( $0.1$ – $9,000$  Hz) sampled at 32 kHz.  
615 Recording then continued without laser pulses as the mice explored a familiar context, a novel  
616 context and a novel animal. Recording sites were later verified histologically with electrolytic  
617 lesions as described above and the position of the optic fiber was also verified from the track.

618 **Data analysis.** Spikes were manually sorted with off-line software SpikeSort3D (Neuralynx).  
619 The mean firing rate of each neuron was calculated using the first 5-min of data recorded in each  
620 exposure condition. When two Familiar Context epochs were recorded in one recording, the  
621 firing rates of each neuron in both epochs were averaged to be the mean. Putative excitatory  
622 neurons (pEN) and inhibitory interneurons (pIN) were separated by the K-means clustering  
623 algorithm. In addition to a threshold of meaning firing rate (neurons with firing rate  $<10$  Hz in all  
624 exposures were identified to be pEN), another two parameters, half-width of the spike and trough  
625 to peak duration, were taken into account. pENs have longer trough-to-peak durations and half-  
626 widths compared to pINs.

627 To determine whether a neuron's firing rate in the Novel Context or Novel Animal epoch was  
628 significantly different from that in the previous Familiar Context epoch, the spike train was  
629 circularly shifted by a random distance after removing the time gap between the two epochs by  
630 swapping a single spike at the joint. This shuffling process was repeated 1,000 times and the  
631 firing rate difference between the two shuffled epochs was calculated, to generate a *P*-value:

$$632 \quad P = (n+1)/(r+1)$$

633 where *r* represents the total number of shuffles (1,000) and *n* is equal to the number of shuffles  
634 with a firing rate difference between the two epochs greater than or equal to the unshuffled  
635 value. Cells with a *P* < 0.05 were considered to have a significantly different firing rate from that  
636 in the Familiar Context epoch.

637 For calculating the change of the novelty response with time, firing rates of individual neurons in  
638 the three different exposures were binned in 30-s bins and normalized to the average Familiar  
639 Context firing rate of all identified neurons.

640 To identify individual DG- or CA2-projecting neurons by antidromic spiking, we used a  
641 combination of criteria including (a) early latency (< 20 ms from the onset of the light pulse), (b)  
642 high fidelity (in response to > 50% of light pulses), and (c) mean light-evoked waveform closely  
643 resembling the spontaneous waveform. Mean firing rates of the optogenetically identified cells in  
644 the Familiar Context, Novel Context and Novel Animal epochs were calculated and compared.

645 **Behavioral tests and optogenetics.** For the contextual memory test, a one-day contextual  
646 habituation protocol was used. Mice expressing ChR2, eNpHR or EYFP and with fibers  
647 delivering light to the DG or CA2 went through six contextual exposure session: Each was 5 min  
648 with 15 min intersession intervals. Mice were first placed in a novel high-walled square  
649 enclosure containing 2 objects and their movement was tracked by ANY-maze (Stoelting) as  
650 they explored for 5 min (Session A1). Following a 15 min intersession interval, mice were  
651 returned to the same context for three additional 5 min sessions (A2, A3 and A4) with 15  
652 minutes intervals in between. Only during Session A4 light (circuit activation: 473 nm, 10 Hz, 30  
653 ms pulse duration, 10 mW; circuit inhibition: 532 nm, constant, 5 mW) was bilaterally delivered  
654 to CA2 or DG for circuit manipulation. After a 15 minutes interval, mice were placed to a novel  
655 context for 5 min exploration with light bilaterally delivered to CA2 or DG (Session B1). Again,

656 after a 15-minute interval, mice were placed back to the context for a final 5 min session in the  
657 absence of light delivery (Session B2).

658 For social memory tests, a direct interaction test was used. Mice were first habituated in a  
659 context for ten days (15 min per day). On the day of social familiarity test, subject mice were  
660 exposed to a novel juvenile male mouse in the familiar context where the mice had been  
661 habituated and allowed to interact for 5 min without light stimulation and interaction time was  
662 scored. Following a 1 hour inter-trial interval the mice were re-exposed to the same juvenile  
663 demonstrator, with the appropriate wavelength light (circuit activation: 473 nm, 10 Hz, 30 ms  
664 pulse duration, 10 mW; circuit inhibition: 532 nm, constant, 5 mW) bilaterally delivered to CA2  
665 or DG for circuit manipulation, and interaction time was scored. Habituation index was  
666 calculated as (time spent exploring the familiar mouse minus time spent exploring the stimulus  
667 mouse) / (time spent exploring the familiar mouse plus time spent exploring the stimulus mouse).  
668 In a social novelty test, the same procedure was used except that in the second trial mice were  
669 required to interact with a novel juvenile male mouse with light delivered. Habituation index was  
670 calculated as (time spent exploring the novel mouse minus time spent exploring the stimulus  
671 mouse) / (time spent exploring the novel mouse plus time spent exploring the stimulus mouse).

672 **In vitro electrophysiology.** For stereotaxic viral injection, animals were anaesthetized with  
673 ketamine (100 mg/kg) and xylazine (7 mg/kg). The adeno-associated virus  
674 AAV9.EF1a.DIO.hChR2(H134R).EYFP was used at  $3 \times 10^8$  vg/mL,  
675 AAV.retro.DIO.hChR2.EYFP was used at  $1.5 \times 10^{11}$  vg/mL. 500 nL of virus was injected into  
676 the brain of 4-week-old male csf2rb2-Cre (SuM-Cre) mice at 100 nL/min and left at the injection  
677 site for 10 min following infusion. The loci of the injection sites were as follows: for medial  
678 SuM, -2.8 mm anterior-posterior relative to bregma (A/P); 0 mm medial-lateral relative to  
679 midline (M/L); 4.75 mm dorsal-ventral relative to surface of the brain (D/V). For CA2, -1.7 A/P,  
680 1.9 mm M/L, 1.6 mm D/V. For DG, -2.0 A/P, 1.2 mm M/L, 2.1 mm D/V.

681 Transverse hippocampal slices were prepared at least 3 weeks after viral injection and whole-cell  
682 patch-clamp recordings were performed from CA2 PNs and DG GCs. Animals were  
683 anaesthetized with ketamine (100 mg/kg) and xylazine (7 mg/kg), and perfused transcardially  
684 with a N-methyl-D-glucamin-based (NMDG) cutting solution containing the following (in mM):  
685 NMDG 93, KCl 2.5, NaH<sub>2</sub>PO<sub>4</sub> 1.25, NaHCO<sub>3</sub> 30, HEPES 20, glucose 25, thiourea 2, Na-

686 ascorbate 5, Na-pyruvate 3, CaCl<sub>2</sub> 0.5, MgCl<sub>2</sub> 10. Brains were then rapidly removed, hippocampi  
687 were dissected and placed upright into an agar mold and sliced with a vibratome (Leica  
688 VT1200S) in the same cutting solution into 400 μm thick transverse slices at 4°C. Slices were  
689 transferred to an immersed-type chamber and maintained in artificial cerebro-spinal fluid  
690 (ACSF) containing the following (in mM) : NaCl 125, KCl 2.5, NaH<sub>2</sub>PO<sub>4</sub> 1.25, NaHCO<sub>3</sub> 26,  
691 glucose 10, Na-pyruvate 2, CaCl<sub>2</sub> 2, MgCl<sub>2</sub> 1. Slices were incubated at 32°C for approximately  
692 20 min then maintained at room temperature for at least 45 min prior to transfer to a recording  
693 chamber perfused with ACSF at 5 mL/min at 30°C. Whole-cell patch-clamp recordings were  
694 performed with a cesium-based intracellular solution containing the following (in mM): Cs-  
695 methyl sulfonate 130, KCl 5, EGTA-KOH 0.1, HEPES 10, NaCl 2, MgATP 5, Na<sub>2</sub>GTP 0.4, Na<sub>2</sub>-  
696 phosphocreatine 10 and biocytin (4 mg/mL). ChR2 expressed in SuM axonal fibers was excited  
697 by 488 nm light delivered by a LED attached to the epifluorescence port of the microscope.  
698 Light stimulation trains consisted of 2 to 10 pulses that were 0.5 ms long, delivered at 10 Hz and  
699 repeated every 10–20 s for at least 20 sweeps. When blocking action potentials with TTX, light  
700 pulse duration was increased to 5 ms to allow direct depolarization of pre-synaptic SuM  
701 terminals (Extended Data Fig. 10). We used a light intensity of 25 mW/mm<sup>2</sup> which was  
702 experimentally determined to be the minimal intensity allowing TTX-sensitive maximal  
703 responses in all cell types. Data were obtained using a Multiclamp 700B amplifier, sampled at 10  
704 kHz and digitized using a Digidata 1550B interface. Series resistance was < 20 MOhms and was  
705 continuously monitored in voltage-clamp mode. Liquid junction potential offset of  
706 approximately 10 mV was not corrected for. Pharmacological agents were added to ACSF at the  
707 following concentrations (in μM): 10 NBQX and 50 D-APV to block AMPA and NMDA  
708 receptors, 1 SR95531 and 2 CGP55845A to block GABAA and GABAB receptors, 100 4-  
709 Aminopyridine (4-AP) to block K<sub>v</sub>1 potassium channels, 0.2 TTX to prevent action potential  
710 generation.

711 Midbrains containing the injection site were always examined post-hoc to ensure that infection  
712 was restricted to the SuM. Post-hoc reconstruction of neuronal morphology and SuM axonal  
713 projections were performed on slices and midbrain tissue following overnight incubation in 4%  
714 PFA in PBS. Midbrain sections were sagittally re-sliced to 100 μm thick sections. Because  
715 internal recording solutions contained Cs<sup>+</sup>, we examined the cellular morphology and location of  
716 all cells post-hoc to ensure data were from CA2 PNs and DG GCs (Extended Data Fig. 10).

717 Slices were permeabilized with 0.2% Triton in PBS and blocked overnight with 3% goat serum  
718 in PBS with 0.2% Triton. Primary antibody incubation was carried out in 3% goat serum in PBS  
719 overnight at 4°C. Channelrhodopsin-2 was detected by chicken primary antibody to GFP  
720 (Abcam; 1:10,000 dilution) and Alexa Fluor 488-conjugated goat-anti chicken secondary (Life  
721 Technologies, 1:300 dilution). Incubations with secondary antibodies, Alexa Fluor 594-  
722 conjugated streptavidin (Life Technologies, 1:500 dilution) and Far-red Neurotrace (Life  
723 Technologies, 1:300 dilution), were carried out in block solution for 4 hours at room  
724 temperature. After a mounting with ProlongDiamond, images were collected with a Zeiss 710 or  
725 Leica SP5 laser-scanning confocal microscope.

726 Electrophysiological recordings were analyzed using IGORpro (Wavemetrics) and Clampfit  
727 (Molecular devices) software. For accurate measurements of the kinetics and latencies of post-  
728 synaptic responses, the following detection process was used. For each cell, average traces were  
729 used to create a template waveform that was then fitted to individual traces and measurements  
730 were performed on the fitted trace. When only amplitudes of responses were measured, standard  
731 average peak detection was used. Results are reported  $\pm$  s.e.m. Statistical significance was  
732 assessed using unpaired- or paired-Student's T test, Mann-Whitney U test, Wilcoxon signed-rank  
733 test, or Kolmogorov-Smirnoff test where appropriate.

734 **Statistics and reproducibility.** All statistical analyses were performed with GraphPad Prism 7,  
735 Excel 2010 (Microsoft) or custom MATLAB codes. The sample sizes were chosen based on  
736 common practice in similar animal experiments<sup>7,10,11,18,19,28,29,33-36</sup>. Experimenters were blind to  
737 genotype, region targeted and optical protocol during injections and experiments, as well as  
738 during histological and behavioral analysis. For all experiments mice were randomly assigned as  
739 to which virus they received, the injection location and protocol used. Data were first tested for  
740 normality. When normality or equal variance between samples was achieved, parametric tests  
741 such as one-way ANOVA with Turkey's post hoc test or *t*-test were used. Where normality or  
742 equal variance of samples failed, nonparametric tests such as Wilcoxon signed rank test were  
743 performed. All values were presented as mean  $\pm$  s.e.m. **Representative example data in all figures**  
744 **was selected from multiple biological replicates; Fig. 1b: 1 of 4 mice, Fig. 1f: 6 example neurons**  
745 **of 119 units recorded from 17 mice, Fig. 2a, b: 1 of 10 mice, Fig. 2c: 1 of 3 mice, Fig. 2d, e: 1 of**  
746 **6 mice, Fig. 3c, g, i: 1 of 3 mice, Fig. 3r: 4 example neurons of 29 units recorded from 19 mice,**

747 Extended Data Fig. 1b: 1 of 4 mice, Extended Data Fig. 2b: 1 of 17 mice, Extended Data Fig. 3c,  
748 d: 1 of 3 mice, Extended Data Fig. 4a: 1 of 10 mice, Extended Data Fig. 4b: 1 of 3 mice,  
749 Extended Data Fig. 5a, b: 1 of 3 mice, Extended Data Fig. 6a: 1 of 6 mice, Extended Data Fig.  
750 6b: 1 of 3 mice, Extended Data Fig. 6e, f: 1 of 2 mice, Extended Data Fig. 7a: 1 of 6 mice,  
751 Extended Data Fig. 7b: 1 of 7 mice, Extended Data Fig. 8c, e: 1 of 3 mice, Extended Data Fig.  
752 8h: 1 of 8 mice, Extended Data Fig. 8k: 1 of 11 mice, Extended Data Fig. 11c, e: 1 of 4 mice,  
753 Extended Data Fig. 11d, f: 1 of 5 mice.

