Local circuit allowing hypothalamic control of hippocampal area CA2 activity and consequences for CA1

Vincent Robert, Ludivine Therreau, Vivien Chevaleyre, Eude Lepicard, Cécile
Viollet, Julie Cognet, Arthur Jy Huang, Roman Boehringer, Denis Polygalov,
Thomas Mchugh, et al.

Local circuit allowing hypothalamic control of hippocampal area CA2 activity and consequences for CA1

Vincent Robert[1], Ludivine Therreau[1], Vivien Chevaleyre[1,2], Eude Lepicard[1], Cécile Viollet[1], Julie Cognet[1], Arthur J.Y. Huang[3], Roman Boehringer[3], Denis Polygalov[3], Thomas McHugh[3], and Rebecca A. Piskorowski[1,2*]

[1] Université de Paris, INSERM UMR1266, Institute of Psychiatry and Neuroscience of Paris, Team Synaptic Plasticity and Neural Networks, 102-108 rue de la Santé, 75014, Paris, France

[2] GHU Paris Psychiatrie and Neurosciences, 75014 Paris, France

[3] Laboratory for Circuit and Behavioral Physiology, RIKEN Center for Brain Science, 2-1 Hirosawa, Wakoshi, Saitama, Japan

* Corresponding author email: rebecca.piskorowski@u-paris.fr

15   **Abstract**

16   The hippocampus is critical for memory formation. The hypothalamic supramammillary

17   nucleus (SuM) sends long-range projections to hippocampal area CA2. While the SuM-CA2

18   connection is critical for social memory, how this input acts on the local circuit is unknown.

19   We found that SuM axon stimulation elicited mixed excitatory and inhibitory responses in area

20   CA2 pyramidal neurons (PNs). Parvalbumin-expressing basket cells were largely responsible

21   for the feedforward inhibitory drive of SuM over area CA2. Inhibition recruited by the SuM

22   input onto CA2 PNs increased the precision of action potential firing both in conditions of low

23   and high cholinergic tone. Furthermore, SuM stimulation in area CA2 modulated CA1 activity,

24   indicating that synchronized CA2 output drives a pulsed inhibition in area CA1. Hence, the

25   network revealed here lays basis for understanding how SuM activity directly acts on the local

26   hippocampal circuit to allow social memory encoding.

27

Introduction

The hippocampus is critical for memory formation and spatial navigation (Buzsáki and Moser, 2013; Eichenbaum and Cohen, 2014), yet basic questions persist regarding the underlying circuitry and cellular components. While area CA2 has been shown to play a significant role in several hippocampal processes including social memory formation (Hitti and Siegelbaum, 2014; Stevenson and Caldwell, 2014) sharp-wave ripple generation (Oliva et al., 2016a) and spatial encoding (Kay et al., 2016), information about the local circuitry and cellular mechanisms allowing these functions is lacking. There is mounting evidence that generalizations cannot be made from the rich understanding of areas CA1 and CA3, as neurons in area CA2 have been shown to have unique molecular expression profiles (Cembrowski et al., 2016; Lein et al., 2004), morphology (Bartesaghi and Ravasi, 1999; No, 1934) and cellular properties (Robert et al., 2020; Srinivas et al., 2017; Sun et al., 2014). Notably, and in contrast to area CA1, CA2 pyramidal neurons do not undergo high frequency stimulation-induced synaptic plasticity (Dasgupta et al., 2020; Zhao et al., 2007). Rather, the excitability of this region is tightly controlled by a highly plastic network of inhibitory neurons (Leroy et al., 2017; Nasrallah et al., 2015; Piskorowski and Chevaleyre, 2013). When active, CA2 pyramidal neurons (PNs) can strongly drive area CA1 (Chevaleyre and Siegelbaum, 2010; Kohara et al., 2014; Nasrallah et al., 2019), thereby influencing hippocampal output. Furthermore, CA2 neurons also project to area CA3, where they recruit inhibition (Boehringer et al., 2017; Kohara et al., 2014) and act to control hippocampal excitability. Thus, CA2 neurons are poised to have long-reaching effects in the hippocampus, and a better understanding of the regulation of neuronal activity in this region is needed.

The hypothalamic supramammillary (SuM) nucleus sends projections to both area CA2 and the dentate gyrus (DG) (Haglund et al., 1984; Vertes, 1992). These long-range connections have been shown in several species including rodents, primates and humans (Berger et al., 2001; Haglund et al., 1984; Wyss et al., 1979) where they are present in early hippocampal development. The SuM has been found to be active during a wide variety of conditions including novel environment exposure (Ito et al., 2009), reinforcement learning (Ikemoto, 2005; Ikemoto et al., 2004), food anticipation (May et al., 2019), and during REM sleep and arousal (Pedersen et al., 2017; Renouard et al., 2015). This nucleus is also known for participating in hippocampal theta rhythm (Pan and McNaughton, 2002, 1997), possibly by its direct projection to the hippocampus or by modulation of the medial septum (Borhegyi et al., 1998; Vertes and Kocsis, 1997), and regulating spike-timing between hippocampus and the cortex (Ito et al.,

61   2018). Disruption of SuM neuron activity with pharmacological methods (Aranda et al., 2008;

62   Shahidi et al., 2004) or lesions (Aranda et al., 2006) has been reported to disrupt hippocampal

63   memory. Serotonin depletion of the SuM leads to deficiencies in spatial learning in the Morris

64   water maze, and results in altered hippocampal theta activity (Gutiérrez-Guzmán et al., 2012;

65   Hernández-Pérez et al., 2015). Salient rewarding experiences also activate the SuM, as

66   evidenced by cFos expression in monoaminergic SuM neurons by consumption of rewarding

67   food (Plaisier et al., 2020). Furthermore, the rewarding aspects of social aggression have been

68   shown to involve an excitatory circuit between the hypothalamic ventral premammillary

69   nucleus and the SuM (Stagkourakis et al., 2018). It has recently been shown that there are two

70   separate populations of cells in the SuM that target either CA2 or the DG (Chen et al., 2020).

71   In the DG, the SuM terminals release both glutamate and GABA (Boulland et al., 2009; Chen

72   et al., 2020; Hashimotodani et al., 2018; Pedersen et al., 2017; Soussi et al., 2010). The SuM-

73   DG projection has been recently shown to play a role in modulating DG activity in response to

74   contextual novelty (Chen et al., 2020) and spatial memory retrieval (Li et al., 2020). In contrast,

75   functional studies of the SuM-CA2 projection have found that this connection is entirely

76   glutamatergic (Chen et al., 2020). It was recently discovered that the CA2-projecting SuM

77   neurons are active during social novelty exposure, and their selective stimulation prevents

78   expression of a memory of a familiar conspecific (Chen et al., 2020). These findings strongly

79   suggest that the SuM-CA2 connection conveys a social novelty signal to the hippocampus.

80   Furthermore, recent *in vivo* recordings from the SuM in anaesthetized rats reported that a subset

81   of SuM neurons were active earlier than CA2 and other hippocampal cells during SWR (Vicente

82   et al., 2020), indicating a possible role for the SuM-CA2 projection in shaping area CA2 activity

83   prior to SWR onset.

84   Even with the anatomical and *in vivo* data, the properties and consequences of SuM activation

85   on area CA2 activity remain unexplored. In this study, we use a combination of approaches to

86   specifically examine the effects of SuM input stimulation on neuronal activity in hippocampal

87   area CA2. Here, we show that the SuM-evoked post-synaptic excitation of CA2 PN is controlled

88   by SuM-driven inhibition. We identified PV-expressing basket cells as the neuronal population

89   most strongly excited by SuM input in area CA2, and thus likely responsible for the feedforward

90   inhibition evoked by SuM in CA2 PNs. We found that recruitment of this inhibition enhances

91   the precision of AP firing by area CA2 PNs in conditions of low and high cholinergic tone.

92   Finally, we observed that the resulting synchronized CA2 PN activity drives inhibition in area

93 CA1, thereby providing a circuit mechanism through which SuM can modulate hippocampal

94 excitability by controlling area CA2 output.

95

96 **Results**

97 <u>SuM axons provide excitatory glutamatergic input to pyramidal neurons in area CA2 and CA3a</u>

98 Its small size and cellular heterogeneity have made the SuM a difficult region to study. It has

99 been shown that the source of vesicular glutamate transporter 2 (VGluT2)-immunopositive

100 boutons in area CA2 originate from the SuM (Halasy et al., 2004). In order to more closely

101 examine the SuM-CA2 long-range connection, we injected a retrograde canine adenovirus type

102 2 (CAV-2) into area CA2 of the hippocampus to permit the expression of Cre-recombinase

103 (Cre) in hippocampal-projecting SuM neurons, and an adeno-associated virus (AAV) was

104 injected into the SuM to allow the expression of EGFP under the control of Cre (Supplemental

105 Figure 1A). In 5 animals the injection of retrograde CAV-2 was sufficiently targeted to area

106 CA2, as indicated by the presence of EGFP-expressing SuM axonal fibers primarily in this

107 hippocampal area (Supplemental Figure 1B). We stained for calretinin to define the boundaries

108 of the SuM nucleus (Pan and Mcnaughton, 2004). Consistent with recent findings using

109 retrograde AAV vectors (Chen et al., 2020), we observed that CA2-projecting cells express

110 calretinin and are located in the medial SuM (Supplemental figure 1C-D). These cells were

111 located bilaterally, ventral to the fiber bundles that traverse the SuM (Supplemental Figure 1C).

112 Furthermore, we confirmed that these cells also stain for VGluT2 (Supplemental figure 1E).

113 In order to better understand the cellular targets and consequences of SuM input activity in area

114 CA2, we injected an AAV to express channelrhodopsin(H143R)-YFP (ChR2-EYFP) under the

115 control of Cre into the SuM of a transgenic mouse line with Cre expression controlled by the

116 VGluT2 promoter, the Tg(Slc17ab-icre)10Ki line (Borgius et al., 2010) (Supplemental Figure

117 1F). In parallel, we used the Csf2rb2-Cre mouse line that selectively expresses Cre in the SuM

118 (Chen et al., 2020) (Figure 1A). We found that with both transgenic mouse lines we could

119 reproducibly restrict expression of ChR2-EYFP in the SuM and avoid infecting nearby

120 hypothalamic regions that also project to the hippocampus (Figure 1A, Supplemental Figure

121 1F). Furthermore, with both lines of transgenic mice, we observed identical patterns of SuM

122 fiber localization in the hippocampus. EYFP-containing SuM axons were found throughout the

123 granule cell layer of the DG and in area CA2 (Figure 1B) where they clustered around the

124 pyramidal layer (*stratum pyramidale*, SP). The SuM fiber projection area was clearly restricted

125   to area CA2, as defined by expression of the CA2-specific markers PCP4 (Supplemental Figure
126   1B) and RGS14 (Figure 1B), and did not spread to neighboring areas CA3 and CA1. In order
127   to maximize the precision of our experiments, we frequently only achieved partial infection of
128   the SuM, as indicated by the sparseness of ChR2-EYFP-containing fibers in comparison to the
129   number of VGluT2-stained boutons in this region (Supplemental Figure 1G-H).

130   We performed whole-cell current and voltage clamp recordings of PNs across the hippocampal
131   CA regions and activated projecting axons with pulses of 488 nm light in acute hippocampal
132   slices. Following all recordings, we performed post-hoc anatomical reconstructions of recorded
133   cells and axonal fibers, as well as immunohistochemical staining for CA2-area markers.
134   Additionally, injection sites were examined *post hoc* to ensure correct targeting of the SuM.

135   We observed that photostimulation of SuM axons elicited excitatory post-synaptic responses in
136   63 % of PNs (n = 166 of 263 cells) located in area CA2. PNs in this region shared similar overall
137   dendritic morphologies and electrophysiological properties (Table 1) but differed along two
138   criteria. First, in *stratum lucidum* where the DG mossy fibers (MF) project, some PNs clearly
139   had thorny excrescences (TE) while others had very smooth apical dendrites (Figure 1C-D).
140   Based on the presence of TEs, we classified cells as CA2 or CA3a PNs (unequivocal distinction
141   was possible for 148 neurons). Second, the distribution of the locations of PN soma along the
142   radial axis of the hippocampus allowed us to cluster them as deep (closer to *stratum oriens*, SO)
143   or superficial (closer to *stratum radiatum*, SR) subpopulations (unequivocal distinction was
144   possible for 157 neurons). We found that the SuM-PN connectivity was not different between
145   CA2 and CA3 PNs (Table 2, χ^2 test for CA2 and CA3 PNs, p = 0.572) or between deep and
146   superficial PNs (Table 2, χ^2 test for deep and superficial PNs, p = 0.946). Light-evoked
147   excitatory post-synaptic potentials (EPSPs) and excitatory post-synaptic currents (EPSCs)
148   recorded at -70mV were of fairly small amplitude (Figure 1C-D) that were similar regardless
149   of the PN type or somatic location (Table 2, Mann-Whitney U test for CA2 and CA3 PNs, p =
150   0.409; Mann-Whitney U test for deep and superficial PNs, p = 0.306). Because no significant
151   differences in post-synaptic responses to SuM input stimulation were observed between CA2
152   and CA3 PNs as well as between deep and superficial PNs, data from all PNs was pooled for
153   the rest of the study. The small amplitude of SuM input-evoked post-synaptic responses in PNs
154   was not due to suboptimal stimulation of SuM axons as EPSC amplitudes rapidly reached a
155   plateau when increasing light intensity (Supplemental Figure 2A-B). We are confident that this
156   transmission is due to action potential-generated vesicle release because all transmission was
157   blocked   following   application   of   the   sodium   channel   blocker   tetrodotoxin   (TTX)

158 (Supplemental Figure 2B). The pure glutamatergic nature of the SuM input was confirmed by

159 the complete block of light-evoked synaptic transmission following the application of the

160 AMPA and NMDA receptors antagonists NBQX and D-APV (Supplemental Figure 2C;

161 amplitudes were -16 ± 4.8 pA in control and -1.8 ± 0.3 pA in NBQX & D-APV, n = 6; Wilcoxon

162 signed-rank test, p = 0.03). These data confirm that SuM inputs provide long-range

163 glutamatergic excitation to CA2 and CA3 PNs in area CA2.

164 <u>PNs in area CA2 receive mixed excitatory and inhibitory responses from the SuM input</u>

165 Using whole-cell voltage clamp recordings in area CA2 and the dentate gyrus (DG), we have

166 previously shown that the CA2-targeting and DG-targeting SuM neurons have contrasting

167 neurotransmitter modalities (Chen et al., 2020). Our results and other have demonstrated that

168 glutamate and GABA are co-released at SuM-DG synapses (Boulland et al., 2009; Chen et al.,

169 2020; Hashimotodani et al., 2018; Pedersen et al., 2017; Soussi et al., 2010), but that the SuM-

170 CA2 synapses are exclusively glutamatergic (Chen et al., 2020). We have previously shown

171 that SuM input stimulation in area CA2 evokes a very large inhibitory post synaptic current

172 (IPSC) that is entirely due to feed-forward inhibition based on the delayed response latencies

173 of IPSCs as compared to EPSCs, the complete block of IPSCs by NBQX and APV, and the

174 complete abolition of IPSCs but sparing of EPSCs in the presence of TTX and 4-amino pyridine

175 (Chen et al., 2020). Because photostimulation of SuM input elicited excitatory post-synaptic

176 potentials (PSPs) of fairly small amplitude in area CA2 PNs held at -70 mV (Figure 1C4 and

177 D4), we asked if the amplitude of SuM input stimulation-evoked PSPs in PNs could be

178 controlled by feed-forward inhibition. Interestingly, blocking inhibitory transmission with the

179 $GABA_A$ and $GABA_B$ receptor antagonists SR95531 and CGP55845A led to a significant

180 increase of light-evoked PSP amplitude recorded in area CA2 PNs (Figure 2A-C; amplitudes

181 of the first response were 0.18 ± 0.05 mV in control and 0.24 ± 0.05 mV in SR95531 &

182 CGP55845A, n = 14; Wilcoxon signed-rank tests, p = 0.004 for the first PSP, p = 0.013 for the

183 second PSP, p < 0.001 for the third PSP). Thus, this result demonstrates a negative control of

184 SuM-driven excitation by feedforward inhibition.

185 Given the combination of direct excitation and feed-forward inhibition from SuM inputs onto

186 CA2 pyramidal cells, we asked how this input would summate with other synaptic inputs in the

187 CA2 dendritic arbor. Hippocampal area CA2 receives synaptic input from CA3 in *stratum*

188 *radiatum* (SR). Stimulation of CA3 inputs evokes a very strong feed-forward inhibition, such

189 that it is exceptionally difficult to evoke action potential firing in CA2 pyramidal neurons when

190 inhibitory transmission is intact (Chevaleyre and Siegelbaum, 2010; Nasrallah et al., 2015;

191   Piskorowski and Chevaleyre, 2013). Additionally, CA2 PNs receive synaptic input from the

192   entorhinal cortex in *stratum lacunosum molecular* (SLM). These inputs are very distal but

193   relatively less attenuated in CA2 PNs in comparison to distal inputs in CA1 (Chevaleyre and

194   Siegelbaum, 2010; Srinivas et al., 2017). In order to answer how the SuM input interacts with

195   the CA3 and entorhinal inputs in area CA2, we electrically stimulated synaptic inputs in SR and

196   SLM in the presence and absence of simultaneous SuM fiber stimulation (Figure 2D). In

197   summary, we found that when the CA2 PNs were kept at -70 mV, SuM input stimulation paired

198   with SR or SLM input had a net depolarizing effect. We measured the amplitudes of the light-

199   evoked SuM PSP, the electrically evoked PSP of either SR or SLM stimulation and the paired

200   SuM and electrical PSP (Figure 2E). For SR input stimulation, we found no significant

201   difference between the observed paired SR + SuM amplitude and the calculated linear

202   summated amplitude (SR alone + SuM alone) (Figure 2F). This was observed for all 4 pulses

203   of input summations delivered at 10 Hz. However, for the SLM input stimulation, the observed

204   paired amplitude was significantly smaller than the linear summation of the two inputs (SLM

205   alone + SuM alone) for the first stimulus (n = 10; T test, p = 0.014) (Figure 2F). This observation

206   is expected, as the attenuation of distal dendritic SLM inputs causes the peak of the PSP to be

207   delayed relative to the more somatic SuM input. Thus, the SuM input paired with either SR or

208   SLM input stimulation has minor depolarizing effect on the PSP in CA2 PNs. However, the

209   SuM input might have different effect on the SR and SLM inputs depending on the precise

210   timing of their activation.

211   We also examine the summation ratio for a train of 4 PSPs at 10 Hz from SR and SLM synaptic

212   inputs stimulation with and without simultaneous SuM input stimulation (Figure 2G-H). We

213   observed a significant reduction of the summation ratio as measured by the ratio of the n-th

214   pulse to the first (Pn/P1) for both SR (n = 10; repeated-measures ANOVA, p = 2.3x10^{-4}) and

215   SLM (n = 10; repeated-measures ANOVA, p = 8.5x10^{-4}). This observation that concomitant

216   SuM activity is reducing the level of facilitation of several pulses in a train indicates that the

217   short-term dynamics of the SuM-driven excitation and feed-forward inhibition are playing a

218   role to prevent cellular excitation from other inputs.

219   <u>Basket cells are strongly recruited by the SuM input</u>

220   Because the hippocampus hosts a variety of interneurons (INs) that are involved in controlling

221   specific aspects of PN excitability, we wished to establish which kind of IN was targeted by the

222   SuM input to area CA2. We performed whole-cell recordings from INs in this area and assessed

223   post-synaptic excitatory responses to SuM axons stimulation in these cells (Figure 3). In

224   contrast with previous reports of an exclusive innervation of PNs by SuM (Maglóczky et al.,
225   1994), we observed robust light-evoked excitatory transmission from SuM axons in 35 out of
226   62 interneurons (INs) with soma located in SP. Following biocytin-streptavidin staining and
227   anatomical reconstructions of recorded INs (allowing unequivocal identification in 48 neurons),
228   we were able to classify INs based on their physiological properties, somatic location and
229   axonal arborization location. We classified 22 cells as basket cells (BCs) because their axonal
230   arborizations were restricted to SP (Figure 3A). BCs fired APs at high frequency either in bursts
231   or continuously upon depolarizing current injection and showed substantial repolarizing sag
232   current when hyperpolarized (Figure 4A, Table 3). Light-evoked EPSCs and PSPs were readily
233   observed in the vast majority of BCs (Figure 3A, 3C and 3D, Table 4) and reached large
234   amplitudes in some instances. An additional 26 INs with soma in SP were classified as non-
235   BCs because their axon did not target SP (Figure 3B). In our recordings, these cells fired in
236   bursts and showed little sag during hyperpolarizing current injection steps (Table 3). We
237   consistently observed no or very minor light-evoked excitatory transmission onto non-BCs
238   (Figure 3B-C, Table 4). Furthermore, we recorded from 17 INs that had soma in *stratum oriens*
239   (SO) and 9 in *stratum radiatum* (SR). Like non-BCs, these INs did not receive strong excitation
240   from SuM fibers (Table 4). This data is consistent with the conclusion that SuM input
241   preferentially forms excitatory synapses onto basket cells in area CA2.

242   To fully assess the strength of SuM inputs onto the different cell types, we examined the
243   following parameters for each population: the connectivity, success rate, amplitude, potency,
244   kinetics, and latencies of EPSCs as well as the resulting depolarization of the membrane
245   potential. First, SuM inputs preferentially innervated BCs as evidenced by a higher connectivity
246   of EPSCs in BCs than in PNs or other INs (Table 4). Importantly, excitatory responses had
247   short latencies with limited jitter (Table 4) indicating that the connection was monosynaptic in
248   all cell types. When voltage-clamping cells at -70 mV, light-evoked EPSCs could be compared
249   between different cell populations. However, not every photostimulation gave rise to an EPSC
250   leading to an average success rate that tended to be highest in BCs (Table 4). In addition, BCs
251   appeared to receive more excitation from the SuM input than other cells types, as the amplitude
252   of EPSCs was larger in BCs than in PNs (Table 4). EPSCs recorded in BCs also had faster
253   kinetics than in PNs (Table 4). Interestingly, combining the success rate of EPSCs with their
254   respective amplitudes to compute the potency of the SuM synapses revealed that it was
255   significantly larger in BCs than in PNs and non-BCs (Figure 3C; potencies were -12 ± 1.6 pA
256   for PNs, n = 166; -29 ± 7.8 pA for BCs, n = 18; -5.9 ± 1.5 pA for non-BCs, n = 13; Kruskal-

Wallis test with Dunn-Holland-Wolfe *post hoc* test, p = 0.022). Consequently, EPSPs recorded at -70 mV were of larger amplitude in BCs than in PNs and non-BCs (Figure 3D; amplitudes were 0.44 ± 0.06 mV for PNs, n = 20; 1.71 ± 0.57 mV for BCs, n = 10; 0.53 ± 0.07 mV for non-BCs, n = 4; Kruskal-Wallis test with Dunn-Holland-Wolfe *post hoc* test, p < 0.001). When recording cell-attached or current-clamping BCs at their resting membrane potential (V_M), photostimulation of SuM axons was able to evoke AP firing (Figure 3E) in multiple instances (n = 7 of 13). However, this was never observed in PNs (n = 0 of 78), non-BCs (n = 0 of 16), SR INs (n = 0 of 9) or SO INs (n = 0 of 8). These results show that SuM projections to area CA2 preferentially provide excitation to BCs that are likely responsible of the feedforward inhibition observed in PNs. This is in accordance with an efficient control of area CA2 PNs excitation by the SuM inhibitory drive as axons from BCs deliver the feedforward inhibition to the peri-somatic region of PNs, effectively shunting incoming PSPs from both the SuM and from dendritic-targeted inputs in SR and SLM.

Parvalbumin-expressing basket cells mediate the feedforward inhibition driven by SuM

In the hippocampus, BCs express either cholecystokinin (CCK) or parvalbumin (PV) (Klausberger and Somogyi, 2008). We found that in response to a 1 second depolarizing pulse, most BCs that received strong SuM excitatory input displayed very fast AP firing with little accommodation in the AP firing frequency (Figure 4A-B, Table 3). This firing behavior is similar to what has been reported for fast spiking PV-expressing BCs in CA1 (Pawelzik et al., 2002). In contrast, CCK-expressing BCs show a lower firing frequency and more accommodation during the train (Pawelzik et al., 2002). This result suggests that BCs connected by the SuM may be expressing PV. To directly confirm this hypothesis, we performed *post hoc* immunostaining of recorded interneurons that received strong excitation from SuM input. Because of the dialysis inherent to the whole-cell recording conditions, we encountered difficulty staining for multiple cells. However, PV-immunoreactivity could unequivocally be detected in either the soma or dendrites of 7 connected BCs (Figure 4C). Therefore, this data demonstrates that at least a fraction of the recorded BCs connected by the SuM is expressing PV.

Hence, to address whether the lack of PV staining in some cells was a consequence of dialysis or resulted from the fact that non-PV+ BCs are also connected, we made use of different strategies to differentiate PV+ and CCK+ INs. First, we wished to genetically confirm that PV+ INs are involved in the SuM-driven feedforward inhibition of area CA2 PNs. We used inhibitory Gi-DREADD to selectively inhibit PV+ INs in area CA2 while monitoring

feedforward IPSCs from area CA2 PNs in response to SuM stimulation. To achieve that, we injected AAVs expressing a Cre-dependent hM4D(Gi) inhibitory Gi-DREADD in area CA2 of PV-Cre mice together with AAVs expressing ChR2 with a pan-neuronal promoter in the SuM (Figure 5A). While we were able to obtain very specific expression of DREADD in PV+ INs, only a fraction of PV+ INs had detectable DREADD expression as quantified by immunohistochemistry (Figure 5B; fraction of PV+ INs expressing DREADDs in CA2 = 75 ± 3.5 %, n = 13). We observed a substantial reduction of SuM-evoked IPSC amplitude recorded in area CA2 PNs upon application of 10 μM of the Gi-DREADD ligand CNO (Figure 5C; amplitudes were 847 ± 122 pA in control and 498 ± 87 pA in CNO hence a 42 ± 6.0 % block, n = 13; paired-T test, p < 0.001). Although we never measured a complete block of inhibitory responses, this result unequivocally places PV+ INs as mediators of the SuM feedforward inhibition of area CA2 PNs. The incomplete block of IPSCs observed in these experiments indicates that either additional non-PV+ INs are recruited by SuM input or that our silencing of PV-mediated feedforward inhibition is incomplete. This could be a consequence of partial infection of PV+ INs in area CA2 by AAVs carrying DREADDs and partial silencing of DREADD-expressing PV+ INs by CNO. To address the latter, we performed whole-cell recordings from Gi-DREADD-expressing CA2 PV+ INs labelled with mCherry and monitored the variations in V_M level and action potential firing to SuM input stimulation before and after CNO application (Supplemental Figure 3A). We found that CNO application caused a significant hyperpolarization of Gi-DREADD-expressing CA2 PV+ INs, albeit modest in magnitude (Supplemental Figure 3B-D; V_M were -55.3 ± 2.3 mV in ACSF and -61.8 ± 2.7 mV in CNO hence a -6.5 ± 2.4 mV hyperpolarization, n = 6; Wilcoxon signed-rank test, p = 0.031). While this confirmed the relevance of our silencing strategy, it highlighted the possibility that Gi-DREADD-expressing CA2 PV+ INs may not be fully silenced by CNO. Indeed, we observed residual SuM-evoked AP firing in these cells after CNO application (Supplemental Figure 3D-E). These data indicate that synaptically evoked somatic AP firing is not fully blocked by CNO in Gi-DREADD-expressing CA2 PV+ INs. Because it is difficult to distinguish between partial silencing of PV INs by Gi-DREADDs or recruitment of other types of INs in the SuM-driven feedforwards inhibition, we adopted other complementary strategies to answer this question.