754 **Figure 1 | SuM responds to contextual and social novelty.** **a**, Following habituation mice are  
755 exposed to a novel context or a novel animal. **b**, c-Fos immunostaining in the SuM of mice after  
756 exposure to a familiar context, a novel context or a novel animal. Scale bar, 200  $\mu\text{m}$ . **c**,  
757 Contextual and social novelty induce significant c-Fos expression in the SuM. One-way  
758 ANOVA with Tukey's post hoc test,  $n = 4$  mice for all groups, **\*\*\*\* $P < 0.0001$** . Open circles  
759 represent values of individual mice. **d**, Behavioral recording protocol; sequential exposure to a  
760 familiar context, a novel context and a novel animal. **e**, Tetrodes targeting SuM. **f**, Example  
761 raster plots. Cells 1 and 2 were activated in a novel context, 3 and 4 were activated by a novel  
762 animal, and 5 and 6 responded to both contextual and social novelty. FR, firing rate. **g**, Mean FR  
763 of all pEN units ( $n = 119$  cells from 17 mice) during novelty. One-way repeated measures  
764 ANOVA with Tukey's post hoc test, **\* $P = 0.0240$ , \*\*\*\* $P < 0.0001$** . **h**, **i**, Scatter plots of the FRs  
765 of all pEN units during novel contextual (**h**) and novel social (**i**) exposures, plotted against FRs  
766 during familiar contextual exposure. Colored dots indicate neurons with significant increase in  
767 FR. Pie chart summarizes fraction of population. **j**, Venn diagram of overlap between  
768 subpopulations with significantly increased activity during novel contextual and novel social  
769 exposures. **k**, SuM neurons with a significant FR increase after exposure to a novel context (blue,  
770  $n = 26$  units) or animal (red,  $n = 25$  units) displayed habituation. Black curve represents FR  
771 change of the union of the above two subsets of neurons ( $n = 39$  units) in a familiar context. All  
772 error bars and shaded areas show mean  $\pm$  s.e.m.

773

774 **Figure 2 | SuM-Cre mice reveal robust SuM-hippocampal projections.** **a**, Left: SuM  
775 injection of Cre-dependent AAV in SuM-Cre mice resulted in SuM-restricted eYFP (green)  
776 expression and strong terminal labeling in the DG and CA2 of the hippocampus. Right: Extent of  
777 axonal terminals (eYFP, green) of SuM neurons in hippocampus along the rostrocaudal axis,  
778 referenced to adapted atlas images. Scale bars, 1 mm. **b**, Cre-dependent eYFP expression in the  
779 SuM of SuM-Cre mice. Numbers indicate posterior distances from bregma. Scale bars, 200  $\mu\text{m}$ .  
780 **c**, Left: Coronal hippocampal section showing axonal terminals of SuM neurons (eYFP, green)  
781 and hippocampal neurons immunostained with anti-PCP4 (red). Scale bar, 500  $\mu\text{m}$ . Right:  
782 Magnified images from boxes on the left highlighting SuM axons targeting granule cells in the  
783 DG and pyramidal neurons in CA2, both PCP4 positive (red). Scale bars, 50  $\mu\text{m}$ . **d**, **e**, Bilateral

784 injection of retroAAV-DIO-mCherry to hippocampal DG and retroAAV-DIO-EYFP to CA2 of  
785 the SuM-Cre mice selectively labels DG-projecting SuM neurons with mCherry and CA2-  
786 projecting SuM neurons with eYFP. Axons targeting the hippocampus are shown in **d** (scale bar,  
787 500  $\mu\text{m}$ ), and soma in the SuM are shown in **e** (scale bars, 200  $\mu\text{m}$ ). Numbers in **e** indicate  
788 posterior distances from bregma.

789

790 **Figure 3 | DG- and CA2-projecting SuM neurons respond to contextual and social novelty,**  
791 **respectively.** **a**, Experimental design to examine the overlapping SuM populations that respond  
792 to two novelty exposures. **b**, Double transgenic mice (SuM-Cre/c-Fos-tTA) were injected with  
793 AAV-TRE-DIO-EYFP. **c**, Images of overlapping populations in the SuM activated by two  
794 novelty exposures. On the right of each image is a magnified view of the area within the white  
795 box (200  $\times$  200  $\mu\text{m}$ ). **d**, eYFP+/c-Fos+ overlap SuM cell counts for the conditions shown in **c**.  
796 One-way ANOVA with Tukey's post hoc test,  $n = 3$  mice for all groups, **\*\*\*\* $P < 0.0001$** . **e**,  
797 Protocol to examine the projection specificity of SuM populations that respond to novel  
798 contextual or social exposure. **f**, **h**, Strategy to simultaneously label DG- (**f**) or CA2- (**h**)  
799 projecting SuM cells using retroAAV-DIO-mCherry and novelty-responsive cells using AAV-  
800 TRE-DIO-EYFP with the SuM-Cre/c-Fos-tTA mice. **g**, **i**, Images of overlapping populations in  
801 the SuM that were activated by contextual or social novelty and projected to the DG or CA2. On  
802 the right of each image is a magnified view of the area within the white box (200  $\times$  200  $\mu\text{m}$ ). **j**,  
803 eYFP+/mCherry+ overlap SuM cell counts for the conditions shown in **g** and **i**. One-way  
804 ANOVA with Tukey's post hoc test,  $n = 3$  mice for all groups, **\*\*\* $P = 0.0004$ , \*\*\*\* $P < 0.0001$** .  
805 **k**, Protocol for a recording session to optogenetically identify DG- or CA2-projecting SuM  
806 neurons. **l**, **o**, Viral injection, optic fiber and tetrodes implantation. **m**, **p**, Peristimulus time  
807 histograms of representative single units photoidentified as SuM-DG (**m**) and SuM-CA2 (**p**)  
808 projectors. **n**, **q**, Waveforms of spontaneous (grey) and light-evoked antidromic (blue) spikes of  
809 the single units in **n** and **q**. **r**, Raster plots of sample neurons. Cells 1 and 2 projecting to DG  
810 were more active in a novel context, and Cells 3 and 4 projecting to CA2 preferentially  
811 responded to a novel animal. FR, firing rate. **s**, **t**, Calculated FR changes of DG-projecting (**s**,  $n =$   
812 12 putative excitatory neurons from 8 mice, **\*\* $P = 0.0024$** ) and CA2-projecting (**t**,  $n = 17$   
813 putative excitatory neurons from 11 mice, **\*\*\* $P = 0.0003$** ) SuM neurons in response to

814 contextual and social novelty. FR change = (FR in Contextual or Social minus FR in Familiar) /  
815 (FR in Contextual or Social plus FR in Familiar). Two-tailed Wilcoxon matched-pairs signed  
816 rank test. All error bars show mean  $\pm$  s.e.m. Open circles are values from individual mice. Scale  
817 bars, 200  $\mu$ m.

818

819 **Figure 4 | SuM-hippocampal circuits selectively modulate contextual and social memory. a,**  
820 Protocol used for contextual memory tests. **b–e,** Distance traveled during each contextual  
821 exposure normalized to Session A1 under optogenetic manipulations of SuM-hippocampal  
822 circuits. Light was delivered in Sessions A4 and B1 to the DG or CA2. Two-way ANOVA with  
823 Bonferroni post hoc test,  $n = 11$  mice (DG/ChR2) versus  $n = 11$  (DG/eYFP) in **b** ( $*P = 0.0119$ ,  $F$   
824  $(20, 100) = 2.961$ ),  $n = 11$  (DG/eNpHR) versus  $n = 9$  (DG/eYFP) in **c** ( $****P < 0.0001$ ,  $F(18,$   
825  $90) = 3.490$ ),  $n = 11$  (CA2/ChR2) versus  $n = 9$  (CA2/eYFP) in **d**,  $n = 10$  (CA2/eNpHR) versus  $n$   
826  $= 12$  (CA2/eYFP) in **e**. **f,** Protocol used for social familiarity tests. **g, h,** Habituation index with a  
827 familiar animal under various optogenetic manipulations of SuM-hippocampal circuits. Two-  
828 tailed unpaired  $t$ -tests,  $n = 13$  mice (DG/ChR2) versus  $n = 9$  (DG/eYFP),  $n = 10$  (DG/eNpHR)  
829 versus  $n = 11$  (DG/eYFP) in **g**,  $n = 17$  (CA2/ChR2) versus  $n = 19$  (CA2/eYFP),  $n = 10$   
830 (CA2/eNpHR) versus  $n = 13$  (CA2/eYFP) in **h**,  $***P = 0.0008$ ,  $t(34) = 3.665$ . **i,** Protocol used  
831 for social novelty tests. **j, k,** Habituation index with a novel animal under various optogenetic  
832 manipulations of SuM-hippocampal circuits. Two-tailed unpaired  $t$ -tests,  $n = 13$  mice  
833 (DG/ChR2) versus  $n = 9$  (DG/eYFP),  $n = 10$  (DG/eNpHR) versus  $n = 11$  (DG/eYFP) in **j**,  $n = 13$   
834 (CA2/ChR2) versus  $n = 10$  (CA2/eYFP),  $n = 16$  (CA2/eNpHR) versus  $n = 12$  (CA2/eYFP) in **k**.  
835 n.s., nonsignificant. Error bars show mean  $\pm$  s.e.m. Boxes denote median (centre line), 25th and  
836 75th percentiles (box edges), and full range of values (whiskers).

837

838 **Extended Data Figure 1 | Identification of hypothalamic regions activated by contextual**  
839 **and social novelty. a,** Counts of c-Fos+ cells in hypothalamic regions that show response to  
840 contextual and social novelty.  $*P = 0.0140$ ,  $**P = 0.0023$ ,  $***P = 0.0009$ ,  $****P < 0.0001$ . n.s.,  
841 nonsignificant. Two-way ANOVA with Tukey's post hoc test,  $n = 4$  mice for all groups. Error  
842 bars indicate mean  $\pm$  s.e.m. Open circles are values from individual mice. **b,** Example c-Fos  
843 immunostaining (red) in the hypothalamus of one mouse from a total of 4 replicates at various

844 distances from bregma along the rostrocaudal axis of mice 1.5 h after exposure to a familiar  
845 context, a novel context or a novel animal. AHN, anterior hypothalamic nucleus; DMH, dorsal  
846 medial hypothalamic nucleus; LHA, lateral hypothalamic area; LPO, lateral preoptic area; MM,  
847 medial mammillary nucleus; MPN, medial preoptic nucleus; MPO, medial preoptic area; PH,  
848 posterior hypothalamic nucleus; PVH, paraventricular hypothalamic nucleus; SuM,  
849 supramammillary nucleus; VMH, ventromedial hypothalamic nucleus. Scale bars, 500  $\mu$ m.

850

851 **Extended Data Figure 2 | Histology of recorded animals and firing rate analysis of**  
852 **interneurons.** **a**, Tetrode recordings in the SuM. **b**, An example coronal section of the SuM from  
853 one of 17 mice used for recording with tetrode locations indicated by arrows. Scale bar, 400  $\mu$ m.  
854 **c**, Reconstructed tetrode tip locations (71 in target and 14 off target) from 17 animals overlaid  
855 onto schematic mouse brain slices. Numbers indicate posterior distances from bregma. **d**,  
856 Behavioral protocol for a recording session, including sequential exposure to a familiar context,  
857 a novel context and a novel animal. **e**, Mean FR of all pIN (putative interneuron,  $n = 22$  from 17  
858 mice) units during different exposures. FR, firing rate. One-way repeated measures ANOVA.  
859 n.s., nonsignificant. **f**, **g**, Scatter plots of the FRs of all IN units during novel contextual (**f**) and  
860 novel social (**g**) exposures, plotted against FRs during familiar contextual exposure. Colored dots  
861 indicate interneurons that showed a significant increase in FR. Horizontal and vertical green bars  
862 indicate 10 Hz, the low threshold of FR to classify interneurons. Pie graphs show the percentage  
863 of pIN units that exhibited significant increase, significant decrease, or no change in FR during  
864 the respective novelty exposure. **h**, Venn diagram of overlap between subpopulations with  
865 significantly increased activity during novel contextual and novel social exposures.

866

867 **Extended Data Figure 3 | Generation and characterization of the SuM-Cre mouse line. a,**  
868 Recombination strategy for SuM-specific expression of Cre recombinase driven by the *csf2rb2*  
869 promoter. Diagram shows the schematic of the *csf2rb2* promoter.Cre.FRT.AmpR.FRT construct.  
870 **b,** Example anti-Cre immunostaining in the SuM of the SuM-Cre mouse (one of three replicates).  
871 Dotted white line indicates the anatomical boundary of the SuM. Scale bar, 500  $\mu$ m. **c, d,**  
872 Coronal (**c**) and sagittal (**d**) sections (scale bars, 2 mm) prepared from an adult progeny (age 12  
873 weeks) of SuM-Cre and Rosa-NLSLacZ crosses and immunostained with anti- $\beta$ -galactosidase  
874 ( $\beta$ -gal, recombination marker). Representative images of from all brain regions where  
875 recombination is identified are shown from one mouse of four replicates of each. Dotted white  
876 lines indicate the boundaries of these brain regions. **On the right of each image in c is a**  
877 **magnified view of the area within the white box (scale bars, 1 mm).** BLA, basolateral amygdala;  
878 LHA, lateral hypothalamic area; PAG, periaqueductal gray; PF, parafascicular thalamic nucleus;  
879 PH, posterior hypothalamic nucleus; RL, rostral linear nucleus raphe; SuM, supramammillary  
880 nucleus. Scale bars, 2 mm.

881

882 **Extended Data Figure 4 | Brain-wide mapping of projections from SuM neurons. a,** Coronal  
883 sections prepared from a SuM-Cre mouse injected with AAV-DIO-EYFP into the SuM. AcbSh,  
884 accumbens nucleus, shell; AHN, anterior hypothalamic nucleus; CM, central medial thalamic  
885 nucleus; DG, dentate gyrus; DP, dorsal peduncular cortex; fi, fimbria of hippocampus; IAM,  
886 interanteromedial thalamic nucleus; IG, induseum griseum; LHA, lateral hypothalamic area;  
887 LHb, lateral habenular nucleus; LPO, lateral preoptic area; LS, lateral septal nucleus; MD,  
888 mediodorsal thalamic nucleus; MDI, mediodorsal thalamic nucleus, lateral part; MPO, medial  
889 preoptic area; MS, medial septal nucleus; PH, posterior hypothalamic nucleus; RE, nucleus of  
890 reuniens; SFi, septofimbrial nucleus; SHi, septohippocampal nucleus; SuM, supramammillary  
891 nucleus; vhc, ventral hippocampal commissure. Example is one mouse of four replicates. **b,**  
892 Sagittal sections prepared from a SuM-Cre mouse injected with AAV-DIO-EYFP into the SuM.  
893 Example shown is one mouse of 3 replicates. Scale bars, 500  $\mu$ m.

894

895 **Extended Data Figure 5 | Examination of projections (a) and activity (b) of SuM neurons**  
896 **by clearing and rapid 3D imaging with light sheet microscopy using the ScaleS method. a,**

897 Whole-brain examination of SuM projections. AAV-DIO-EYFP was injected to the SuM of  
898 SuM-Cre mice to label SuM projections by Cre-dependent EYFP expression. Acb, accumbens  
899 nucleus; DG, dentate gyrus; EC, entorhinal cortex; SuM, supramammillary nucleus. Scales: 14.6  
900 mm (x) × 17.1 mm (y) × 7.5 mm (z) for horizontal views and 11.7 mm (x) × 17.6 mm (y) × 9.0  
901 mm (z) for the sagittal view. One example of 3 replicates is shown. **b**, AbScale shows EYFP+/c-  
902 Fos+ neurons in the SuM after exposure to contextual (left, Mouse #252-1263) or social novelty  
903 (right, Mouse #252-1265). 800 μm thick coronal slices were cleared and c-Fos was stained by  
904 AbScale, followed by rapid 3D imaging with laser-scanning confocal microscopy. One example  
905 of 3 replicates is shown. Scale bars in the top panel, 1 cm. Scale bars in other panels, 500 μm.

906

907 **Extended Data Figure 6 | Retrograde tracing of SuM neurons projecting to DG and CA2. a**,  
908 Collateral projections of DG and CA2-projecting SuM neurons. Dual-color Cre-dependent  
909 retrograde AAVs were used to label the cell bodies and axons of SuM neurons projecting to DG  
910 (mCherry, red) and CA2 (EYFP, green) (see also Fig. 2d, e). Weak collateral projections from  
911 DG and CA2-projecting SuM neurons could be seen in the medial (MS) and lateral septal  
912 nucleus (LS) and lateral hypothalamic area (LHA). One example of 6 replicates is shown. Scale  
913 bars, 500 μm. **b**, Dual-color cholera toxin subunit B (CTB) labeling was used to trace the cell  
914 bodies of SuM neurons projecting to DG (CTB 488, green) and CA2 (CTB 594, red). The  
915 majority of the identified SuM cell bodies were found to project solely either to the DG or CA2  
916 with only  $2.6 \pm 0.5\%$  ( $N = 3$  mice,  $n = 708$  neurons) projecting to both. Scale bars, 250 μm. **c, d**,  
917 Strategy for multiplex fluorescent in situ hybridization (RNAscope) to identify VGLUT2 and  
918 VGAT mRNAs present in the cell bodies of DG- and CA2-projecting SuM neurons. Cre-  
919 dependent retrograde AAV was used to label the cell bodies and axons of SuM neurons  
920 projecting to DG (**c**) and CA2 (**d**). **e, f**, DG- (**e**) and CA2-projecting (**f**) SuM neurons were  
921 stained by antisense probes directed against VGLUT2 (570 nm), VGAT (690 nm), and EYFP  
922 (520 nm) mRNAs. Scale bars, 500 μm. **g, h**, Quantification of EYFP+/VGLUT2+/VGAT+ and  
923 EYFP+/VGLUT2+/VGAT- neurons in the DG- (**g**) and CA2-projecting (**h**) SuM neurons.  $n = 2$   
924 animals for each group. All the DG-projecting and CA2-projecting neurons are VGLUT2+. The  
925 majority (average: 71.3%, Animal 1: 72.2%, Animal 2: 70.3%) of DG-projecting neurons are

926 VGAT+, while only a small portion (average: 13.1%, Animal 1: 12.3%, Animal 2: 13.8%) of  
927 CA2-projecting neurons are VGAT+.

928

929 **Extended Data Figure 7 | Validation of the functional segregation of SuM-DG and SuM-**  
930 **CA2 circuits. a, b**, Strategies of whole-cell voltage clamp recording experiments to validate the  
931 physiological segregation of SuM-DG and SuM-CA2 circuits. Retrograde AAV-DIO-ChR2-  
932 EYFP was bilaterally injected into the DG (**a**) or CA2 (**b**) of SuM-Cre mice to infect DG- (**a**) or  
933 CA2-projecting (**b**) SuM cells. Two weeks later, acute transverse hippocampal slices were  
934 prepared and whole-cell voltage clamp recordings were performed in CA2 pyramidal neurons  
935 (PNs, **a**) or DG granule cells (GCs, **b**) in order to determine if the DG-projecting SuM neurons  
936 also innervated CA2 cells (**a**), and vice versa (**b**). All recorded cells were filled with biocytin for  
937 post-hoc identification. Lower panels are confocal images of transverse hippocampal slices  
938 recorded with Nissl (blue), ChR2-EYFP expressing SuM fibers (green), and biocytin-filled (red)  
939 CA2 PN (**a**) or DG GC (**b**). Scale bars, 250  $\mu\text{m}$ . **c, d**, Example light-evoked IPSCs (10 traces,  
940 light red; average trace, red) and light-evoked EPSCs (10 traces, grey; average trace, black)  
941 recorded with 5 light pulses in CA2 PNs (**c**) and DG GCs (**d**). No inhibitory or excitatory light-  
942 evoked current was detected for any of the 17 CA2 PNs from 6 mice and 19 DG GCs from 7  
943 mice, suggesting that DG-projecting SuM cells do not innervate CA2, and vice versa. **e, f**,  
944 Strategies of behavioral experiments to examine the effect from light bleed-through on the  
945 optogenetic dissection of SuM-DG and SuM-CA2 circuits. RetroAAV-DIO-ChR2 was  
946 bilaterally injected into the DG (**e**) or CA2 (**f**) of SuM-Cre mice to infect DG- (**e**) or CA2-  
947 projecting (**f**) SuM cells. Optic fiber was bilaterally implanted in the uninjected region of each  
948 mouse: DG-ChR2 with fibers in CA2 (**e**) or CA2-ChR2 with fibers in DG (**f**). **g–j**, Contextual (**g**,  
949 **h**) and social (**i**, **j**) memory tests. Light was delivered to the uninfected region of the  
950 hippocampus: DG-ChR2 with light delivery to CA2 (**g**, **i**) or CA2-ChR2 with light delivery to  
951 DG (**h**, **j**). Two-way ANOVA with Bonferroni post hoc test,  $n = 8$  mice (DG/ChR2) versus  $n = 8$   
952 (DG/eYFP) in **g**,  $n = 8$  mice (CA2/ChR2) versus  $n = 8$  (CA2/eYFP) in **h**. Two-tailed unpaired  $t$ -  
953 tests,  $n = 8$  mice (DG/ChR2) versus  $n = 8$  (DG/eYFP) in **i**,  $n = 8$  mice (CA2/ChR2) versus  $n = 8$   
954 (CA2/eYFP) in **j**. n.s., nonsignificant. Error bars show mean  $\pm$  s.e.m. **Boxes denote median**  
955 **(centre line), 25th and 75th percentiles (box edges), and full range of values (whiskers).**

956

957

958 **Extended Data Figure 8 | Circuit-specific c-Fos mapping and optogenetic identification and**

959 **recording. a**, Experimental design to examine the projection specificity of SuM populations that

960 respond to novel contextual or novel social exposure. **b, d**, Strategy to label DG- (**b**) or CA2- (**d**)

961 projecting SuM cells using retroAAV-DIO-EYFP with the SuM-Cre mice. **c, e**, Images of

962 overlapping populations in the SuM that had contextual or social novelty-induced c-Fos

963 expression and projected to the DG or CA2. On the right of each image is a magnified view of

964 the area within the white box ( $200 \times 200 \mu\text{m}$ ). One example of 3 replicates is shown. **f**,

965 EYFP+/c-Fos+ overlap SuM cell counts for the conditions shown in **c** and **e**. DG-projecting SuM

966 neurons were preferentially activated by contextual novelty (DG-projecting:  $53.3 \pm 6.1\%$  versus

967 CA2-projecting:  $37.1 \pm 0.4\%$ ,  $*P = 0.0429$ , one-way ANOVA with Tukey's post hoc test,  $n = 3$

968 mice for both groups), whereas CA2-projecting neurons responded more to social novelty (CA2-

969 projecting:  $50.2 \pm 1.8\%$  versus DG-projecting:  $25.4 \pm 2.7\%$ ,  $**P = 0.0042$ , one-way ANOVA

970 with Tukey's post hoc test,  $n = 3$  mice for both groups). All error bars show mean  $\pm$  s.e.m. Open

971 circles are values from individual mice. Scale bars,  $200 \mu\text{m}$ . **g, j**, Strategies for optogenetic

972 identification and recording DG- (**g**) and CA2-projecting (**j**) neurons in the SuM. **h, k**, Example

973 coronal sections of the SuM with tetrode locations indicated by arrows. Scale bars,  $200 \mu\text{m}$ . **i, l**,

974 Histology of recorded animals for circuit-specific optogenetic identification and recording.

975 Reconstructed tetrode tip locations overlaid onto schematic mouse brain slices are shown.

976 Numbers indicate posterior distances from bregma. 35 tetrodes were in target and 8 out of target

977 from 8 mice for recording DG-projecting neurons, while 49 tetrodes were in target and 9 out of

978 target from 11 mice for recording CA2-projecting neurons.

979

980 **Extended Data Figure 9 | Behavioral tests for contextual memory. a**, Familiar (A1-A4 in **b**)

981 and novel contexts (B1 and B2 in **b**) used for contextual memory tests. **b**, Behavioral protocol.

982 **c–e**, Example traveling trajectories of animals during each contextual exposure described in **b**

983 under different optogenetic manipulations of the SuM-DG circuit. **f–i**, Percentage of distances

984 traveled during each contextual exposure under various optogenetic manipulations. Light was

985 delivered in Sessions A4 and B1 to the DG or CA2. The no-light data were used as the control,

986 in supplementary to the EYFP controls in Fig. 4b–e. Two-way ANOVA with Bonferroni post  
987 hoc test,  $n = 11$  mice (DG/ChR2/light) versus  $n = 11$  (DG/ChR2/no light) in **f** (\*\* $P = 0.0013$ ,  $F$   
988  $(20, 100) = 2.882$ ),  $n = 11$  (DG/eNpHR/light) versus  $n = 11$  (DG/eNpHR/no light) in **g** (\*\*\*\* $P <$   
989  $0.0001$ ,  $F(20, 100) = 2.402$ ),  $n = 11$  (CA2/ChR2/light) versus  $n = 11$  (CA2/ChR2/no light) in **h**,  
990  $n = 10$  (CA2/eNpHR/light) versus  $n = 10$  (CA2/eNpHR/no light) in **i**. All error bars show  
991 mean  $\pm$  s.e.m.