We used a pharmacological strategy to selectively manipulate PV+ INs by targeting their GABA release machinery. In the neocortex, P/Q-type voltage-gated calcium channels are necessary for GABA release from PV+ fast-spiking INs onto PNs (Zaitsev et al., 2007). In

contrast, N-type calcium channels are primarily involved in GABA release from CCK+ INs (Hefft and Jonas, 2005). Thus, we recorded SuM input-evoked EPSCs and IPSCs in CA2 PNs before and after application of the P/Q-type voltage-gated calcium channels specific blocker ω-agatoxin TK (200 nM) (Figure 5D). We observed a near-complete block of IPSCs upon ω-agatoxin TK application (Figure 5D1, IPSC amplitudes were 245.5 ± 92.6 pA in control and 35.0 ± 15.4 pA in ω-agatoxin TK hence a 81.8 ± 3.9 % block, n = 5; paired-T test, p < 0.001), suggesting a major contribution from PV+ INs to SuM-driven feedforward inhibition consistent with our previous results. However, we observed that excitatory transmission from SuM axons was also partially blocked by ω-agatoxin TK application, as SuM input-evoked EPSCs were significantly reduced although not abolished (Figure 5D2, EPSC amplitudes were -51.8 ± 5.9 pA in SR95531 & CGP55845A and -26.5 ± 5.4 pA after ω-agatoxin TK hence a 49.6 ± 5.6 % block, n = 6; paired-T test, p < 0.001). This observation indicates that glutamate release from SuM axons relies on P/Q-type voltage-gated calcium channels to some degree, thereby complicating the interpretation of the reduction of IPSC amplitude in CA2 PNs.

It has previously been demonstrated that PV+ BC transmission can be strongly attenuated by mu opioid receptor activation (MOR) while CCK+ BC transmission is insensitive to MOR activation (Glickfeld et al., 2008). Thus, we recorded from PNs in area CA2 and examined the sensitivity of light-evoked IPSCs to the application of the MOR agonist DAMGO (Figure 5E). We found that there was a near complete block of the light-evoked IPSC amplitude following 1 μM DAMGO application (Figure 5E1; IPSC amplitudes were 343 ± 123 pA in control and 31 ± 12.4 pA in DAMGO hence a 88 ± 5.0 % block, n = 6 PNs; Wilcoxon signed-rank test, p = 0.031), while direct excitatory transmission remained unaffected (Figure 5E2; EPSC amplitudes were -6.7 ± 1.1 pA in SR95531 & CGP55845A and -5.6 ± 0.9 pA after DAMGO, n = 17 PNs; Wilcoxon signed-rank test, p = 0.19). While this result is in agreement with our DREADD and ω-agatoxin TK results showing a major contribution of PV+ INs to the SuM-driven feedforward inhibition, it should be noted that the dichotomy between PV+ versus CCK+ INs sensitivity to DAMGO has not been directly verified in area CA2.

It has recently been shown that delta opioid receptors (DORs) are specifically expressed in a fraction of PV+ cells in the hippocampus (Erbs et al., 2012). Furthermore, PV+ INs in area CA2 are the substrate of an iLTD of feedforward inhibition from CA3 mediated by delta opioid receptor (DOR) activation (Nasrallah et al., 2019; Piskorowski and Chevaleyre, 2013). Therefore, we sought to further refine our characterization of the SuM feedforward inhibition by assessing its sensitivity to DOR activation (Figure 5F). Application of 0.5 μM of the DOR

agonist DPDPE led to a long-term reduction of light-evoked IPSCs recorded in area CA2 PNs, similar to the iLTD seen by CA3 input stimulation (Figure 5F1; IPSC amplitudes were 168 ± 28 pA in control and 64 ± 22 pA in DPDPE hence a 61 ± 14 % block by DPDPE, n = 7; paired-T test, p = 0.015), while leaving direct EPSCs unaffected (Figure 5F2; EPSC amplitudes were -4.0 ± 1.6 pA in SR95531 & CGP55845A and -3.1 ± 1.1 pA after DPDPE, n = 7; Wilcoxon signed-rank test, p = 0.22). Further confirming the PV+ nature of INs responsible for the SuM feedforward inhibition, this result reveals that both the local CA3 and long-range SuM inputs converge onto an overlapping population of INs to inhibit area CA2 PNs, thus enabling cross-talk between these routes through synaptic plasticity of PV+ INs. However, since DORs are only expressed in a fraction of PV+ INs and therefore only reduces but does not fully block PV+ IN-mediated GABA release (Nasrallah et al., 2019; Piskorowski and Chevaleyre, 2013), it is difficult to know if the remaining SuM-evoked IPSCs are from PV+ INs not expressing DOR or from other INs recruited by the SuM input.

Altogether, these 4 methods strongly suggest that SuM inputs selectively recruit PV+ interneurons to inhibit CA2 PNs. Although individually each method does not conclusively demonstrate that SuM input exclusively targets PV+ INs, the consistent reduction of SuM-driven feedforward inhibition of CA2 PNs observed with every approach allows us to conclude that PV+ cells are predominantly targeted by SuM inputs in area CA2.

The feedforward inhibitory drive from SuM controls pyramidal neuron excitability

Given SuM axonal stimulation triggers an excitatory-inhibitory sequence in post-synaptic PNs, we asked which effect would prevail on PN excitability. In order to assess this, we mimicked an active state in PNs by injecting constant depolarizing current steps sufficient to sustain AP firing during 1 second while photostimulating SuM axons at 10 Hz (Figure 6A-B). We observed that recruitment of SuM inputs significantly delayed the onset of the first AP (Figure 6C; latency to the first AP were 221 ± 19.9 ms in control and 233 ± 19.1 ms with photostimulation, hence a 12.1 ± 4.3 ms increase upon photostimulation, n = 12; paired-T test, p = 0.016). In addition, given SuM neurons display theta-locked firing *in vivo*, we asked if rhythmic inhibition driven by SuM inputs in area CA2 could pace AP firing in PNs by defining windows of excitability. Indeed, photostimulation of SuM axons at 10 Hz led to a significant decrease of variability in the timing of AP firing by PNs (Figure 6D-E; standard deviations of the first AP timing were 36.9 ± 11 ms in control and 24.7 ± 7.4 ms with photostimulation, hence a 12.3 ± 5.3 ms decrease upon photostimulation, n = 12; Wilcoxon signed-rank tests, p < 0.001 for the first AP, p = 0.008 for the second AP, p = 0.004 for the third AP). Both the delay of AP onset

and the reduction of AP jitter stemmed from the feedforward inhibition recruited by SuM inputs as application of GABA$_A$ and GABA$_B$ receptor antagonists abolished these effects of SuM stimulation (Figure 6C-E; latency to the first AP were 232 ± 19.8 ms in SR95531 & CGP55845A and 235 ± 18.0 ms with photostimulation, n = 6; Wilcoxon signed-rank test, p = 0.44; standard deviations of the first AP timing were 11.9 ± 2.0 ms in SR95531 & CGP55845A and 7.1 ± 1.5 ms with photostimulation, n = 6; Wilcoxon signed-rank tests, p = 0.22 for the first AP, p = 0.16 for the second AP, p = 0.09 for the third AP). These results reveal that the purely glutamatergic SuM input, by recruiting feedforward inhibition, has an overall inhibitory effect on PN excitability and can influence the timing and jitter of area CA2 PN action potential firing.

One drawback of these results is that the injection of current steps to evoke action potential firing is not entirely representative of CA2 PN activity, as there is no synaptic input leading to AP firing. It has been reported that the AP discharge of SuM neurons *in vivo* is phase-locked to the hippocampal theta rhythm (Bernat Kocsis and Vertes, 1994). Because theta rhythm is a brain state characterized by elevated levels of acetylcholine, we approximately mimicked these conditions in the hippocampal slice preparation by bath application of 10 μM of the cholinergic agonist carbachol (CCh). Under these conditions, CA2 PNs depolarize and spontaneously fire rhythmic bursts of APs, and the properties of these AP bursts are tightly controlled by excitatory and inhibitory synaptic transmission (Robert et al., 2020). Thus, we decided to examine how SuM input stimulation influenced CA2 PN firing under these conditions.

Because SuM neurons fire in bursts at theta frequency *in vivo* (Kirk et al., 1996), and because the elevated cholinergic tone accompanying theta can activate muscarinic receptors that alter the synaptic release properties of many synapses in the brain, we examined how synaptic transmission from the SuM input to area CA2 was affected by the application of 10 μM carbachol (CCh) (Supplemental Figure 4A) (Kirk et al., 1996; B Kocsis and Vertes, 1994). With GABA receptors blocked to first assess the SuM excitatory transmission only, we observed that CCh decreased the amplitude and increased the PPR of SuM-evoked EPSCs in CA2 PNs (Supplemental Figure 4B). This suggests a decrease of glutamate release by SuM axons induced by CCh. We found similar results for SuM-evoked feedforward inhibitory transmission to CA2 PNs as IPSC amplitude was decreased and PPR increased with CCh application (Supplemental Figure 4C). Next, we examined the relative short-term dynamics of SuM-evoked excitatory and inhibitory transmission to CA2 PNs. For this, both EPSCs and IPSCs were recorded from the same individual CA2 PNs upon repeated SuM input stimulation with 5 pulses delivered at 10 Hz before and after CCh application (Supplemental Figure 4D-G). We observed that both SuM-

422   evoked EPSCs and IPSCs underwent short-term depression, as evidenced by a decrease in

423   amplitude along the pulse train as well as amplitude ratios between subsequent pulses over the

424   first pulse (Pn/P1) (Supplemental Figure 4D-F, Supplemental Table 1). It is worth noting that

425   the Pn/P1 ratio was similar for EPSCs and IPSCs and that the E/I ratio did not significantly

426   change with repeated SuM input stimulation (Supplemental Figure 4F-G, Supplemental Table

427   1). This indicates that the SuM influence over CA2 PN may remain overall inhibitory during

428   prolonged SuM input activation. Similarly influencing both EPSCs and IPSCs, application of

429   10 μM CCh affected these short-term dynamics of the SuM-CA2 PN transmission by

430   decreasing the amplitude of the initial response (Supplemental Figure 4D-E, Supplemental

431   Table 1) but limiting the subsequent short-term depression of SuM-evoked PSCs amplitude

432   (Supplemental Figure 4D-F, Supplemental Table 1). Interestingly, the overall effect of repeated

433   SuM input stimulation on post-synaptic responses in area CA2 PNs was even more biased

434   towards inhibition after CCh application as the E/I ratio of PSCs during the pulse train was

435   lower in CCh as compared to control (Supplemental Figure 4G, Supplemental Table 1),

436   possibly because of a lesser depression of IPSCs as compared to EPSCs (Supplemental Figure

437   4D-F, Supplemental Table 1) which could be due to a CCh-induced depolarization of INs

438   mediating SuM-evoked feedforward inhibition. Altogether, these observations match with our

439   findings of the SuM input having an overall inhibitory influence over area CA2, and suggest

440   that this effect might be more gradual over time but even stronger in conditions of elevated

441   cholinergic tone.

442   Under these conditions of elevated cholinergic tone, we asked how the spontaneous AP bursting

443   activity of CA2 PNs would be affected by activation of the SuM input by triggering 10 second-

444   long trains of 0.5 ms light pulses delivered at 10 Hz to stimulate SuM axons at the onset of

445   bursts (Figure 7A). Because of the intrinsic cell-to-cell variability of bursting kinetics, we

446   photostimulated SuM inputs only during interleaved bursts in the same cells. To do this, bursts

447   were detected automatically with an online threshold detection system that started the

448   photostimulation pulse train after the first AP of every alternating burst, starting with the second

449   burst (Figure 7A-B). For analysis, the number of APs and bursting kinetics could be compared

450   within the same cell. We observed a significant decrease in the number of APs fired during a

451   burst when SuM inputs were photostimulated as compared to interleaved control bursts (Figure

452   7C-D; numbers of APs per burst were 15.2 ± 2.3 in control and 6.9 ± 1.3 with photostimulation,

453   n = 7; paired-T test, p = 0.031). In control bursts, the AP firing rate of CA2 PNs initially

454   increases, and then progressively decreases. In the photostimulation bursts, the initial increase

of AP firing frequency was absent, and the subsequent AP firing frequency was reduced (Figure 7E; 2-way ANOVA on firing rate over time in light-on vs light-off conditions; light factor, p < 0.001; time factor, p < 0.001; light x time factor, p = 0.052).