992

993 **Extended Data Figure 10 | Synaptic transmission from SuM terminals to DG granule cells**  
994 **(GCs) and CA2 pyramidal neurons (PNs).** **a**, Identification of direct glutamatergic  
995 transmission in CA2 from SuM inputs. (left) Diagram illustrating the whole-cell recordings of  
996 hippocampal pyramidal neurons (PNs) and SuM fiber stimulation in acute slice preparation.  
997 Cesium-based intracellular solution was used in the recording pipette and all cells were recorded  
998 in voltage-clamp mode with a membrane potential held at  $-70$  mV in order to record excitatory  
999 glutamatergic transmission. (right) Diagram mapping the location of all PNs recorded in the  
1000 hippocampus with closed circles indicating connected cells and open circles indicating  
1001 unconnected cells with no detected transmission. Cells were classified as CA1 ( $n = 11$ , all  
1002 unconnected), CA2 ( $n = 46$  connected,  $n = 30$  unconnected) and CA3 ( $n = 19$  connected,  $n = 29$   
1003 unconnected) based on morphology (the presence of thorny excrescences for CA3) and location.  
1004 Circle color indicates cell type with CA2 PNs black, CA3 PNs brown and CA1 PNs orange. **b**,  
1005 Identification of feed-forward inhibitory transmission in CA2 from SuM inputs. (left) Diagram  
1006 illustrating the local circuitry involved in the feed-forward inhibition recruited by SuM inputs in  
1007 the hippocampus. The same recording configuration was used as shown in **a**, except that cells  
1008 were held at  $+10$  mV in order to measure feed-forward inhibitory transmission. (right) Diagram  
1009 mapping PNs in the hippocampus that receive feed-forward transmission from light-evoked SuM  
1010 inputs with unconnected cells shown as open circles and connected cells as filled circles. No  
1011 CA1 PNs received feed-forward inhibition ( $n = 11$ ) while over half of CA2 PNs received  
1012 detectable feed-forward inhibition ( $n = 30$  connected,  $n = 18$  unconnected) as well as a fraction  
1013 of CA3 PNs ( $n = 20$  connected,  $n = 18$  unconnected). **c**, Identification of excitatory and  
1014 inhibitory transmission to DG granule cells (GCs) from SuM inputs. (left) Diagram illustrating  
1015 the experimental setup to measure local excitatory and inhibitory circuitry recruited by SuM

1016 inputs in the DG. The same recording configuration was used as shown in **a** and **b**. Cells were  
1017 held at -70 mV to measure excitatory transmission and then at +10 mV. Inhibitory transmission  
1018 was measured before and after the addition of 10  $\mu$ M NBQX & 50  $\mu$ M APV to distinguish feed-  
1019 forward and direct inhibitory transmission. (right) Diagram mapping GCs in the DG that receive  
1020 excitatory and inhibitory (both feed-forward and direct) transmission from light-evoked SuM  
1021 inputs. Blue filled circles mark the location of cells that received both excitatory and inhibitory  
1022 transmission ( $n = 21$ ). All cells received both direct and feed-forward inhibition. Yellow-filled  
1023 circles mark cells that did not receive detectable excitatory transmission, but both direct and  
1024 feed-forward inhibitory transmission ( $n = 3$ ). Data for panels **a-c**. were collected from 156  
1025 slices from 92 mice. Abbreviations: so, stratum oriens; sp, stratum pyramidale; sr, stratum  
1026 radiatum; mf, mossy fiber; ml, molecular layer; gcl, granule cell layer. **d**, Inverted epifluorescent  
1027 image of a hippocampal slice with a DG GC and a CA2 PN. Each neuron had been recorded and  
1028 filled with biocytin which was stained with streptavidin-conjugated fluorophore. **e**, Dendritic  
1029 (blue) and axonal (red) reconstruction of the cells in **d** with the hippocampal regions demarcated.  
1030 **f**, Diagram illustrating the local circuitry and whole-cell recording configuration of DG GCs in  
1031 acute brain slices prepared from SuM-Cre mice injected with AAV-DIO-ChR2-EYFP. SuM  
1032 axonal terminals were illuminated by a 0.5 ms 488 nm light stimulation. IN, interneuron. **g**,  
1033 Sample traces of light-evoked EPSCs recorded at -70 mV (individual traces in grey, average  
1034 trace in black) and IPSCs recorded at +10 mV (individual traces in light red, average trace in red)  
1035 in a same DG GC under voltage clamp. The blue line denotes when the light stimulus was  
1036 applied. **h**, Normalized cumulative distribution of latencies for DG GC EPSCs (black) and IPSCs  
1037 (red), both displaying response latencies consistent with direct monosynaptic transmission ( $4.1 \pm$   
1038  $0.3$  ms for EPSCs,  $n = 20$  GCs;  $4.16 \pm 0.3$  ms for IPSCs,  $n = 22$  GCs; Student's *t*-test,  $P = 0.94$ ;  
1039 Kolmogorov-Smirnov test,  $P = 0.97$ ). **i**, **j**, Sample traces (**i**) and time course of amplitudes (**j**,  
1040 IPSCs only,  $n = 22$  GCs from 8 mice) of light-evoked EPSCs (black) and IPSCs (red) recorded  
1041 in DG GCs before and after application of 10  $\mu$ M NBQX & 50  $\mu$ M APV (grey), and further  
1042 application of 1  $\mu$ M SR95531 & 2  $\mu$ M CGP55845A (green). AMPA and NMDA receptor  
1043 blockers completely blocked EPSCs ( $13 \pm 3.1$  pA,  $n = 20$  GCs), but only partially blocked IPSCs  
1044 (by 36%,  $86 \pm 21$  pA before,  $31 \pm 7.2$  pA following NBQX and APV addition, **\*\* $P = 0.0037$** ,  
1045 Wilcoxon signed-rank test,  $n = 22$  GCs from 8 mice), indicating that there is feed-forward  
1046 inhibition recruited by SuM inputs, accompanied by a larger amount of direct inhibitory

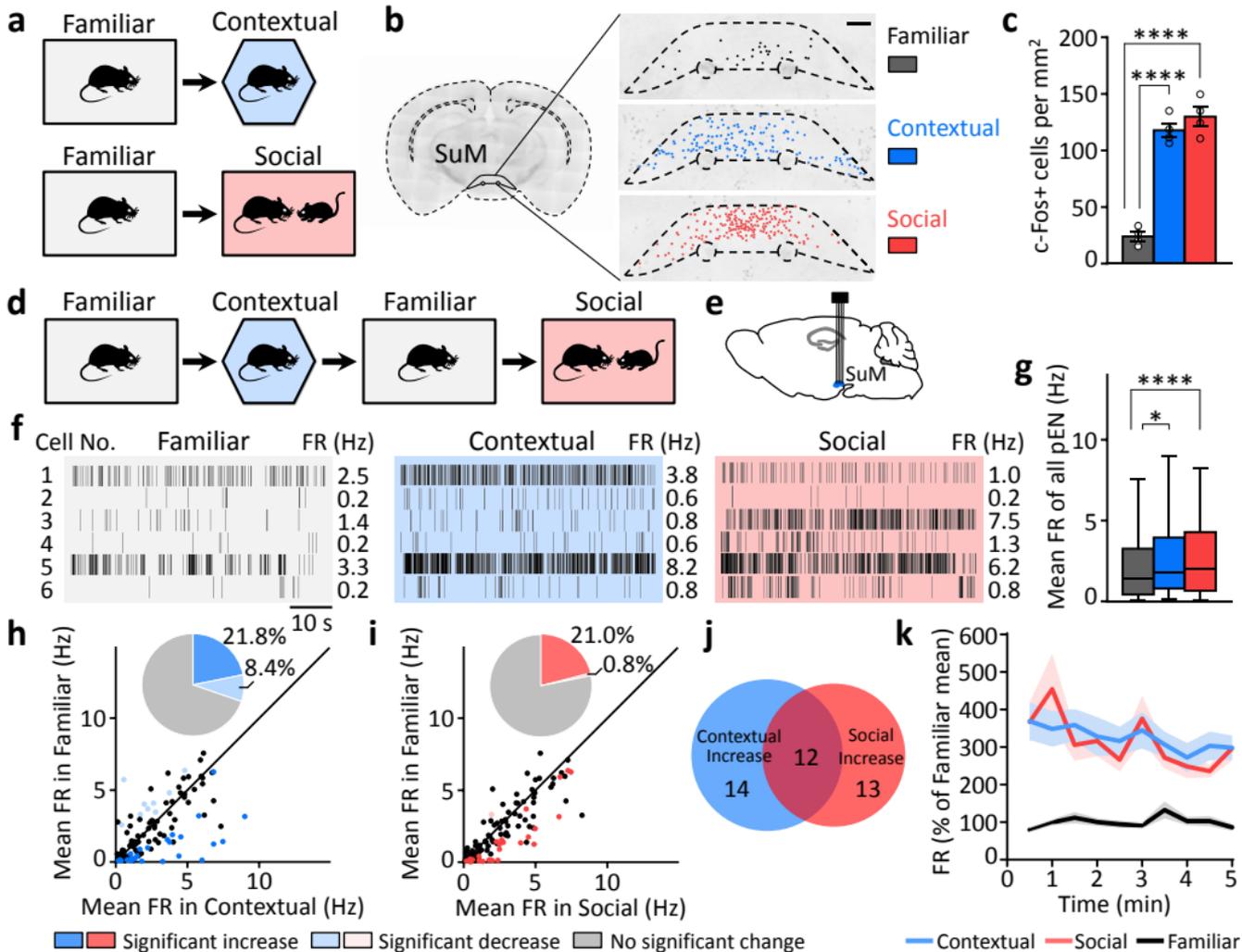
1047 transmission. The remaining light-evoked IPSCs were entirely blocked by the subsequent  
1048 addition of GABAA and GABAB receptor blockers, suggesting that DG-projecting neurons in  
1049 the SuM are capable of simultaneously releasing both glutamate and GABA. **k**, Diagram similar  
1050 to **f**, showing the local circuitry and whole-cell recording configuration of CA2 PNs. **l**, Sample  
1051 traces of EPSCs recorded at  $-70$  mV (individual traces in grey, average trace in black) and  
1052 IPSCs recorded at  $+10$  mV (individual traces in light red, average trace in red) in a same CA2  
1053 PN under voltage clamp. Note the increased latency of IPSCs onset compared to EPSCs. **m**,  
1054 Normalized cumulative distribution of latencies for CA2 PN EPSCs (black) and IPSCs (red).  
1055 IPSCs displayed significantly different response latencies from the EPSCs, with a longer IPSC  
1056 response latency consistent with bi-synaptic feed-forward inhibition ( $2.9 \pm 0.1$  ms for EPSCs,  $n$   
1057  $= 166$  PNs;  $6.2 \pm 0.4$  ms for IPSCs,  $n = 69$  PNs; \*\*\*\* $P < 0.0001$ , Mann–Whitney U test;  
1058 Kolmogorov–Smirnov test, \*\*\*\* $P < 0.0001$ , data from 92 mice). **n**, **o**, Sample traces (**n**) and  
1059 time course of amplitudes (**o**, IPSCs only,  $n = 7$  from 4 mice) of light-evoked EPSCs (black) and  
1060 IPSCs (red) recorded in CA2 PNs before and after application of  $10 \mu\text{M}$  NBQX &  $50 \mu\text{M}$  APV  
1061 (grey). Both the EPSCs ( $16 \pm 4.8$  pA,  $n = 6$  PNs from 4 mice) and IPSCs ( $167 \pm 40$  pA,  $n = 7$   
1062 PNs from 4 mice) were completely blocked by the application of AMPA and NMDA receptor  
1063 blockers, indicating that the synaptic transmission from the SuM is entirely glutamatergic in  
1064 CA2. Notably, both SuM-DG and SuM-CA2 transmissions recruit a robust feed-forward  
1065 inhibition. **p**, Confirmation of SuM-CA2 transmission by the addition of tetrodotoxin (TTX) and  
1066 4-aminopyridine (4-AP) to isolate transmitter release resulting from direct optical terminal  
1067 depolarization. Application of TTX & 4-AP abolishes IPSCs and spares EPSCs in CA2  
1068 pyramidal neurons (PNs), consistent with mono-synaptic excitation and di-synaptic inhibition.  
1069 Control sample traces are shown in black (EPSC) and red (IPSC), while traces following  
1070 application of TTX & 4-AP are shown in grey. **q**, Time course of light-evoked EPSC and IPSC  
1071 amplitudes upon application of  $0.2 \mu\text{M}$  TTX &  $100 \mu\text{M}$  4-AP in which a longer light stimulus  
1072 was used to directly depolarize the SuM axonal terminal. Initial amplitudes were  $20.2 \pm 6.1$  pA  
1073 for EPSCs and  $110 \pm 50$  pA for IPSCs, and  $9.3 \pm 7.7$  pA for EPSCs and  $4.8 \pm 0.7$  pA for IPSCs  
1074 following TTX & 4-AP, indicating a  $61 \pm 24$  % block of EPSCs and  $88 \pm 4.2$  % block of IPSCs  
1075 by TTX & 4-AP.  $P = 0.22$  for EPSCs,  $P = 0.016$  for IPSCs, Wilcoxon signed-rank test,  $n = 7$   
1076 PNs from 3 mice. All error bars show mean  $\pm$  s.e.m.

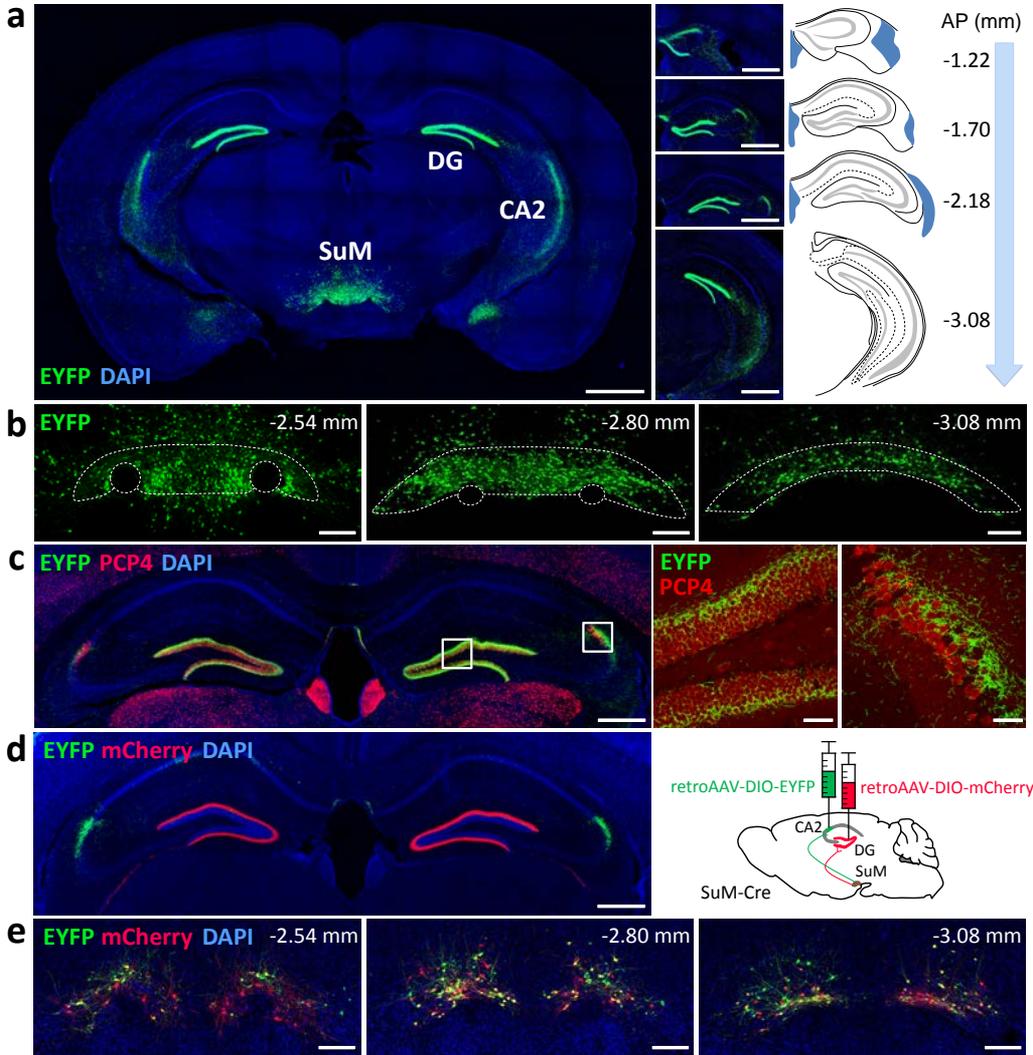
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1078 **Extended Data Figure 11 | Retrograde tracing of upstream inputs to the DG- and CA2-**  
1079 **projecting SuM neurons. a, b,** Strategies of projection-specific retrograde tracing to map the  
1080 upstream inputs to the DG- (**a**) and CA2-projecting (**b**) neurons. Retrograde AAV helper viruses  
1081 expressing the rabies G protein and the TVA receptor are injected into DG (**a**) or into CA2 (**b**),  
1082 and three weeks later EnvA pseudotyped rabies virus expressing mCherry is injected into the  
1083 SuM. This allows for the retrograde labeling of upstream neurons that send efferent axons to  
1084 DG- or CA2-projecting SuM cells by the rabies virus. **c, d,** Coronal sections showing DG- (**c**)  
1085 and CA2-projecting (**d**) starter cells in the SuM. Cells in red (mCherry) are TVA+ cells that  
1086 express rabies virus (one example of four mice for DG injections, one example of five mice for  
1087 CA2 injections). Cells in green (GFP) express oG (optimized glycoprotein). The  
1088 mCherry+/GFP+ overlapping cells (yellow) are starter cells where the *trans*-complementation of  
1089 RVdG with oG results in the production of oG+RVdG that spreads trans-synaptically to input  
1090 cells. Scale bar, 1 mm. **e, f,** Coronal sections with trans-synaptically labeled input cells upstream  
1091 to the DG- (**e**) and CA2-projecting (**f**) SuM neurons. Scale bars, 1 mm. **g, h,** Quantification of  
1092 inputs from various brain regions to the DG- (**g**,  $n = 4$  mice) and CA2-projecting (**h**,  $n = 5$  mice)  
1093 SuM neurons. Both populations received extensive inputs from subcortical regions including the  
1094 hypothalamus, brainstem, septum and nucleus accumbens. However, the inputs to the DG-  
1095 projecting population were comparatively biased to brain regions in the reward and motor  
1096 systems, such as the VTA, SI, AcbSh, LS and MS, while the CA2 projectors received  
1097 proportionally greater inputs from neurons in socially engaged regions, particularly the PVH ( $*P$   
1098  $= 0.0159$ , Mann-Whitney U test) and MPO. All error bars show mean  $\pm$  s.e.m. LS, lateral septal  
1099 nucleus; MS, medial septal nucleus; SI, substantia innominata; AcbSh, accumbens nucleus, shell;  
1100 ZI, zona incerta; LHA, lateral hypothalamic area; MPO, medial preoptic area; LPO, lateral  
1101 preoptic area; PVH, paraventricular hypothalamic nucleus; PH, posterior hypothalamic nucleus;  
1102 PAG, periaqueductal gray; MRN, midbrain reticular nucleus; VTA, ventral tegmental area;  
1103 Raphe: DR, dorsal raphe nucleus & MnR, median raphe nucleus.

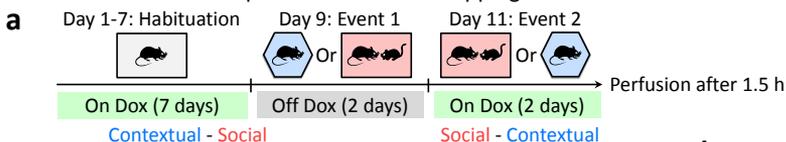
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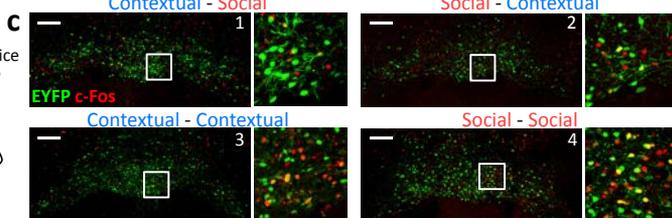
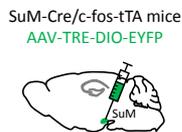




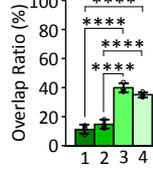
## Event-specific c-Fos+ cells mapping



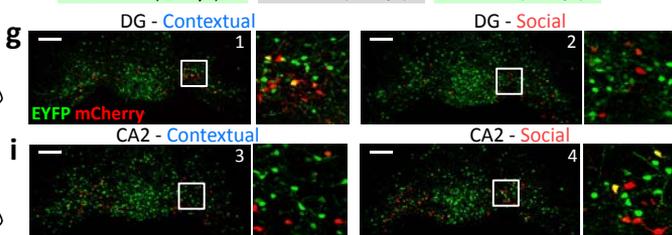
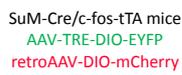
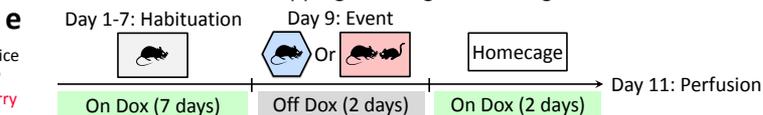
**b**



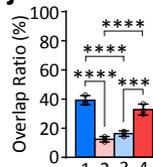
**d**



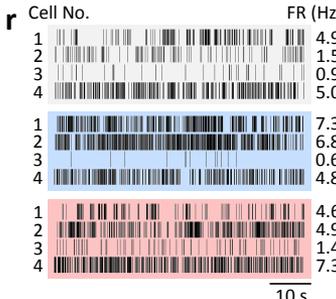
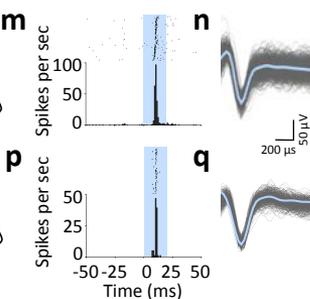
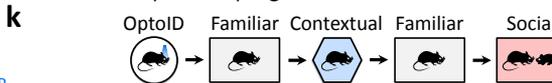
## c-Fos+ cells mapping & retrograde tracing



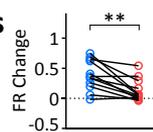
**j**



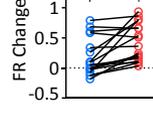
## Circuit-specific optogenetic identification and recording