In the presence of CCh, CA2 PNs undergo a depolarization of the membrane potential that is followed by a period of AP firing as the membrane potential remains depolarized for several seconds, and then slowly hyperpolarizes until the next bursting event (Robert et al., 2020). We observed that photostimulation of SuM inputs resulted in a striking reduction in the amount of time the membrane potential remained depolarized, and this is likely why the burst duration was significantly shorter in bursts with SuM photo-stimulation (Figure 7F-G; burst duration was 4.0 ± 1.1 s in control and 1.6 ± 0.5 s with photostimulation, n = 7; paired-T test, p = 0.037). The rate and level of V_M repolarization following bursts were not significantly changed by SuM input photostimulation (V_M repolarization rate was -3.3 ± 0.6 mV/s in control and -3.6 ± 0.7 mV/s with photostimulation, n = 7; paired-T test, p = 0.601; post-burst V_M was -62.8 ± 1.7 mV in control and -62.0 ± 2.0 mV with photostimulation, n = 7; paired-T test, p = 0.173), however the inter-burst time interval was reduced. Indeed, AP bursts with SuM input activation were followed more rapidly by another burst of APs than the ones without SuM input activation (Figure 7B and 7H; time until next burst was 93 ± 14 s in control and 59 ± 17 s with photostimulation, n = 7; paired-T test, p = 0.001), which could be due to both short-term depression of inhibitory transmission after repeated activation during the SuM input photostimulation train and reduced activation of hyperpolarizing and shunting conductances during bursts shortened by SuM input photostimulation. Thus, in our preparation, SuM input activation is able to modify the spontaneous bursting activity of CA2 PNs under conditions of high cholinergic tone.

As SuM input controls burst firing of action potentials and likely paces activity in area CA2, we wondered how the subsequent output of CA2 PNs would affect their post-synaptic targets. Because CA2 PNs strongly project to CA1 PNs, this activity is likely to influence CA1 encoding and hippocampal output. Thus, we examined the consequences of SuM-CA2 input stimulation on area CA1 both *in vivo* and in acute slices treated with CCh to induce spontaneous activity (Figure 8).

ChR2-EYFP was expressed in the SuM of Csf2rb2-Cre mice in a Cre-dependent manner and the mice were implanted with a microdrive targeting tetrodes to region CA1 and an optical fiber to the SuM terminals in CA2 (Figure 8A). Mice were placed in a small box (familiar context) and left free to explore as blue (473 nm) laser light pulses (50 ms pulse width) were applied to

488 the SuM terminals at 10 Hz. Across 23 recording sessions in five mice we found that the

489 activation of SuM terminals in CA2 resulted in a significant and reproducible change in the

490 multiunit spiking activity recorded in the pyramidal cell layer of CA1 on 34 of 55 tetrodes. The

491 firing rate change was similar across individual tetrodes (Figure 8B-C), with a decrease in the

492 normalized firing rate starting shortly after laser onset and continuing for about 10 ms, followed

493 immediately by a rebound-like increase to about 20 % greater than baseline firing rate (Figure

494 8B-C).

495 In order to get a better mechanistic understanding of this observation, we set out to decipher

496 how SuM activity in area CA2 influences CA1 in the hippocampal slice preparation. To this

497 end, we used the same photostimulation protocol used *in vivo* that consisted of light stimulation

498 trains of 50 ms-long pulses delivered at 10 Hz for 1 second, repeated every 10 seconds for 2

499 minutes and interleaved with light-off sweeps of the same duration, with the microscope

500 objective centered on area CA2. Whole-cell patch-clamp recordings of CA1 PNs were obtained

501 in acute hippocampal slices superfused with CCh and subjected to this light stimulation protocol

502 (Figure 8D). We asked what synaptic events may be responsible for the decreased firing of CA1

503 units observed 10 – 20 ms after light onset *in vivo* (Figure 8A-C). Whole-cell recordings of

504 CA1 PNs showed an absence of EPSCs time-locked to the photostimulation in all but one case

505 (n = 11/12) (Figure 8E-F). In contrast, we often (n = 7/12) observed light-evoked IPSCs in CA1

506 PNs occurring 10 – 20 ms after light onset (Figure 8G-H). Therefore, the reduction in firing of

507 CA1 units *in vivo* is likely caused by increased inhibitory inputs onto CA1 PNs within 10 – 20

508 ms of SuM fiber stimulation over area CA2. This result highlights a contribution of SuM input

509 to controlling CA2 output that regulate CA1 activity *in vivo* and provides a mechanistic

510 interpretation of this observation at the circuit level.

511

512 **Discussion**

513 In this study, we provide direct evidence for a functional connection between the hypothalamus

514 and the hippocampus. Using stereotaxic injection of viral vectors in combination with

515 transgenic mouse lines to express channelrhodopsin in a projection-specific manner, we have

516 been able to selectively stimulate SuM axons in area CA2 of the hippocampus, allowing for the

517 direct examination of synaptic transmission. This approach yielded novel functional

518 information about the SuM input post-synaptic targets and the overall physiological

519 consequences of its activation. We found that, in contrast to previous anatomical reports, SuM

520 input forms synapses onto both PNs and INs in area CA2. The excitatory drive evoked by light-

stimulation of SuM input was significantly larger for BC INs, which we demonstrate are likely
PV+. The resulting feedforward inhibition recruited by SuM input stimulation enhanced the
precision of AP timing of CA2 PNs in conditions of low and high cholinergic tone relevant to
different brain states. The modified CA2 output evoked poly-synaptic inhibition in area CA1,
likely responsible for a decrease in firing rate of CA1 units *in vivo*. Overall, we demonstrate
that SuM input controls CA2 output to area CA1 by recruiting feedforward inhibition.

SuM input to area CA2 forms a microcircuit where PV+ basket cells strongly inhibit pyramidal neurons

Glutamatergic innervation of area CA2 by the SuM has been previously described by tracing
studies (Kiss et al., 2000; Soussi et al., 2010) and presumed to form synapses exclusively onto
PNs (Maglóczky et al., 1994). Our experimental strategy allowed for the direct examination of
the post-synaptic targets of SuM glutamatergic axons. Our results confirm that PNs in area CA2
indeed receive excitatory synapses from SuM axons. However, in contrast to what had been
proposed in previous studies, we observed that SuM inputs target not only PNs but also INs in
area CA2. Importantly, we identified a specific subpopulation of INs as BCs which were the
cell type most potently excited by SuM. These BCs could fire action potentials upon SuM input
photostimulation leading to a substantial feedforward inhibition of neighboring PNs. We found
that at resting membrane potentials, the mixed excitatory/inhibitory SuM input resulted in a net
depolarization of the membrane potential in CA2 PNs. However, when the SuM input was
paired with either inputs in SR or SLM, we observed a decrease in the summation ratios of
trains of synaptic inputs consistent with a perisomatic shunting inhibition driven by SuM in
area CA2. Furthermore, we found that with elevated cholinergic tone, recruitment of BCs by
SuM controlled PNs excitability and shaped spontaneous burst firing. This finding
demonstrates that SuM activity can pace action potential firing in PNs through recruitment of
feedforward inhibition.

The population of INs potently excited by SuM transmission display many features that
motivate us to classify them as PV+ BCs. They have somas located in the somatic layer, have
densely packed perisomatic-targeted axons, are fast spiking and show PV immuno-reactivity.
The selective expression of GiDREADD in PV+ cells allows for selective silencing that reduces
SuM-driven feedforward inhibition of area CA2 PNs. With these techniques, however, we were
unable to sufficiently silent PV+ cells in area CA2, leaving open the possibility that another
population of basket cell is targeted by SuM input. The feedforward inhibitory transmission
recruited by SuM stimulation is highly sensitive to MOR activation. While this supports our

554   hypothesis that PV+ cells are targeted by SuM input, MORs are not entirely exclusive to PV+

555   cells (Stumm et al., 2004). We also show that the SuM-recruited feedforward inhibition is

556   sensitive to DOR activation. Unlike MORs, DORs have been shown to be specific to PV+ cells

557   in area CA2, however, only a sub-population of PV+ INs express this receptor (Nasrallah et al.,

558   2019; Piskorowski and Chevaleyre, 2013) leaving open the possibility that the remaining IPSCs

559   evoked by SuM stimulation are not from PV+ cells. We also show in this work that SuM-evoked

560   inhibitory currents are blocked by the application of ω-agatoxin TK, indicating that these

561   recruited INs express P/Q-type CaV channels, consistent with PV+ BCs (Zaitsev et al., 2007).

562   However, we also saw that ω-agatoxin TK also blocked gluatamatergic transmission from SuM

563   inputs, preventing a simple interpretation of these results. Thus, while there is ample evidence

564   that SuM inputs target PV+ BCs in area CA2, we cannot exclude the possibility that other

565   populations of BCs, such as CCK+ INs are also targeted by these inputs. PV+ BCs in the

566   hippocampus have been shown to be modulated by CCK (Lee et al., 2011) which would have

567   very interesting implications for the effect of SuM activity in area CA2. Furthermore, it was

568   recently shown that PV+ BCs actively inhibit CCK+ BCs, enabling a complementary

569   perisomatic inhibitory system that allows for brain-state dependent activity during behavior

570   (Dudok et al., 2021)

571   Recent studies have indicated that the SuM input to CA2 plays a key role in social novelty

572   discrimination (Chen et al., 2020). Our findings are very consistent with the finding that DOR-

573   mediated inhibitory synaptic plasticity of PV+ INs in area CA2 is required for social recognition

574   memory (Domínguez et al., 2019). Furthermore, exposure to a novel conspecific induces a

575   DOR-mediated plasticity in this same inhibitory network in area CA2 (Leroy et al., 2017). Thus,

576   our finding that SuM input acts via PV+ interneurons fits with previous studies and provides a

577   link between social novelty detection and local CA2 hippocampal inhibitory plasticity.

578   Overall, the local circuitry and consequences of SuM input to area CA2 contrasts with the SuM-

579   DG path (Hashimotodani et al., 2018; Li et al., 2020; Mizumori et al., 1989; Nakanishi et al.,

580   2001). Previously, we have shown that unlike the SuM-DG synapse, the SuM-CA2 synapse is

581   entirely glutamatergic (Chen et al., 2020). In this study we use both a VGluT2-Cre and SuM-

582   Cre mouse lines to demonstrate how the combination of direct excitation and feedforward

583   inhibition regulates CA2 PN AP firing. Our data shows that SuM activity results in

584   synchronized feedforward inhibition from CA2 to CA1 which decreases CA1 PN firing. While

585   our results are very intriguing given the importance of area CA2 in propagation of hippocampal

586   network activity (Oliva et al., 2016a), further questions remain. CA2 PNs also receive

587   excitatory input from DG cells via the mossy fibers (Kohara et al., 2014; Llorens-Martín et al.,

588   2015). It has been postulated that by increasing DG excitability, the SuM may also be indirectly

589   acting on CA2 (Silkis and Markevich, 2020). These circuits merit further exploration.

590

591   <u>Consequences of SuM input on area CA2 output</u>

592   Recent work has demonstrated a strong excitatory drive from area CA2 to CA1 (Chevaleyre

593   and Siegelbaum, 2010; Kohara et al., 2014; Nasrallah et al., 2019). Consequently, modification

594   of CA2 output through synaptic plasticity (Nasrallah et al., 2019) or neuromodulation (Tirko et

595   al., 2018) affects CA1 activity. This observation is critical when considering social memory

596   formation, which is known to depend on CA2 output (Hitti and Siegelbaum, 2014; Stevenson

597   and Caldwell, 2014) and is likely encoded in downstream ventral CA1 (Okuyama et al., 2016).

598   CA2-targeting cells in the SuM have recently been shown to be highly active during novel

599   social exploration (Chen et al., 2020). From our results, we hypothesize that this novel social

600   signal from the SuM, acts via the PV+ inhibitory network in area CA2 to control the timing of

601   CA2 output onto area CA1.

602   By recruiting feedforward inhibition, SuM activity paces and temporally constrains AP firing

603   from CA2 PNs undergoing depolarization. More critically, in conditions of elevated cholinergic

604   tone relevant to SuM activity *in vivo*, CA2 PNs depolarize and fire bursts of APs that can be

605   shaped by SuM input both by controlling AP firing as well as membrane depolarization. While

606   this result was obtained by triggering SuM input stimulation to the onset of burst firing by CA2

607   PNs, *in vivo* and acute slice experiments revealed a consistent influence of CA1 PN AP firing

608   by SuM input to area CA2 regardless of the timing of SuM input stimulation relative to CA2

609   PN AP burst firing. These results demonstrate a powerful control of SuM input over CA2 output

610   when PNs are spontaneously firing bursts of APs, a firing mode that is most efficient at

611   influencing CA1 activity (Tirko et al., 2018). Optogenetic experiments have recently shown

612   that CA2 PNs can drive a strong feedforward inhibition in area CA1 (Kohara et al., 2014;

613   Nasrallah et al., 2019). Although SuM input likely does not directly drive feedforward

614   inhibition in area CA1 (Chen et al., 2020), the recruitment of feedforward inhibition in area

615   CA2 by SuM input activation could curtail the time window of spontaneous firing in CA2 PNs

616   and effectively lead to a synchronized drive of feedforward inhibition by area CA2 over area

617   CA1. We postulate that the concerted IPSC that we detect in area CA1 with SuM fiber

618   photostimulation in area CA2 corresponds to the large decrease in firing that is observed in

619   CA1 multi-unit recordings *in vivo*. Thus, these data provide evidence for a long-range control

620   of CA2 bursting activity and the consequences in downstream area CA1 in conditions of high
621   cholinergic tone that accompanies theta oscillations *in vivo* during which SuM is active.

622

623   <u>Relevance of the SuM input to area CA2 for hippocampal oscillations</u>

624   The activity of hippocampal neurons is orchestrated by brain rhythms, notably theta and gamma
625   oscillations that are prominent during exploration and linked to the learning and memory
626   functions of the hippocampus (Buzsáki, 2002; Buzsáki and Wang, 2012; Colgin, 2016). The
627   SuM is active during these brain states and contributes to theta oscillations in the hippocampus
628   (Kirk et al., 1996; Kirk and McNaughton, 1993; B Kocsis and Vertes, 1994; McNaughton et
629   al., 1995; Pan and McNaughton, 2002, 1997; Thinschmidt et al., 1995). Here, we show that the
630   SuM controls area CA2 output to CA1 by recruiting PV+ BCs, which are important for both
631   theta and gamma oscillations (Fuchs et al., 2007; Gulyás et al., 2010; Korotkova et al., 2010;
632   Mann and Mody, 2010). Through its perisomatic mono-synaptic excitation and PV+ BC-
633   mediated di-synaptic inhibition of CA2 PNs, the SuM likely contributes to enforcing theta-
634   locked windows of excitability shaping CA2 PNs output. Area CA2 can influence CA1 activity
635   not only by direct projections but also through its interactions with both CA3 (Boehringer et
636   al., 2017) and EC (Chevaleyre and Siegelbaum, 2010; Rowland et al., 2013) which are major
637   contributors to CA1 theta and gamma oscillations (Buzsáki, 2002; Colgin, 2016). CA2 axons
638   target both CA1 *stratum oriens* and *radiatum* (Nasrallah et al., 2019) , thus the CA2 projections
639   to CA1 likely contributes to the theta and slow gamma oscillations observed in these strata in
640   CA1 (Belluscio et al., 2012; Colgin et al., 2009; Schomburg et al., 2014). Indeed, CA2 PNs
641   show theta- and gamma-modulation of their activity (Fernandez-Lamo et al., 2019; Oliva et al.,
642   2016b), and chemogenetic manipulations of their excitability bidirectionally influences
643   hippocampal low gamma power (Alexander et al., 2018). Further, chronic block of CA2 output
644   transmission leads to hippocampal hyperexcitability and disrupts CA1 theta phase preference
645   and spatial coding (Boehringer et al., 2017). Therefore, by providing a theta-locked input
646   shaping CA2 PN activity, the SuM is poised to contribute to oscillatory activity in downstream
647   brain regions receiving CA2 input. Indeed, chemogenetic activation or silencing of SuM
648   glutamatergic neurons respectively increases or decreases theta and gamma power in the EEG
649   (Pedersen et al., 2017). Further, the SuM is involved in coordinating activity between the
650   prefrontal cortex, the thalamus and area CA1 as evidenced by a loss of theta coherence amongst
651   these regions upon SuM optogenetic silencing during a spatial task requiring action planning

652 (Ito et al., 2018). Altogether, these studies point to the SuM as a crucial component in the

653 regulation of hippocampal oscillations and our findings shed light on an aspect of this circuit.

654

655 <u>Gating of area CA2 activity by PV+ INs and significance for pathologies</u>

656 The density of PV+ INs in area CA2 is strikingly higher than in neighboring areas CA3 and

657 CA1 (Botcher et al., 2014; Piskorowski and Chevaleyre, 2013). This population of INs has been

658 shown to play a powerful role in controlling the activation of CA2 PNs by CA3 inputs

659 (Nasrallah et al., 2015). We show in this study that long-range inputs from the SuM can strongly

660 recruit PV+ BCs, which in turn inhibit PNs in this area. Hence, both intra-hippocampal inputs

661 from CA3 and long-range inputs from the SuM converge onto PV+ INs to control CA2 PN

662 excitability and output.

663 Postmortem studies have reported losses of PV+ INs in area CA2 in pathological contexts

664 including bipolar disorder (Benes et al., 1998), Alzheimer's disease (Brady and Mufson, 1997),

665 and schizophrenia (Benes et al., 1998; Knable et al., 2004). Consistent with these reports, in a

666 mouse model of the 22q11.2 deletion syndrome, a major risk factor for schizophrenia in

667 humans, we found a loss of PV staining and deficit of inhibitory transmission in area CA2 that

668 were accompanied by impairments in social memory (Piskorowski et al., 2016). We postulate

669 that the PV+ INs altered during pathological conditions may be the same population of PV+

670 BCs recruited by long-range SuM inputs. Indeed, the DOR-mediated plasticity onto PV+ INs

671 is altered in the 22q11.2 deletion syndrome mouse model, and we show here that a fraction of

672 the PV+ INs targeted by the SuM also express DOR. Thus, the loss of function of PV+ INs in

673 area CA2 could disrupt proper long-range connection between the hippocampus and the

674 hypothalamus and possibly contribute to some of the cognitive impairments observed in

675 schizophrenic patients and animal models. Further, pharmacological mouse models of

676 schizophrenia have reported increased c-fos immunoreactivity in the SuM as well as memory

677 impairments (Castañé et al., 2015). Although several alterations in these models of

678 schizophrenia could lead to deficits of hippocampal-dependent behavior, abnormalities of the

679 SuM projection onto area CA2 appear as a potential mechanism that warrants further

680 investigation.

681

682 **Materials & Methods**

Key Resources Table

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
genetic reagent (Mus. musculus)	Tg(Slc17ab-icre)10Ki	(Borgius et al., 2010)	Tg(Slc17ab-icre)10Ki ; VGluT2-cre	
genetic reagent (Mus. musculus)	csf2rb2-Cre	(Chen et al., 2020)	csf2rb2-Cre ; SuM-cre	
genetic reagent (Mus. musculus)	Pvalbtm1(cre)Arbr/J (PV-Cre)	Jackson	Stock No. 017320	
genetic reagent (adeno-associated virus)	AAV9.EF1a.DIO.hChR2(H134R).EYFP	Addgene	Addgene20298	
genetic reagent (adeno-associated virus)	AAV9.hSynapsin.EGFP.WPRE.bGH	Addgene	Addgene 51502	
genetic reagent (adeno-associated virus)	AAV.Synapsin.DIO.hM4D(Gi).mCherry	McHugh Laboratory, Riken		
genetic reagent (adeno-associated virus)	AAV2/9.hSyn.hChR2(H134R).EYFP.WPRE.hGH	Addgene	Addgene 26973	
genetic reagent (Canine adeno virus)	CAV2-cre	Platforme de Vectorologie de Montpellier	CAV Cre	

antibody	anti-RGS14 (mouse monoclonal)	NeuroMab	73-422	1:300
antibody	anti-GFP (chicken polyclonal)	Abcam	ab13970	1:10,000
antibody	anti-VGluT2 (guinea pig polyclonal)	Millipore	AB22	1:10000
antibody	anti-parvalbumin (rabbit polyclonal)	Swant	PV27	1:2000
antibody	anti- PCP4 (rabbit polyclonal)	Sigma	HPA005792	1:600
antibody	anti-Calretinin (mouse monoclonal)	Millipore	MAB1568	1:500
antibody	anti-mCherry (rat monoclonal	Life technologies	M11217	1:5000
other	far-red neurotrace	Life technologies	N21483	1:300
peptide, recombinant protein	Alexa-546-conjugated streptavidin	Life Technologies	S11225	1:500
peptide, recombinant protein	Biocytin	HelloBio	HB5035	4mg / mL

chemical compound, drug	NBQX	HelloBio	HB0443	10 μM
chemical compound, drug	D-APV	HelloBio	HB0225	50 μM
chemical compound, drug	SR95531	Tocris	1262	1 μM
chemical compound, drug	CGP55845A	Tocris	1248	2 μM
chemical compound, drug	DPDPE	Alfa Aesar	J66293	0.5μM
chemical compound, drug	DAMGO	Tocris	1171	1 μM
chemical compound, drug	clozapine N-oxide (CNO)	HelloBio	HB1807	10 μM
chemical compound, drug	Tetrodotoxin (TTX)	Tocris	1078	0.2 μM
chemical compound, drug	Carbamoylcholine chloride (CCh)	Tocris	2810	10 μM
chemical compound, drug	ω-agatoxin TK	Alomone labs	STA-530	200 nM
software, algorithm	Matlab	Mathworks	www.mathworks.com	
software, algorithm	Igor Pro	Wavemetrics	www.wavemetrics.com	
software, algorithm	OriginPro	OriginLab Corporation	www.originlab.com	

software, algorithm	pClamp	Molecular Devices	www.molecul ardevices.com	
software, algorithm	Axograph	Axograph	www.axograp h.com	

683

All procedures involving animals were performed in accordance with institutional regulations (French Ministry of Research and Education protocol #12406-2016040417305913). Animal sample sizes were estimated using power tests with standard deviations and ANOVA values from pilot experiments. A 15 % failure rate was assumed to account for stereotaxic injection errors and slice preparation complications. Every effort was made to reduce animal suffering.

Use of the Tg(Slc17ab-icre)10Ki mouse line: we used the Tg(Slc17ab-icre)10Ki mouse line that was previously generated (Borgius et al., 2010) and expresses the Cre recombinase under control of the slc17a6 gene coding the vesicular glutamate transporter isoform 2 (VGluT2).

Use of the csf2rb2-Cre mouse line: We used the csf2rb2-Cre mouse line that was recently generated (Chen et al., 2020) and expresses the Cre recombinase under control of the csf2rb2 gene that shows selective expression in the SuM.

Use of the Pvalbtm1(cre)Arbr/J mouse line: we used the Pvalbtm1(cre)Arbr/J mouse line that was previously generated (Hippenmeyer et al., 2005) and expresses the Cre recombinase under control of the Pvalbm gene coding parvalbumin (PV).

Stereotaxic viral injection: Animals were anaesthetized with ketamine (100 mg/kg) and xylazine (7 mg/kg). The adeno-associated viruses AAV9.EF1a.DIO.hChR2(H134R).EYFP and AAV9.hSynapsin.EGFP.WPRE.bGH were used at $3x10^8$ vg, the AAV.Synapsin.DIO.hM4D(Gi).mCherry was used at $3.6x10^9$ vg and the AAV2/9.hSyn.hChR2(H134R).EYFP.WPRE.hGH was used at $3.7x10^{13}$ vg. The retrograde tracer CAV2-cre virus was used at $2.5x10^{12}$ vg. 500 nL of virus was unilaterally injected into the brain of 4 week-old male wild type C57BL/6, Tg(Slc17ab-icre)10Ki (VGluT2-Cre), csf2rb2-cre (SuM-Cre) or Pvalbtm1(cre)Arbr/J (PV-Cre) mice at 100 nL/min and the injection cannula was left at the injection site for 10 min following infusion. In the case of AAV.Synapsin.DIO.hM4D(Gi)-mCherry injection in PV-Cre mice, bilateral injections were performed in dorsal CA2. The loci of the injection sites were as follows: anterior–posterior relative to bregma: -2.8 mm for SuM, -1.6 mm for CA2; medial-lateral relative to midline: 0

710   mm for SuM, 1.9 mm for CA2; dorsal-ventral relative to surface of the brain: 4.75 mm for SuM,

711   1.4 mm for CA2.

712   <u>Electrophysiological recordings:</u> Transverse hippocampal slices were prepared at least 3 weeks

713   after viral injection and whole-cell patch-clamp recordings were performed from PNs and INs

714   across the hippocampal CA regions. In the case of PV-Cre mice injected with

715   AAV.Synapsin.DIO.hM4D(Gi)-mCherry, slices were prepared 6 weeks after viral injection.

716   Animals were deeply anaesthetized with ketamine (100 mg/kg) and xylazine (7 mg/kg), and

717   perfused transcardially with a N-methyl-D-glucamin-based (NMDG) cutting solution

718   containing the following (in mM): NMDG 93, KCl 2.5, NaH_2PO_4 1.25, $NaHCO_3$ 30, HEPES

719   20, glucose 25, thiourea 2, Na-ascorbate 5, Na-pyruvate 3, $CaCl_2$ 0.5, $MgCl_2$ 10. Brains were

720   then rapidly removed, hippocampi were dissected out and placed upright into an agar mold and

721   cut into 400 μm thick transverse slices (Leica VT1200S) in the same cutting solution at 4 °C.

722   Slices were transferred to an immersed-type chamber and maintained in artificial cerebro-spinal

723   fluid (ACSF) containing the following (in mM) : NaCl 125, KCl 2.5, NaH_2PO_4 1.25, $NaHCO_3$

724   26, glucose 10, Na-pyruvate 2, $CaCl_2$ 2, $MgCl_2$ 1. Slices were incubated at 32°C for

725   approximately 20 min then maintained at room temperature for at least 45 min prior to patch-

726   clamp recordings performed with either potassium- or cesium-based intracellular solutions

727   containing the following (in mM): K- or Cs-methyl sulfonate 135, KCl 5, EGTA-KOH 0.1,

728   HEPES 10, NaCl 2, MgATP 5, Na_2GTP 0.4, Na_2-phosphocreatine 10 and biocytin (4 mg/mL).

729   ChR2 was excited by 488 nm light delivered by a LED attached to the epifluorescence port of

730   the microscope. Light stimulations trains consisted of 2-10 pulses, 0.5 ms long, delivered at 10

731   Hz, repeated every 20 s for at least 20 sweeps. Stimulating pipettes filled with ACSF were

732   placed in *stratum radiatum* (SR) of CA1 to antidromically excite CA3-CA2 synapses and in

733   *stratum lacunosum moleculare* (SLM) to stimulate distal dendritic inputs in area CA2. Synaptic

734   currents were evoked with a constant voltage stimulating unit (Digitimer Ltd.) set at 0.1 msec

735   at a voltage range of 5 to 10 V. For the patch-clamp recordings in area CA1 with stimulation of

736   SuM axons in area CA2, 50 ms long light stimulation pulses were delivered every 10 seconds.

737   We used a light intensity of 25 mW/mm² which was experimentally determined as the lowest

738   irradiance allowing TTX-sensitive maximal responses in all cell types and conditions. Data

739   were obtained using a Multiclamp 700B amplifier, sampled at 10 kHz and digitized using a

740   Digidata. The pClamp10 software was used for data acquisition. Series resistance were < 20

741   MOhm and were not compensated in voltage-clamp, bridge balance was applied in current-

742   clamp. An experimentally determined liquid junction potential of approximately 9 mV was not

743   corrected for. Pharmacological agents were added to ACSF at the following concentrations (in

744   μM): 10 NBQX and 50 D-APV to block AMPA and NMDA receptors, 1 SR95531 and 2

745   CGP55845A to block $GABA_A$ and $GABA_B$ receptors, 1 DAMGO to activate μ-opioid receptors

746   (MOR), 0.5 DPDPE to activate δ-opioid receptors (DOR), 10 clozapine N-oxide (CNO) to

747   activate hM4D(Gi) DREADDs, 10 CCh to activate cholinergic receptors, 0.2 tetrodotoxin

748   (TTX) to prevent sodic action potential generation, 200 nM ω-agatoxin TK to block P/Q-type

749   voltage-gated calcium channels.