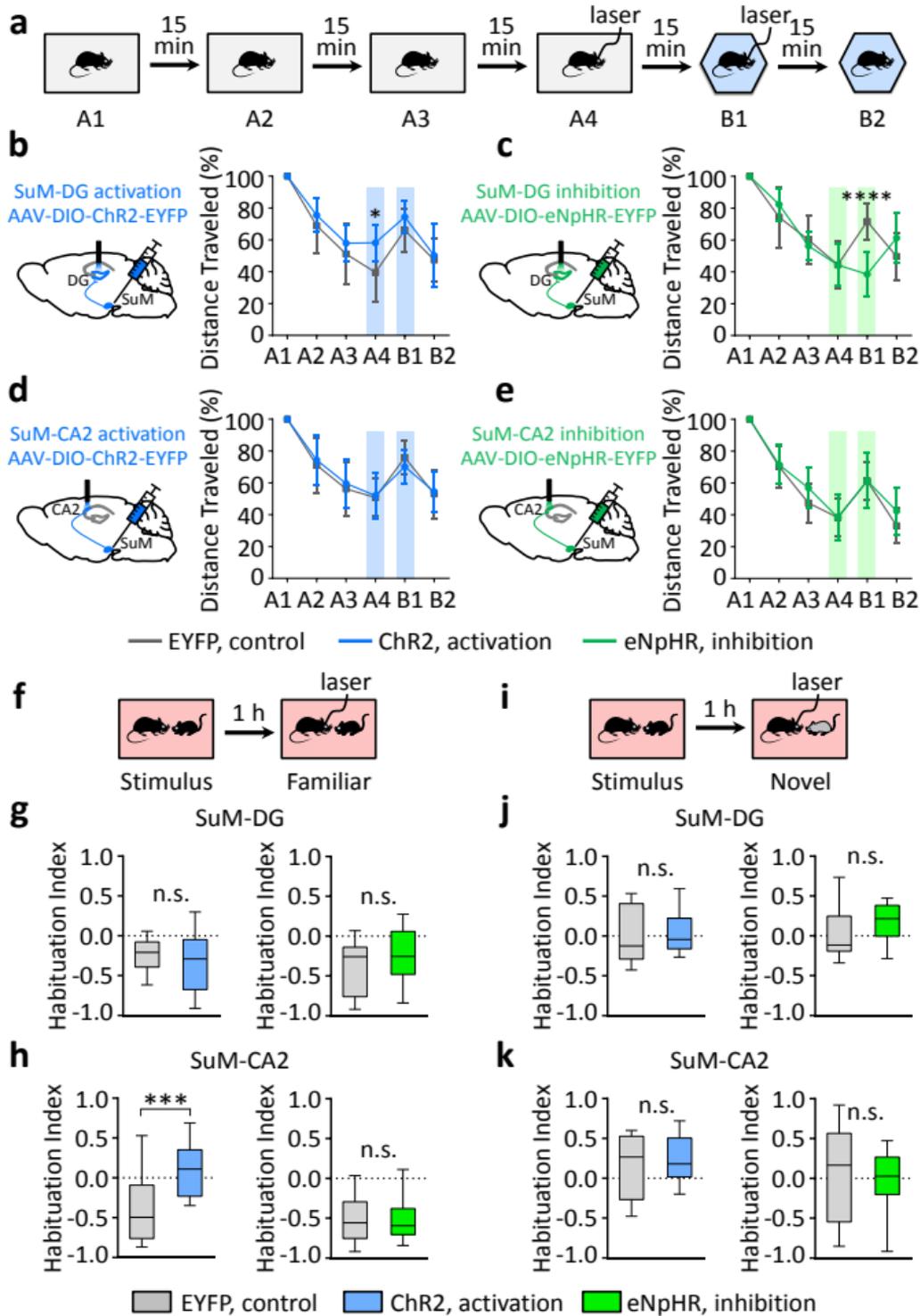


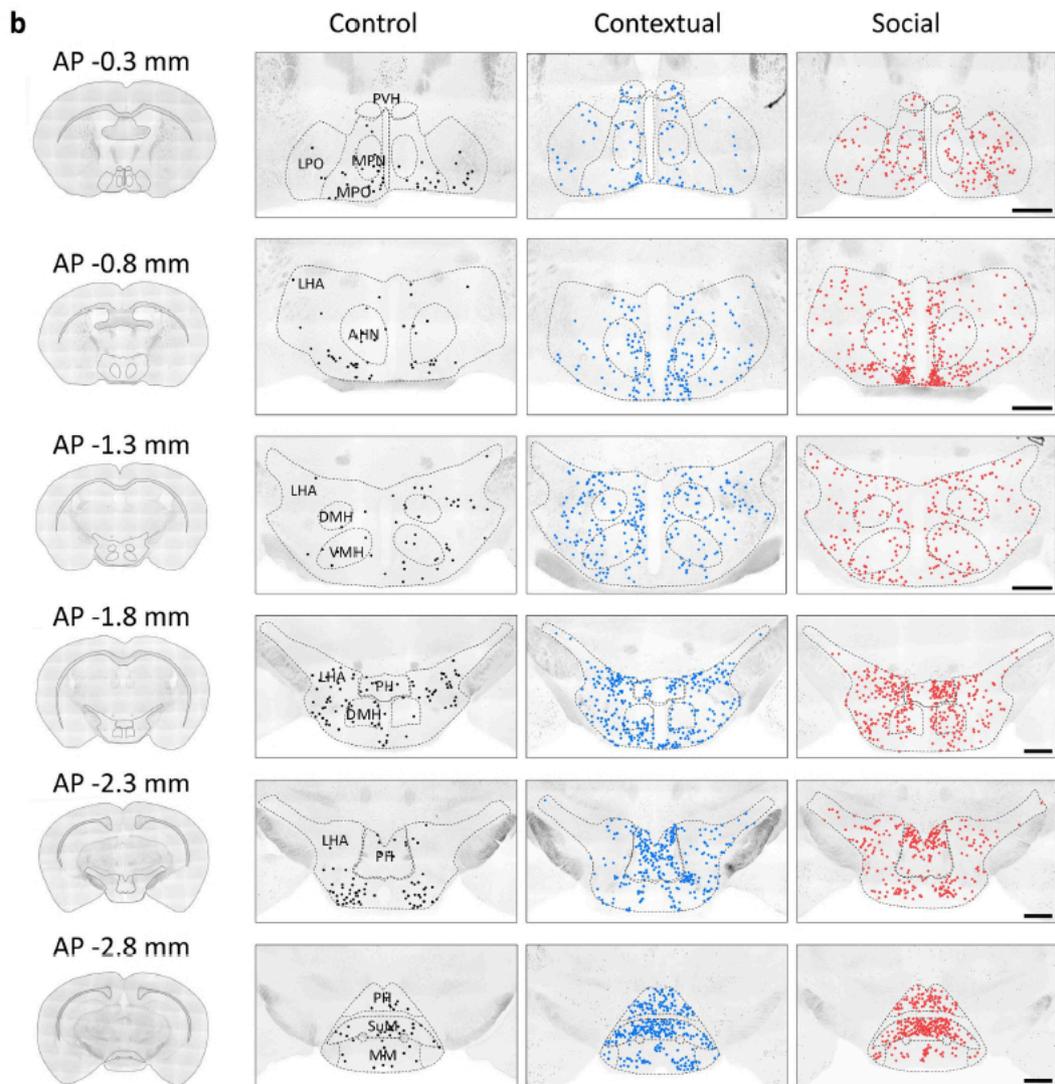
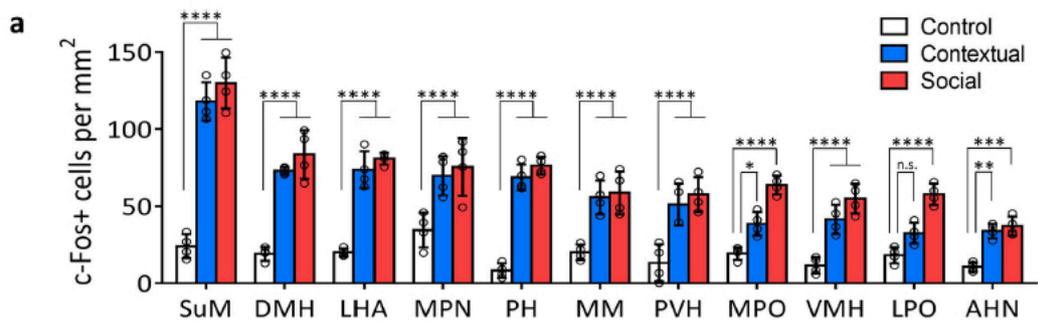
**s** ○ Contextual ○ Social

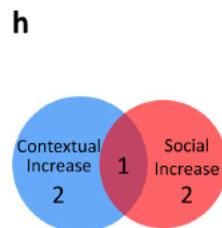
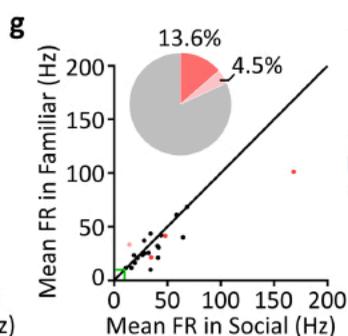
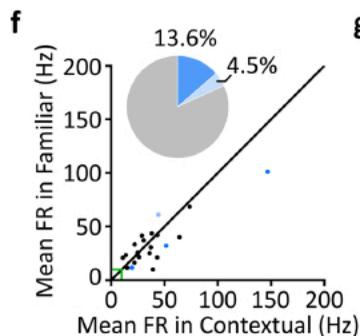
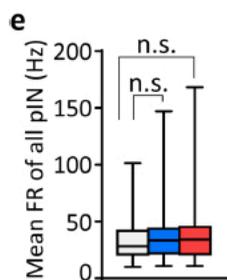
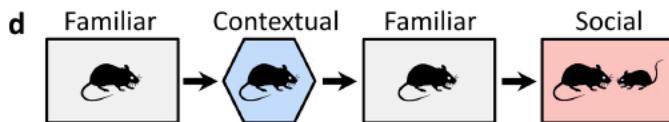
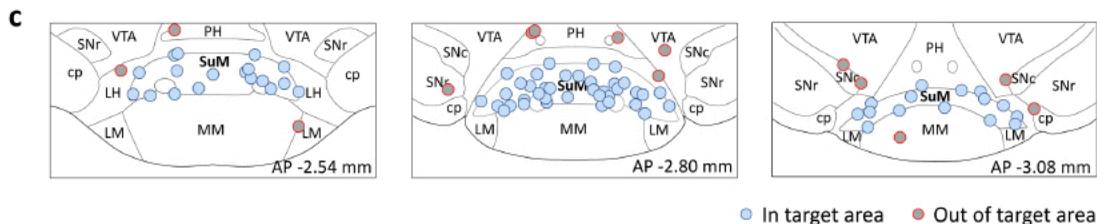
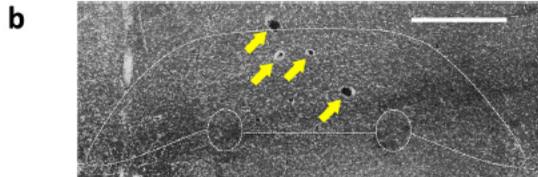
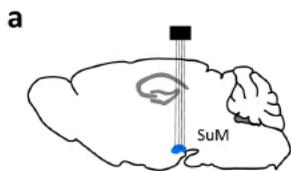


**t**

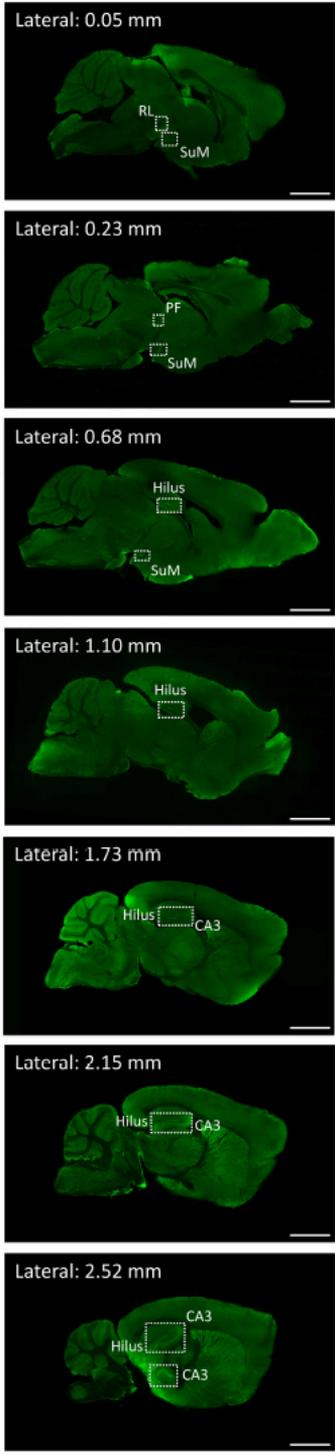
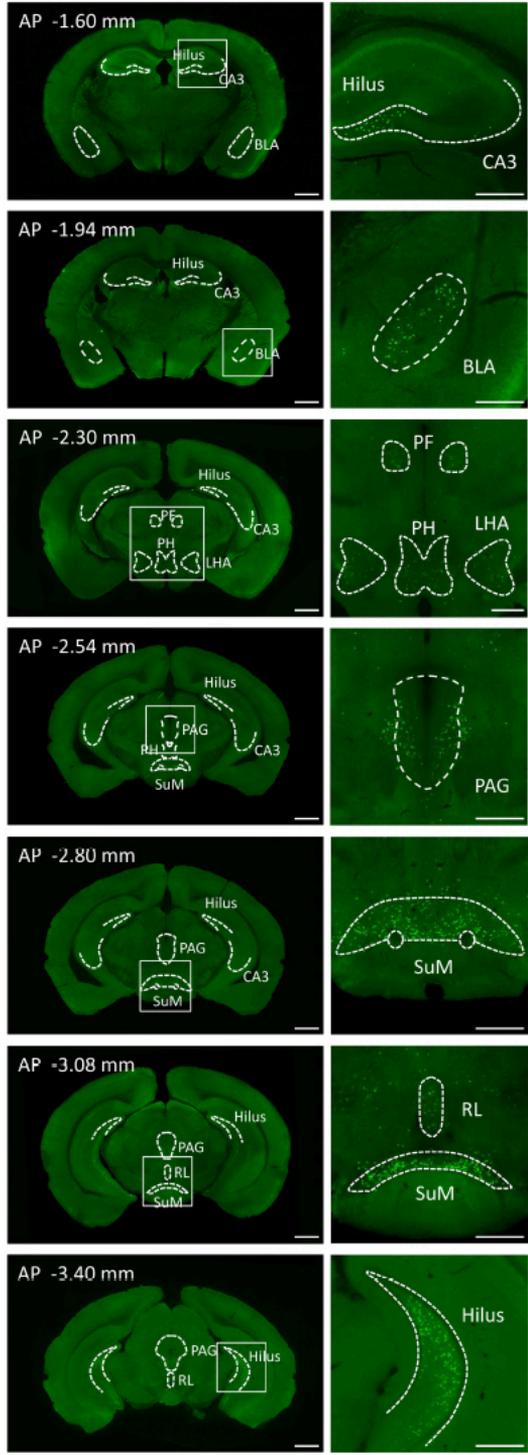
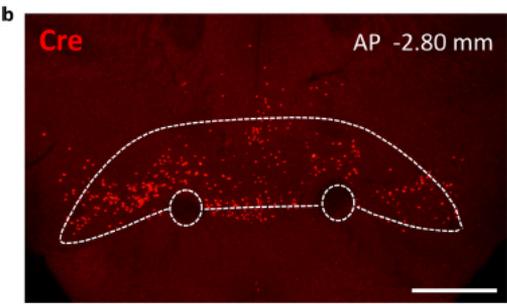
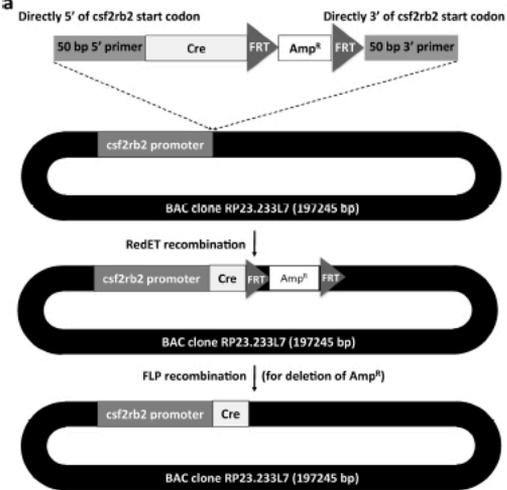