750   <u>Surgery for *in vivo* recordings:</u> All surgeries were performed in a stereotaxic frame (Narishige).

751   Csf2rb2-cre male mice from 3 to 6 months of age were anaesthetized using 500 mg/kg Avertin.

752   pAAV.DIO.hChR2(H134R).EYFP was injected into the SuM (-2.7 mm AP, $+0.4$ mm ML,

753   -5.0 mm DV) using a 10 μL Hamilton microsyringe (701LT, Hamilton) with a beveled 33

754   gauge needle (NF33BL, World Precision Instruments (WPI)). A microsyringe pump (UMP3,

755   WPI) with controller (Micro4, WPI) were used to set the speed of the injection (100 nl/min).

756   The needle was slowly lowered to the target site and remained in place for 5 min prior to start

757   of the injection and the needle was removed 10 min after infusion was complete. Following

758   virus injection, a custom-built screw-driven microdrive containing six independently adjustable

759   nichrome tetrodes (14 μm diameter), gold-plated to an impedance of 200 to 250 kΩ was

760   implanted, with a subset of tetrodes targeting CA1, and an optic fiber (200 μm core diameter,

761   NA=0.22) targeting CA2 (-1.9 mm AP, $+/-$ 2.2 mm ML, -1.6 mm DV). Following recovery,

762   the tetrodes were slowly lowered over several days to CA1 pyramidal cell layer, identified by

763   characteristic local field potential patterns (theta and sharp-wave ripples) and high amplitude

764   multiunit activity. During the adjustment period the animal was habituated every day to a small

765   box in which recording and stimulation were performed.

766   *In vivo* recording protocol: Recording was commenced following tetrodes reaching CA1. To

767   examine the impact of SuM terminal stimulation in CA2 the mice were returned to the small

768   familiar box and trains of 10 light pulses (473 nm, 10 mW/mm^2 and pulse width 50 ms) were

769   delivered to the CA2 at 10 Hz. The pulse train was repeated every 10 seconds for at least 20

770   times as the animals freely explored the box. Multiunit activity was recorded using a

771   DigitalLynx 4SX recording system running Cheetah v.5.6.0 acquisition software (Neuralynx).

772   Broadband signals from each tetrode were filtered between 600 and 6,000 Hz and recorded

773   continuously at 32 kHz. Recording sites were later verified histologically with electrolytic

774   lesions as described above and the position of the optic fiber was also verified from the track.

775   *In Vivo* data analysis:

776 Spike and event timestamps corresponding to onset of each laser pulse were imported into
777 Matlab (MathWorks) and spikes which occurred 50 ms before and 100 ms after each laser pulse
778 were extracted. Raster plots were generated using a 1 ms bin size. Similar results were obtained
779 using 5 ms and 10 ms bin size (data not shown). Firing rate histograms were calculated by
780 dividing total number of spikes in each time bin by that bin's duration. Each firing rate
781 histogram was normalized by converting it into z-score values. Mean standard deviation values
782 for the z-score calculation were taken from pre-laser pulse time period. To average the response
783 across all mice, for each tetrode the firing rate in each bin was normalized to the average rate
784 in the pre-laser period.

785 <u>Immunochemistry and cell identification:</u>

786 Midbrains containing the injection site were examined post-hoc to ensure that infection was
787 restricted to the SuM.

788 Post-hoc reconstruction of neuronal morphology and SuM axonal projections were performed
789 on slices and midbrain tissue following overnight incubation in 4 % paraformaldehyde in
790 phosphate buffered saline (PBS). Midbrain sections were re-sliced sagittally to 100 μm thick
791 sections. Slices were permeabilized with 0.2 % triton in PBS and blocked overnight with 3 %
792 goat serum in PBS with 0.2 % triton. Primary antibody (Life technologies) incubation was
793 carried out in 3 % goat serum in PBS overnight at 4°C. Channelrhodopsin-2 was detected by
794 chicken primary antibody to GFP (Life technologies) (1:10,000 dilution) and an alexa488-
795 conjugated goat-anti chick secondary. Other primary antibodies used were mouse anti-RGS14
796 (Neuromab) (1:300 dilution), rabbit anti- PCP4 (Sigma) (1:600 dilution), guinea pig anti-
797 VGluT2 antibody (Milipore) (1:10,000 dilution), rabbit anti-parvalbumin antibody (Swant)
798 (1:2000 dilution). Alexa-546-conjugated streptavidin (Life technologies), secondary antibodies
799 and far-red neurotrace (Life technologies) incubations were carried out in block solution for 4
800 hours at room temperature. Images were collected with a Zeiss 710 laser-scanning confocal
801 microscope.

802 Reconstructed neurons were classified as either PNs or INs based on the extension and
803 localization of their dendrites and axons. PNs were classified as deep (closest to *stratum oriens*)
804 or superficial (closest to *stratum radiatum*) based on the radial position of their soma in the
805 pyramidal layer. CA1, CA2 and CA3 PNs were identified based on their somatic localization,
806 dendritic arborization and presence of thorny excrescences (TE). Among INs with somas
807 located in the pyramidal layer (*stratum pyramidale*, SP), discrimination between BCs and non-
808 BCs was achieved based on the restriction of their axons to SP or not, respectively. When

809 available, firing patterns upon injection of depolarizing current step injection, action potential

810 (AP) half-width, amount of repolarizing sag current upon hyperpolarization from -70 mV to -

811 100 mV by current step injection, membrane resistance (R_M) and capacitance (C_M) were

812 additionally used for cell identification. CA2 and CA3a PNs as well as superficial and deep

813 PNs displayed similar firing patterns, AP width, sag current, R_M and C_M; the only statistically

814 difference being a larger R_M of CA3a compared to CA2 PNs which is consistent with previous

815 studies (Chevaleyre and Siegelbaum, 2010; Sun et al., 2017). In contrast, INs had faster firing

816 rates, shorter AP width, higher R_M and lower C_M than PNs. BCs further differed from non-BCs

817 by the presence of a larger sag current. All recorded neurons that could not be unequivocally

818 identified as PNs or INs were excluded from analysis. SuM connectivity to each neuronal

819 population was quantified by dividing the number of cells that displayed a post-synaptic

820 response to SuM input stimulation by the total number of cells sampled for each neuronal

821 population across all recording sessions with successful SuM-CA2 transmission.

822 <u>Data analysis and statistics</u>: Electrophysiological recordings were analyzed using IGORpro

823 (Wavemetrics) and Clampfit (Molecular devices) software. For accurate measurements of the

824 kinetics and latencies of post-synaptic responses, the following detection process was used. For

825 each cell, average traces were used to create a template waveform that was then fitted to

826 individual traces and measurements were performed on the fitted traces. When only amplitudes

827 of responses were needed, traces were baselined and amplitudes were simply measured at the

828 peak of the responses. Results are reported ± SEM. Statistical significance was assessed using

829 χ^2 test, Student's T test, Mann-Whitney U test, Wilcoxon signed-rank test, Kruskal-Wallis test,

830 one-way or two-way ANOVA where appropriate.

831

832 **Author Contributions**

833 RAP, VR & TM designed experiments. RAP, VR, VC, LT, EL, RB, AJYH performed

834 experiments. JC and CV provided technical support. VR, RAP, VC and DP completed analysis.

835 VR and RAP wrote the manuscript with input from all authors.

836

845

846 **References**

847
848

849 Alexander GM, Brown LY, Farris S, Lustberg D, Pantazis C, Gloss B, Plummer NW, Jensen
850   P, Dudek SM. 2018. CA2 neuronal activity controls hippocampal low gamma and ripple
851   oscillations. *eLife* **7**:27. doi:10.7554/elife.38052

852 Aranda L, Begega A, Sánchez-López J, Aguirre JA, Arias JL, Santín LJ. 2008. Temporary
853   inactivation of the supramammillary area impairs spatial working memory and spatial
854   reference memory retrieval. *Physiology Behav* **94**:322–330.
855   doi:10.1016/j.physbeh.2008.01.024

856 Aranda L, Santín LJ, Begega A, Aguirre JA, Arias JL. 2006. Supramammillary and adjacent
857   nuclei lesions impair spatial working memory and induce anxiolitic-like behavior. *Behav*
858   *Brain Res* **167**:156–164. doi:10.1016/j.bbr.2005.09.002

859 Bartesaghi R, Ravasi L. 1999. Pyramidal neuron types in field CA2 of the guinea pig. *Brain*
860   *research bulletin* **50**:263–273.

861 Belluscio MA, Mizuseki K, Schmidt R, Kempter R, Buzsáki G. 2012. Cross-Frequency
862   Phase–Phase Coupling between Theta and Gamma Oscillations in the Hippocampus. *J*
863   *Neurosci* **32**:423–435. doi:10.1523/jneurosci.4122-11.2012

864 Benes FM, Kwok EW, Vincent SL, Todtenkopf MS. 1998. A reduction of nonpyramidal cells
865   in sector CA2 of schizophrenics and manic depressives. *Biol Psychiat* **44**:88–97.
866   doi:10.1016/s0006-3223(98)00138-3

867 Berger B, Esclapez M, Alvarez C, Meyer G, Catala M. 2001. Human and monkey fetal brain
868   development of the supramammillary-hippocampal projections: A system involved in the
869   regulation of theta activity. *J Comp Neurol* **429**:515–529. doi:10.1002/1096-
870   9861(20010122)429:4<515::aid-cne1>3.0.co;2-2

871 Boehringer R, Polygalov D, Huang AJY, Middleton SJ, Robert V, Wintzer ME, Piskorowski
872   RA, Chevaleyre V, McHugh TJ. 2017. Chronic Loss of CA2 Transmission Leads to
873   Hippocampal Hyperexcitability. *Neuron* **94**:642-655.e9. doi:10.1016/j.neuron.2017.04.014

874 Borgius L, Restrepo CE, Leao RN, Saleh N, Kiehn O. 2010. A transgenic mouse line for
875   molecular genetic analysis of excitatory glutamatergic neurons. *Mol Cell Neurosci* **45**:245–
876   257. doi:10.1016/j.mcn.2010.06.016

877 Borhegyi Z, Maglóczky Z, Acsády L, Freund TF. 1998. The supramammillary nucleus
878   innervates cholinergic and GABAergic neurons in the medial septum-diagonal band of
879   Broca complex. *Neuroscience* **82**:1053–1065. doi:10.1016/s0306-4522(97)00301-1

880 Botcher NA, Falck JE, Thomson AM, Mercer A. 2014. Distribution of interneurons in the
881   CA2 region of the rat hippocampus. *Frontiers Neuroanatomy* **8**:104.
882   doi:10.3389/fnana.2014.00104

883 Boulland J-L, Jenstad M, Boekel AJ, Wouterlood FG, Edwards RH, Storm-Mathisen J,
884   Chaudhry FA. 2009. Vesicular glutamate and GABA transporters sort to distinct sets of
885   vesicles in a population of presynaptic terminals. *Cereb Cortex* **19**:241–248.
886   doi:10.1093/cercor/bhn077

887 Brady DR, Mufson EJ. 1997. Parvalbumin-immunoreactive neurons in the hippocampal
888   formation of Alzheimer's diseased brain. *Neuroscience* **80**:1113–1125. doi:10.1016/s0306-
889   4522(97)00068-7

890 Buzsáki G. 2002. Theta oscillations in the hippocampus. *Neuron* **33**:325–340.

891 Buzsáki G, Moser EI. 2013. Memory, navigation and theta rhythm in the hippocampal-
892   entorhinal system. *Nat Neurosci* **16**:130–138. doi:10.1038/nn.3304

893 Buzsáki G, Wang XJ. 2012. Mechanisms of gamma oscillations. *Annu Rev Neurosci* **35**:203–
894   225. doi:10.1146/annurev-neuro-062111-150444

895 Castañé A, Santana N, Artigas F. 2015. PCP-based mice models of schizophrenia: differential
896   behavioral, neurochemical and cellular effects of acute and subchronic treatments.
897   *Psychopharmacology* **232**:4085–4097. doi:10.1007/s00213-015-3946-6

898 Cembrowski MS, Wang L, Sugino K, Shields BC, Spruston N, Marder E. 2016. Hipposeq: a
899   comprehensive RNA-seq database of gene expression in hippocampal principal neurons.
900   *Elife* **5**:e14997. doi:10.7554/elife.14997

901 Chen S, He L, Huang AJY, Boehringer R, Robert V, Wintzer ME, Polygalov D, Weitemier
902   AZ, Tao Y, Gu M, Middleton SJ, Namiki K, Hama H, Therreau L, Chevaleyre V, Hioki H,
903   Miyawaki A, Piskorowski RA, McHugh TJ. 2020. A hypothalamic novelty signal
904   modulates hippocampal memory. *Nature* **586**:270–274. doi:10.1038/s41586-020-2771-1

905 Chevaleyre V, Siegelbaum SA. 2010. Strong CA2 pyramidal neuron synapses define a
906   powerful disynaptic cortico-hippocampal loop. *Neuron* **66**:560–572.
907   doi:10.1016/j.neuron.2010.04.013

908 Colgin LL. 2016. Rhythms of the hippocampal network. *Nat Rev Neurosci* **17**:239–249.
909   doi:10.1038/nrn.2016.21

910 Colgin LL, Denninger T, Fyhn M, Hafting T, Bonnevie T, Jensen O, Moser M-B, Moser EI.
911   2009. Frequency of gamma oscillations routes flow of information in the hippocampus.
912   *Nature* **462**:353–357. doi:10.1038/nature08573