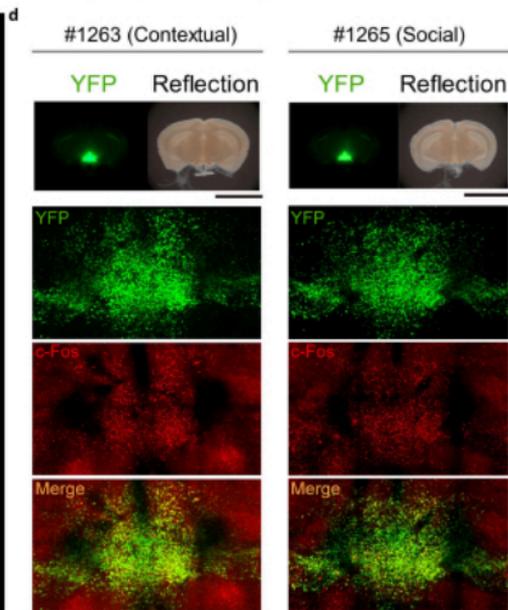
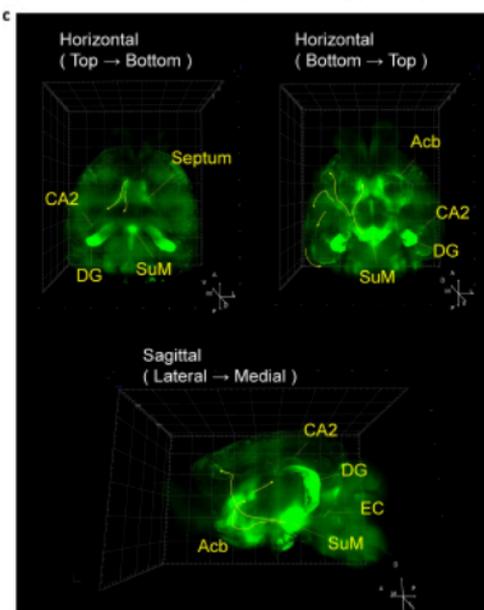
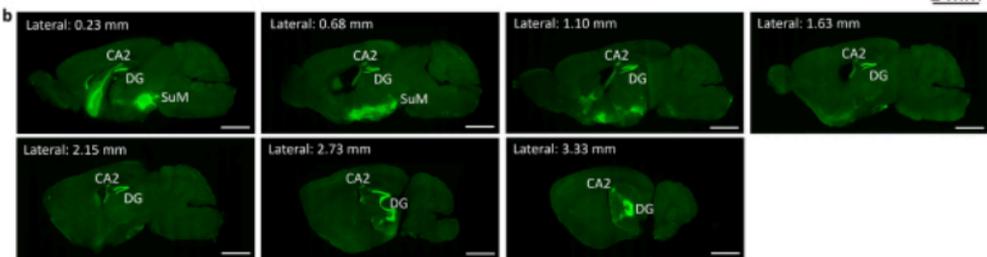
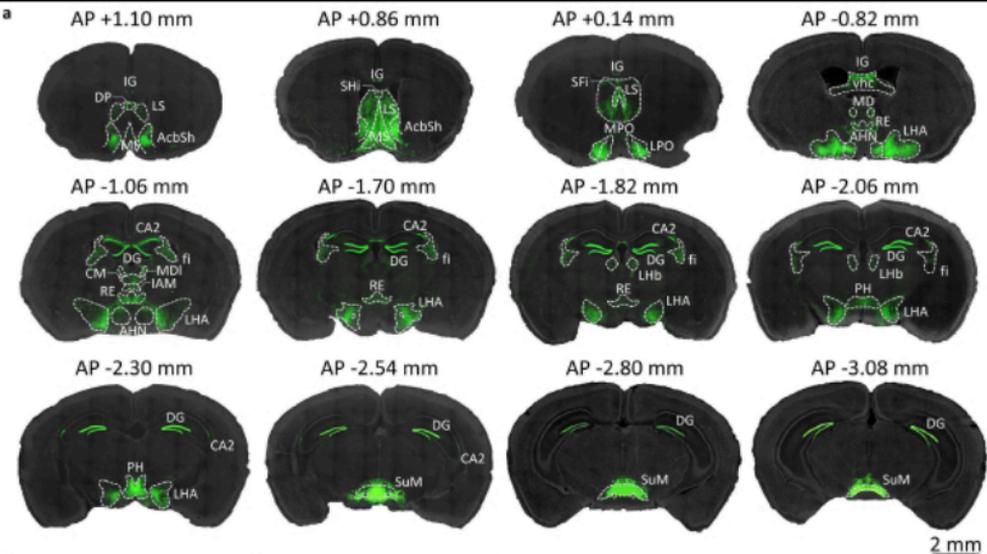




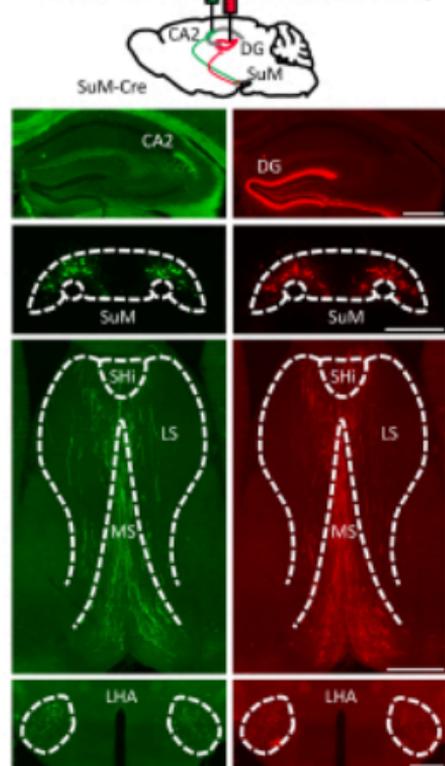


■ Significant increase    ■ Significant decrease    ■ No significant change

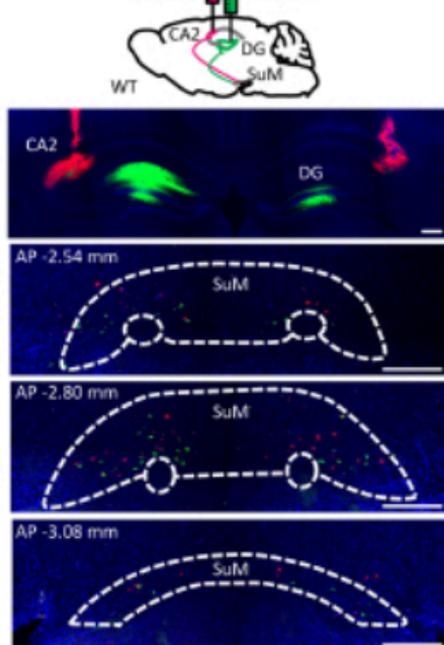




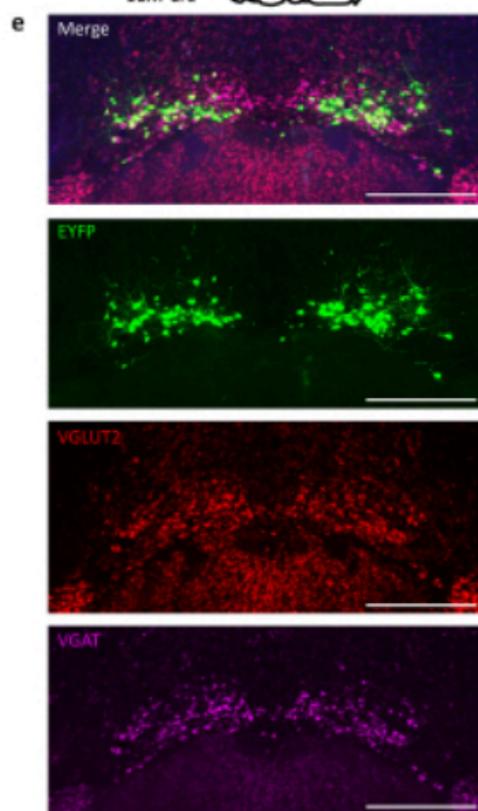
**a** retroAAV-DIO-EYFP retroAAV-DIO-mCherry



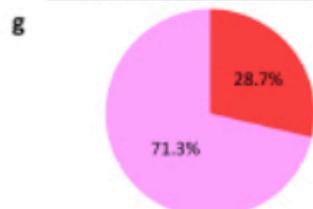
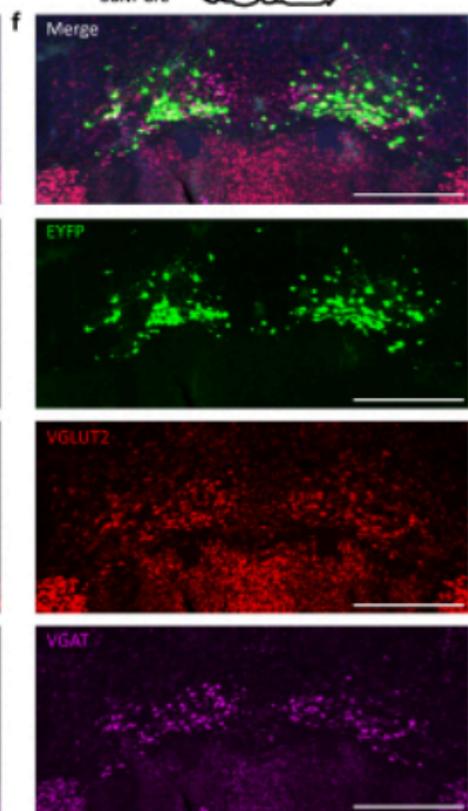
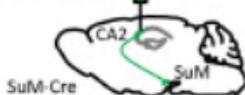
**b** CTB 594 CTB 488