913 Dasgupta A, Lim YJ, Kumar K, Baby N, Pang KLK, Benoy A, Behnisch T, Sajikumar S.
914   2020. Group III metabotropic glutamate receptors gate long-term potentiation and synaptic
915   tagging/capture in rat hippocampal area CA2. *eLife* **9**:919–20. doi:10.7554/elife.55344

916 Domínguez S, Rey CC, Therreau L, Fanton A, Massotte D, Verret L, Piskorowski RA,
917   Chevaleyre V. 2019. Maturation of PNN and ErbB4 Signaling in Area CA2 during
918   Adolescence Underlies the Emergence of PV Interneuron Plasticity and Social Memory.
919   *CellReports* **29**:1099-1112.e4. doi:10.1016/j.celrep.2019.09.044

920 Dudok B, Klein PM, Hwaun E, Lee BR, Yao Z, Fong O, Bowler JC, Terada S, Sparks FT,
921   Szabo GG, Farrell JS, Berg J, Daigle TL, Tasic B, Dimidschstein J, Fishell G, Losonczy A,
922   Zeng H, Soltesz I. 2021. Alternating sources of perisomatic inhibition during behavior.
923   *Neuron* **109**:997-1012.e9. doi:10.1016/j.neuron.2021.01.003

924 Eichenbaum H, Cohen NJ. 2014. Can we reconcile the declarative memory and spatial
925   navigation views on hippocampal function? *Neuron* **83**:764–770.
926   doi:10.1016/j.neuron.2014.07.032

927 Erbs E, Faget L, Scherrer G, Kessler P, Hentsch D, Vonesch J-L, Matifas A, Kieffer BL,
928   Massotte D. 2012. Distribution of delta opioid receptor-expressing neurons in the mouse
929   hippocampus. *Neuroscience* **221**:203–213. doi:10.1016/j.neuroscience.2012.06.023

930 Fernandez-Lamo I, Gomez-Dominguez D, Sanchez-Aguilera A, Oliva A, Morales AV,
931   Valero M, Cid E, Berényi A, Prida LM de la. 2019. Proximodistal Organization of the
932   CA2 Hippocampal Area. *Cell Reports* **26**:1734-1746.e6. doi:10.1016/j.celrep.2019.01.060

933 Fuchs EC, Zivkovic AR, Cunningham MO, Middleton S, Lebeau FEN, Bannerman DM,
934   Rozov A, Whittington MA, Traub RD, Rawlins JNP, Monyer H. 2007. Recruitment of
935   parvalbumin-positive interneurons determines hippocampal function and associated
936   behavior. *Neuron* **53**:591–604. doi:10.1016/j.neuron.2007.01.031

937 Glickfeld LL, Atallah BV, Scanziani M. 2008. Complementary modulation of somatic
938   inhibition by opioids and cannabinoids. *J Neurosci* **28**:1824–1832.
939   doi:10.1523/jneurosci.4700-07.2008

940 Gulyás AI, Szabó GG, Ulbert I, Holderith N, Monyer H, Erdélyi F, Szabó G, Freund TF,
941   Hájos N. 2010. Parvalbumin-containing fast-spiking basket cells generate the field
942   potential oscillations induced by cholinergic receptor activation in the hippocampus. *J*
943   *Neurosci* **30**:15134–15145. doi:10.1523/jneurosci.4104-10.2010

944 Gutiérrez-Guzmán BE, Hernández-Pérez JJ, López-Vázquez MÁ, Fregozo CS, Guevara MÁ,
945   Olvera-Cortés ME. 2012. Serotonin depletion of supramammillary/posterior hypothalamus
946   nuclei produces place learning deficiencies and alters the concomitant hippocampal theta
947   activity in rats. *Eur J Pharmacol* **682**:99–109. doi:10.1016/j.ejphar.2012.02.024

948 Haglund L, Swanson LW, Köhler C. 1984. The projection of the supramammillary nucleus to
949   the hippocampal formation: an immunohistochemical and anterograde transport study with
950   the lectin PHA-L in the rat. *J Comp Neurol* **229**:171–185. doi:10.1002/cne.902290204

951 Halasy K, Hajszan T, Kovács EG, Lam T-T, Leranth C. 2004. Distribution and origin of
952   vesicular glutamate transporter 2-immunoreactive fibers in the rat hippocampus.
953   *Hippocampus* **14**:908–918. doi:10.1002/hipo.20006

954 Hashimotodani Y, Karube F, Yanagawa Y, Fujiyama F, Kano M. 2018. Supramammillary
955   Nucleus Afferents to the Dentate Gyrus Co-release Glutamate and GABA and Potentiate
956   Granule Cell Output. *Cell Reports* **25**:2704-2715.e4. doi:10.1016/j.celrep.2018.11.016

957 Hefft S, Jonas P. 2005. Asynchronous GABA release generates long-lasting inhibition at a
958   hippocampal interneuron-principal neuron synapse. *Nat Neurosci* **8**:1319–1328.
959   doi:10.1038/nn1542

960 Hernández-Pérez JJ, Gutiérrez-Guzmán BE, López-Vázquez MÁ, Olvera-Cortés ME. 2015.
961   Supramammillary serotonin reduction alters place learning and concomitant hippocampal,
962   septal, and supramammillar theta activity in a Morris water maze. *Frontiers Pharmacol*
963   **6**:250. doi:10.3389/fphar.2015.00250

964 Hippenmeyer S, Vrieseling E, Sigrist M, Portmann T, Laengle C, Ladle DR, Arber S. 2005. A
965   Developmental Switch in the Response of DRG Neurons to ETS Transcription Factor
966   Signaling. *Plos Biol* **3**:e159. doi:10.1371/journal.pbio.0030159

967 Hitti FL, Siegelbaum SA. 2014. The hippocampal CA2 region is essential for social memory.
968   *Nature* **508**:88–92. doi:10.1038/nature13028

969 Ikemoto S. 2005. The supramammillary nucleus mediates primary reinforcement via
970   GABA(A) receptors. *Neuropsychopharmacol* **30**:1088–1095. doi:10.1038/sj.npp.1300660

971 Ikemoto S, Witkin BM, Zangen A, Wise RA. 2004. Rewarding effects of AMPA
972   administration into the supramammillary or posterior hypothalamic nuclei but not the
973   ventral tegmental area. *J Neurosci* **24**:5758–5765. doi:10.1523/jneurosci.5367-04.2004

974 Ito HT, Moser EI, Moser M-B. 2018. Supramammillary Nucleus Modulates Spike-Time
975   Coordination in the Prefrontal-Thalamo- Hippocampal Circuit during Navigation. *Neuron*
976   **99**:576-587.e5. doi:10.1016/j.neuron.2018.07.021

977 Ito M, Shirao T, Doya K, Sekino Y. 2009. Three-dimensional distribution of Fos-positive
978   neurons in the supramammillary nucleus of the rat exposed to novel environment.
979   *Neurosci Res* **64**:397–402. doi:10.1016/j.neures.2009.04.013

980 Kay K, Sosa M, Chung JE, Karlsson MP, Larkin MC, Frank LM. 2016. A hippocampal
981   network for spatial coding during immobility and sleep. *Nature* **531**:185–190.
982   doi:10.1038/nature17144

983 Kirk IJ, McNaughton N. 1993. Mapping the differential effects of procaine on frequency and
984   amplitude of reticularly elicited hippocampal rhythmical slow activity. *Hippocampus*
985   **3**:517–525. doi:10.1002/hipo.450030411

986 Kirk IJ, Oddie SD, Konopacki J, Bland BH. 1996. Evidence for differential control of
987   posterior hypothalamic, supramammillary, and medial mammillary theta-related cellular

988   discharge by ascending and descending pathways. *J Neurosci Official J Soc Neurosci*
989   **16**:5547–54.

990   Kiss J, Csáki Á, Bokor H, Shanabrough M, Leranth C. 2000. The supramammillo-
991   hippocampal and supramammillo-septal glutamatergic/aspartatergic projections in the rat:
992   a combined [3H]d-aspartate autoradiographic and immunohistochemical study.
993   *Neuroscience* **97**:657–669. doi:10.1016/s0306-4522(00)00127-5

994   Klausberger T, Somogyi P. 2008. Neuronal diversity and temporal dynamics: the unity of
995   hippocampal circuit operations. *Science* **321**:53–57. doi:10.1126/science.1149381

996   Knable MB, Barci BM, Webster MJ, Meador-Woodruff J, Torrey EF, Consortium SN. 2004.
997   Molecular abnormalities of the hippocampus in severe psychiatric illness: postmortem
998   findings from the Stanley Neuropathology Consortium. *Mol Psychiatr* **9**:609-20–544.
999   doi:10.1038/sj.mp.4001471

1000   Kocsis Bernat, Vertes RP. 1994. Characterization of neurons of the supramammillary nucleus
1001   and mammillary body that discharge rhythmically with the hippocampal theta rhythm in
1002   the rat. *J Neurosci* **14**:7040–7052. doi:10.1523/jneurosci.14-11-07040.1994

1003   Kocsis B, Vertes RP. 1994. Characterization of neurons of the supramammillary nucleus and
1004   mammillary body that discharge rhythmically with the hippocampal theta rhythm in the
1005   rat. *J Neurosci Official J Soc Neurosci* **14**:7040–52.

1006   Kohara K, Pignatelli M, Rivest AJ, Jung H-Y, Kitamura T, Suh J, Frank D, Kajikawa K, Mise
1007   N, Obata Y, Wickersham IR, Tonegawa S. 2014. Cell type-specific genetic and
1008   optogenetic tools reveal hippocampal CA2 circuits. *Nat Neurosci* **17**:269–279.
1009   doi:10.1038/nn.3614

1010   Korotkova T, Fuchs EC, Ponomarenko A, Engelhardt J von, Monyer H. 2010. NMDA
1011   receptor ablation on parvalbumin-positive interneurons impairs hippocampal synchrony,
1012   spatial representations, and working memory. *Neuron* **68**:557–569.
1013   doi:10.1016/j.neuron.2010.09.017

1014   Lee SY, Földy C, Szabadics J, Soltesz I. 2011. Cell-type-specific CCK2 receptor signaling
1015   underlies the cholecystokinin-mediated selective excitation of hippocampal parvalbumin-
1016   positive fast-spiking basket cells. *J Neurosci* **31**:10993–11002.
1017   doi:10.1523/jneurosci.1970-11.2011

1018   Lein ES, Zhao X, Gage FH. 2004. Defining a Molecular Atlas of the Hippocampus Using
1019   DNA Microarrays and High-Throughput In Situ Hybridization. *Journal of Neuroscience*
1020   **24**:3879–3889. doi:10.1523/jneurosci.4710-03.2004

1021   Leroy F, Brann DH, Meira T, Siegelbaum SA. 2017. Input-Timing-Dependent Plasticity in
1022   the Hippocampal CA2 Region and Its Potential Role in Social Memory. *Neuron* **95**:1089-
1023   1102.e5. doi:10.1016/j.neuron.2017.07.036

1024   Li Y, Bao H, Luo Y, Yoan C, Sullivan HA, Quintanilla L, Wickersham I, Lazarus M, Shin Y-
1025   YI, Song J. 2020. Supramammillary nucleus synchronizes with dentate gyrus to regulate

1026 spatial memory retrieval through glutamate release. *eLife* **9**:604–23.
1027 doi:10.7554/elife.53129

1028 Llorens-Martín M, Jurado-Arjona J, Avila J, Hernández F. 2015. Novel connection between
1029 newborn granule neurons and the hippocampal CA2 field. *Exp Neurol* **263**:285–292.
1030 doi:10.1016/j.expneurol.2014.10.021

1031 Maglóczky Z, Acsády L, Freund TF. 1994. Principal cells are the postsynaptic targets of
1032 supramammillary afferents in the hippocampus of the rat. *Hippocampus* **4**:322–334.
1033 doi:10.1002/hipo.450040316

1034 Mann EO, Mody I. 2010. Control of hippocampal gamma oscillation frequency by tonic
1035 inhibition and excitation of interneurons. *Nat Neurosci* **13**:205–212. doi:10.1038/nn.2464

1036 May MVL, Hume C, Sabatier N, Schéle E, Bake T, Bergström U, Menzies J, Dickson SL.
1037 2019. Activation of the rat hypothalamic supramammillary nucleus by food anticipation,
1038 food restriction or ghrelin administration. *Journal of neuroendocrinology* **31**:e12676-14.
1039 doi:10.1111/jne.12676

1040 McNaughton N, Logan B, Panickar KS, Kirk IJ, Pan W, Brown NT, Heenan A. 1995.
1041 Contribution of synapses in the medial supramammillary nucleus to the frequency of
1042 hippocampal theta rhythm in freely moving rats. *Hippocampus* **5**:534–545.
1043 doi:10.1002/hipo.450050605

1044 Mizumori SJ, McNaughton BL, Barnes CA. 1989. A comparison of supramammillary and
1045 medial septal influences on hippocampal field potentials and single-unit activity. *Journal
1046 of Neurophysiology* **61**:15–31.

1047 Nakanishi K, Saito H, Abe K. 2001. The supramammillary nucleus contributes to associative
1048 EPSP-spike potentiation in the rat dentate gyrus in vivo. *Eur J Neurosci* **13**:793–800.
1049 doi:10.1046/j.1460-9568.2001.01446.x

1050 Nasrallah K, Piskorowski RA, Chevaleyre V. 2015. Inhibitory Plasticity Permits the
1051 Recruitment of CA2 Pyramidal Neurons by CA3(1,2,3). *Eneuro* **2**:1–12.
1052 doi:10.1523/eneuro.0049-15.2015

1053 Nasrallah K, Therreau L, Robert V, Huang AJY, McHugh TJ, Piskorowski RA, Chevaleyre
1054 V. 2019. Routing Hippocampal Information Flow through Parvalbumin Interneuron
1055 Plasticity in Area CA2. *Cell Reports* **27**:86-98.e3. doi:10.1016/j.celrep.2019.03.014

1056 No RL de. 1934. Studies on the Structure of the Cerebral Cortex. II. Continuation of the Study
1057 of the Ammonic System. *Journal f Psychologie and Neurologie* 113–175.