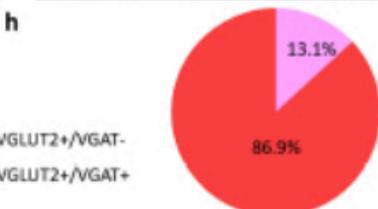
**c** retroAAV-DIO-EYFP

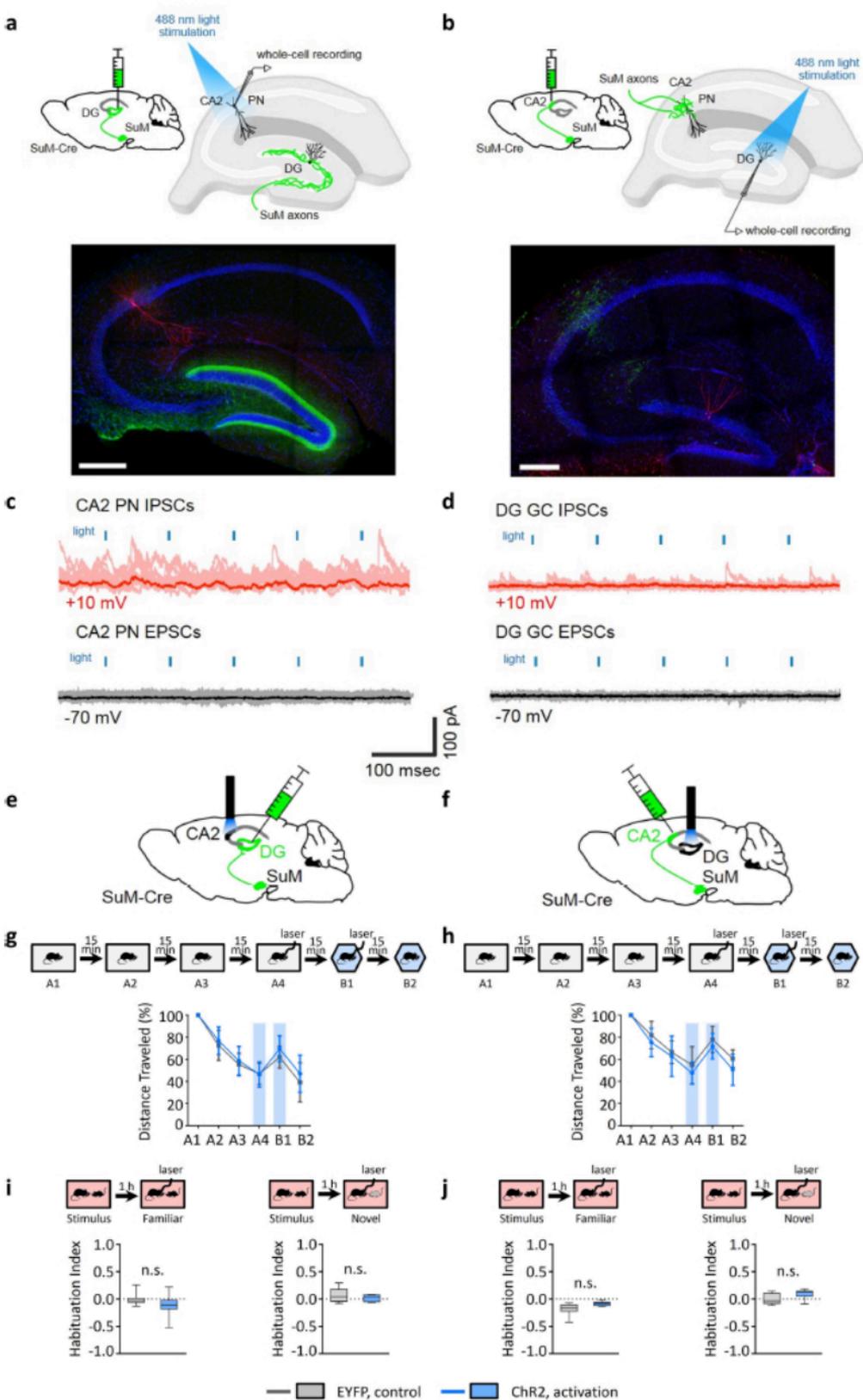


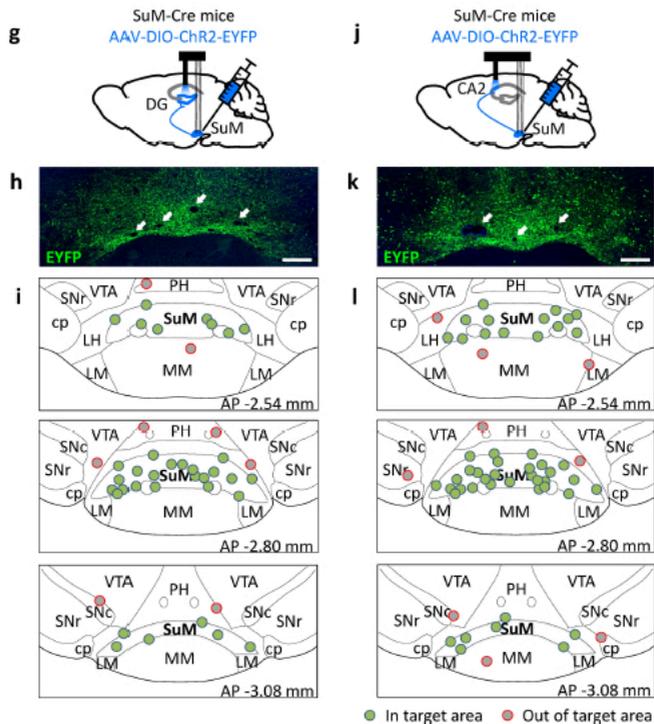
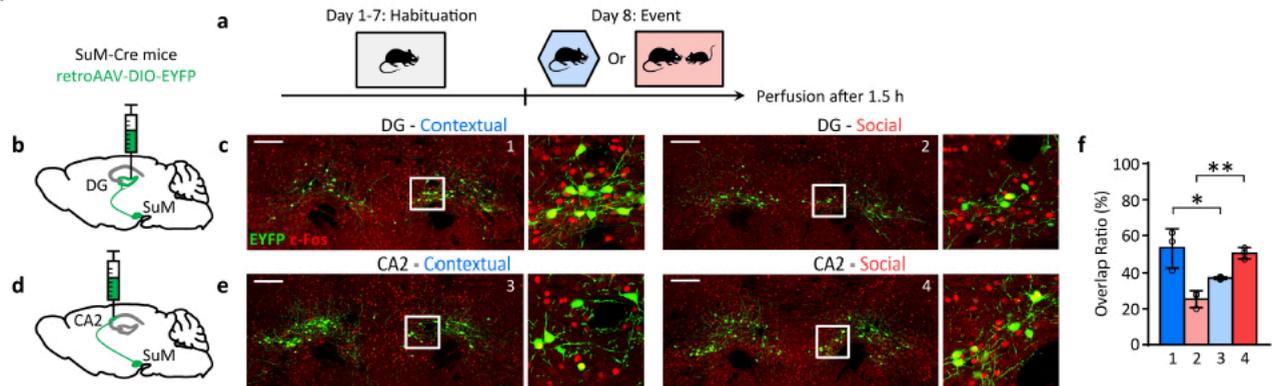
**d** retroAAV-DIO-EYFP

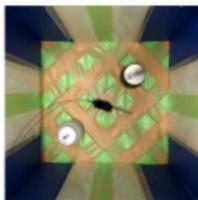


■ EYFP+/VGLUT2+/VGAT-  
■ EYFP+/VGLUT2+/VGAT+

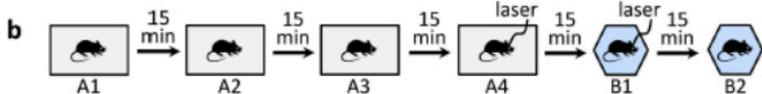
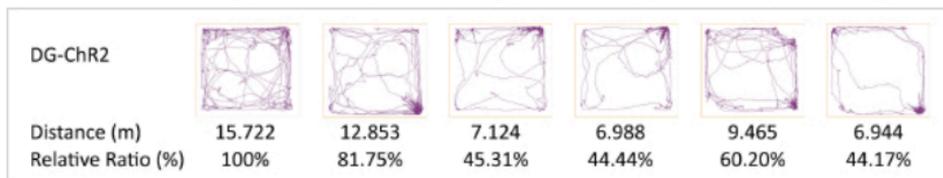
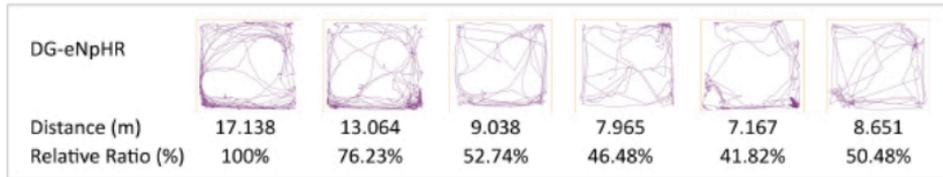
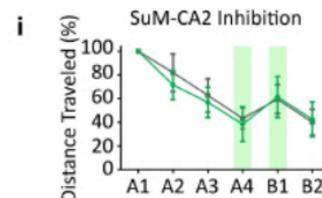
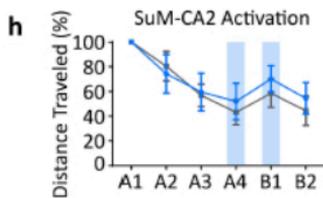
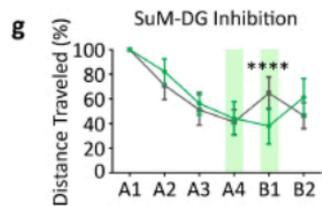
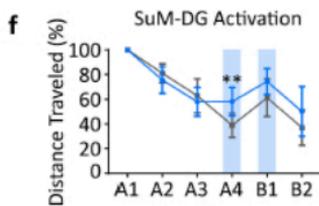
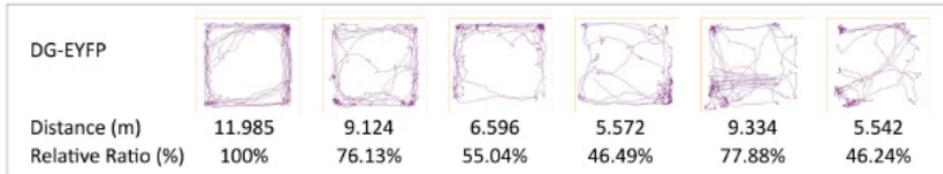






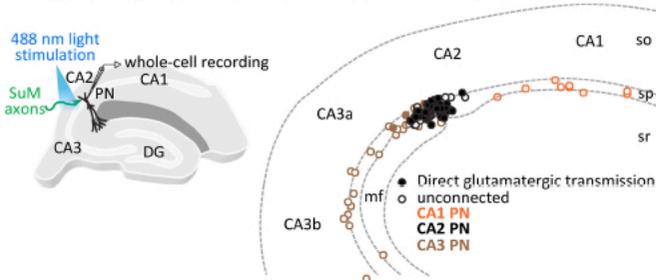
**a** Context A

Context B

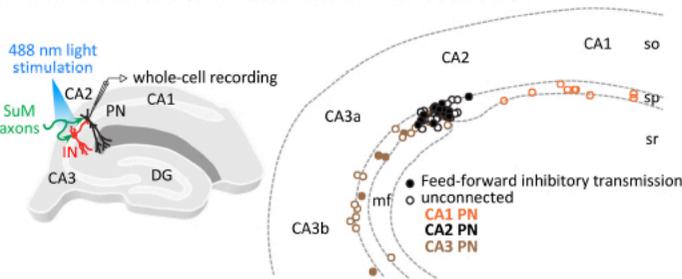
**c****d****e**

■ No laser, control   ■ Chr2, activation   ■ eNpHR, inhibition

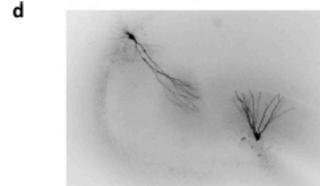
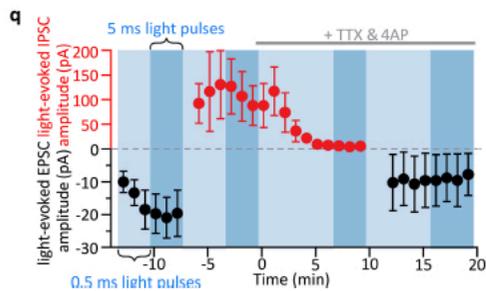
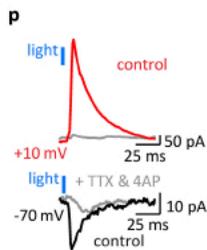
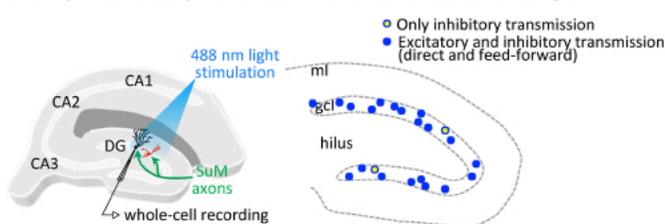
**a** Direct glutamatergic transmission in area CA2 from SuM inputs



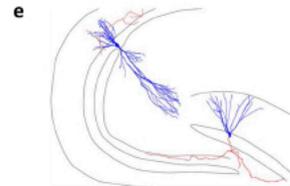
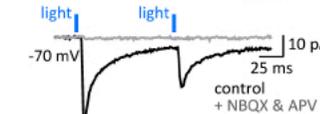
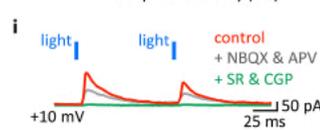
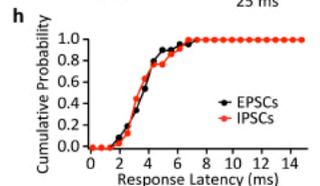
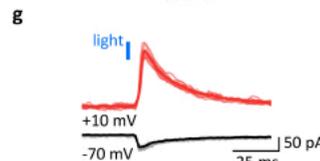
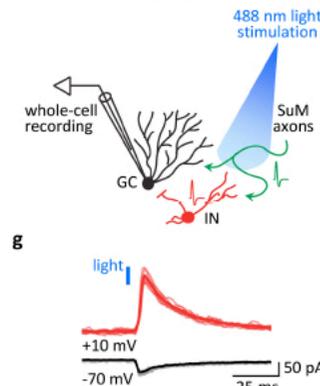
**b** Feed-forward inhibitory transmission in area CA2 from SuM inputs



**c** Excitatory and inhibitory transmission onto DG GC cells from SuM inputs



**f** SuM-DG Transmission



**k** SuM-CA2 Transmission