1058 Okuyama T, Kitamura T, Roy DS, Itohara S, Tonegawa S. 2016. Ventral CA1 neurons store
1059 social memory. *Science* **353**:1536–1541. doi:10.1126/science.aaf7003

1060 Oliva A, Fernández-Ruiz A, Buzsáki G, Berényi A. 2016a. Role of Hippocampal CA2 Region
1061 in Triggering Sharp-Wave Ripples. *Neuron* **91**:1342–1355.
1062 doi:10.1016/j.neuron.2016.08.008

1063   Oliva A, Fernández-Ruiz A, Buzsáki G, Berényi A. 2016b. Spatial coding and physiological
1064   properties of hippocampal neurons in the Cornu Ammonis subregions. *Hippocampus*
1065   **26**:1593–1607. doi:10.1002/hipo.22659

1066   Pan W, McNaughton N. 2002. The role of the medial supramammillary nucleus in the control
1067   of hippocampal theta activity and behaviour in rats. *Eur J Neurosci* **16**:1797–1809.
1068   doi:10.1046/j.1460-9568.2002.02267.x

1069   Pan W-X, Mcnaughton N. 2004. The supramammillary area: its organization, functions and
1070   relationship to the hippocampus. *Prog Neurobiol* **74**:127–166.
1071   doi:10.1016/j.pneurobio.2004.09.003

1072   Pan W-X, McNaughton N. 1997. The medial supramammillary nucleus, spatial learning and
1073   the frequency of hippocampal theta activity. *Brain Res* **764**:101–108. doi:10.1016/s0006-
1074   8993(97)00431-9

1075   Pawelzik H, Hughes DI, Thomson AM. 2002. Physiological and morphological diversity of
1076   immunocytochemically defined parvalbumin- and cholecystokinin-positive interneurones
1077   in CA1 of the adult rat hippocampus. *J Comp Neurol* **443**:346–367. doi:10.1002/cne.10118

1078   Pedersen NP, Ferrari L, Venner A, Wang JL, Abbott SBG, Vujovic N, Arrigoni E, Saper CB,
1079   Fuller PM. 2017. Supramammillary glutamate neurons are a key node of the arousal
1080   system. *Nat Commun* **8**:1–16. doi:10.1038/s41467-017-01004-6

1081   Piskorowski RA, Chevaleyre V. 2013. Delta-opioid receptors mediate unique plasticity onto
1082   parvalbumin-expressing interneurons in area CA2 of the hippocampus. *J Neurosci*
1083   **33**:14567–14578. doi:10.1523/jneurosci.0649-13.2013

1084   Piskorowski RA, Nasrallah K, Diamantopoulou A, Mukai J, Hassan SI, Siegelbaum SA,
1085   Gogos JA, Chevaleyre V. 2016. Age-Dependent Specific Changes in Area CA2 of the
1086   Hippocampus and Social Memory Deficit in a Mouse Model of the 22q11.2 Deletion
1087   Syndrome. *Neuron* **89**:163–176. doi:10.1016/j.neuron.2015.11.036

1088   Plaisier F, Hume C, Menzies J. 2020. Neural connectivity between the hypothalamic
1089   supramammillary nucleus and appetite- and motivation-related regions of the rat brain.
1090   *Journal of neuroendocrinology* jne.12829-31. doi:10.1111/jne.12829

1091   Renouard L, Billwiller F, Ogawa K, Clément O, Camargo N, Abdelkarim M, Gay N, Scoté-
1092   Blachon C, Touré R, Libourel P-A, Ravassard P, Salvert D, Peyron C, Claustrat B, Léger
1093   L, Salin P, Malleret G, Fort P, Luppi P-H. 2015. The supramammillary nucleus and the
1094   claustrum activate the cortex during REM sleep. *Sci Adv* **1**:e1400177–e1400177.
1095   doi:10.1126/sciadv.1400177

1096   Robert V, Therreau L, Davatolhagh MF, Bernardo-Garcia FJ, Clements KN, Chevaleyre V,
1097   Piskorowski RA. 2020. The mechanisms shaping CA2 pyramidal neuron action potential
1098   bursting induced by muscarinic acetylcholine receptor activation. *J Gen Physiol* **152**.
1099   doi:10.1085/jgp.201912462

1100   Rowland DC, Weible AP, Wickersham IR, Wu H, Mayford M, Witter MP, Kentros CG.
1101   2013. Transgenically Targeted Rabies Virus Demonstrates a Major Monosynaptic

1102   Projection from Hippocampal Area CA2 to Medial Entorhinal Layer II Neurons. *J*
1103   *Neurosci* **33**:14889–14898. doi:10.1523/jneurosci.1046-13.2013

1104   Schomburg EW, Fernández-Ruiz A, Mizuseki K, Berényi A, Anastassiou CA, Koch C,
1105   Buzsáki G. 2014. Theta Phase Segregation of Input-Specific Gamma Patterns in
1106   Entorhinal-Hippocampal Networks. *Neuron* **84**:470–485.
1107   doi:10.1016/j.neuron.2014.08.051

1108   Shahidi S, Motamedi F, Naghdi N. 2004. Effect of reversible inactivation of the
1109   supramammillary nucleus on spatial learning and memory in rats. *Brain Res* **1026**:267–
1110   274. doi:10.1016/j.brainres.2004.08.030

1111   Silkis IG, Markevich VA. 2020. Possible Mechanisms of the Influence of the Supramillary
1112   Nucleus on the Functioning of the Dentate Gyrus and the CA2 Field of the Hippocamsus
1113   (Role of Disinhibition). *Neurochem J* **14**:375–383. doi:10.1134/s181971242004011x

1114   Soussi R, Zhang N, Tahtakran S, Houser CR, Esclapez M. 2010. Heterogeneity of the
1115   supramammillary-hippocampal pathways: evidence for a unique GABAergic
1116   neurotransmitter phenotype and regional differences. *Eur J Neurosci* **32**:771–785.
1117   doi:10.1111/j.1460-9568.2010.07329.x

1118   Srinivas KV, Buss EW, Sun Q, Santoro B, Takahashi H, Nicholson DA, Siegelbaum SA.
1119   2017. The Dendrites of CA2 and CA1 Pyramidal Neurons Differentially Regulate
1120   Information Flow in the Cortico-Hippocampal Circuit. *J Neurosci* **37**:3276–3293.
1121   doi:10.1523/jneurosci.2219-16.2017

1122   Stagkourakis S, Spigolon G, Williams P, Protzmann J, Fisone G, Broberger C. 2018. A neural
1123   network for intermale aggression to establish social hierarchy. *Nat Neurosci* **21**:834–842.
1124   doi:10.1038/s41593-018-0153-x

1125   Stevenson EL, Caldwell HK. 2014. Lesions to the CA2 region of the hippocampus impair
1126   social memory in mice. *Eur J Neurosci* **40**. doi:10.1111/ejn.12689

1127   Stumm RK, Zhou C, Schulz S, Höllt V. 2004. Neuronal types expressing mu- and delta-
1128   opioid receptor mRNA in the rat hippocampal formation. *J Comp Neurol* **469**:107–118.
1129   doi:10.1002/cne.10997

1130   Sun Q, Sotayo A, Cazzulino AS, Snyder AM, Denny CA, Siegelbaum SA. 2017.
1131   Proximodistal Heterogeneity of Hippocampal CA3 Pyramidal Neuron Intrinsic Properties,
1132   Connectivity, and Reactivation during Memory Recall. *Neuron* **95**:656-672.e3.
1133   doi:10.1016/j.neuron.2017.07.012

1134   Sun Q, Srinivas KV, Sotayo A, Siegelbaum SA. 2014. Dendritic Na(+) spikes enable cortical
1135   input to drive action potential output from hippocampal CA2 pyramidal neurons. *Elife*
1136   **3**:7750. doi:10.7554/elife.04551

1137   Thinschmidt JS, Kinney GG, Kocsis B. 1995. The supramammillary nucleus: Is it necessary
1138   for the mediation of hippocampal theta rhythm? *Neuroscience* **67**:301–312.
1139   doi:10.1016/0306-4522(95)00045-k

1140 Tirko NN, Eyring KW, Carcea I, Mitre M, Chao MV, Froemke RC, Tsien RW. 2018.
1141   Oxytocin Transforms Firing Mode of CA2 Hippocampal Neurons. *Neuron* **100**:593-
1142   608.e3. doi:10.1016/j.neuron.2018.09.008

1143 Vertes RP. 1992. PHA-L analysis of projections from the supramammillary nucleus in the rat.
1144   *J Comp Neurol* **326**:595–622. doi:10.1002/cne.903260408

1145 Vertes RP, Kocsis B. 1997. Brainstem-diencephalo-septohippocampal systems controlling the
1146   theta rhythm of the hippocampus. *Neuroscience* **81**:893–926.

1147 Vicente AF, Slézia A, Ghestem A, Bernard C, Quilichini PP. 2020. In Vivo Characterization
1148   of Neurophysiological Diversity in the Lateral Supramammillary Nucleus during
1149   Hippocampal Sharp-wave Ripples of Adult Rats. *Neuroscience* **435**:95–111.
1150   doi:10.1016/j.neuroscience.2020.03.034

1151 Wyss JM, Swanson LW, Cowan WM. 1979. Evidence for an input to the molecular layer and
1152   the stratum granulosum of the dentate gyrus from the supramammillary region of the
1153   hypothalamus. *Anat Embryol* **156**:165–176. doi:10.1007/bf00300012

1154 Zaitsev AV, Povysheva NV, Lewis DA, Krimer LS. 2007. P/Q-Type, But Not N-Type,
1155   Calcium Channels Mediate GABA Release From Fast-Spiking Interneurons to Pyramidal
1156   Cells in Rat Prefrontal Cortex. *J Neurophysiol* **97**:3567–3573. doi:10.1152/jn.01293.2006

1157 Zhao M, Choi Y-S, Obrietan K, Dudek SM. 2007. Synaptic plasticity (and the lack thereof) in
1158   hippocampal CA2 neurons. *J Neurosci* **27**:12025–12032. doi:10.1523/jneurosci.4094-
1159   07.2007

1160

1161

1162 **Figure legends**

1163 **Figure 1. Selective functional mapping of SuM neurons that project to hippocampal area**

1164 **CA2.** A. Left, diagram illustrating the injection of AAVs into the SuM. Middle, sagittal image

1165 indicating the infected SuM area expressing hCHR2(H134R)-EYFP (green). Right, expanded

1166 view of injection site in the Csf2rbr-Cre mouse line. B. Left, hCHR2(H134R)-EYFP -

1167 expressing SuM fibers (green) and nissl staining (blue) in the hippocampus. Right, higher

1168 magnification image of area CA2 with hCHR2(H134R)-EYFP -expressing SuM fibers (green)

1169 and nissl staining (blue) and RGS14 staining (magenta) to label area CA2. C. CA2 pyramidal

1170 neurons in the SuM-innervated region receive excitatory transmission. (C1) Example CA2 PN

1171 reconstruction (dendrites in black, axons in grey, hippocampal stratum borders shown in dotted

1172 line, area demarcated in blue corresponds to the expanded image in C2). (C2) Biocytin labeling

1173 of the recorded cell proximal dendrites, scale bar represents 10 μm. (C3) AP firing and

1174   repolarizing sag current in response to steps of +800 and -400 pA current injection. (C4) Light-

1175   evoked EPSPs (top traces, individual traces shown in grey, average trace shown in black) and

1176   EPSCs (bottom traces, individual traces shown in grey, average trace shown in black). D. CA3a

1177   pyramidal neurons in the SuM-innervated region receive excitatory transmission. (D1) Example

1178   CA3 PN reconstruction (dendrites in brown, axons in light brown, hippocampal stratum borders

1179   shown in dotted line, area demarcated in blue corresponds to the expanded image in D2). (D2)

1180   Biocytin labeling of the recorded cell proximal dendrites, note the presence of thorny

1181   excrescences, as indicated by the red arrows; scale bar represents 10 μm. (D3) AP firing and

1182   repolarizing sag current in response to steps of +800 and -400 pA current injection. (D4) Light-

1183   evoked EPSPs (top traces, individual traces shown in grey, average trace shown in black) and

1184   EPSCs (bottom traces, individual traces shown in grey, average trace shown in black).

1185

1186   **Figure 2. SuM input drives inhibition that controls excitation in CA2 PNs.** Whole-cell

1187   current clamp recordings of light-evoked post-synaptic potentials (PSPs) from SuM input

1188   stimulation onto CA2 PNs reveal contribution of feed-forward inhibition in dampening

1189   excitatory input at -70 mV. A. Diagram illustrating whole-cell recording configuration in acute

1190   hippocampal slices. During these experiments, direct current was injected as necessary to

1191   maintain a membrane potential of -70 mV. B. Sample traces of three 10 Hz SuM light-evoked

1192   PSPs in a CA2 PN before and after blocking inhibitory transmission (control shown in black,

1193   SR95531 & CGP55845A in grey). C. Summary graph of light-evoked PSP amplitudes recorded

1194   in PNs before and after application of 1 μM SR95531& 2 μM CGP55845A (individual cells

1195   shown as thin lines, population average shown as thick line, error bars represent SEM, n = 14;

1196   Wilcoxon signed-rank tests, p = 0.004 for the first PSP, p = 0.013 for the second PSP, p < 0.001

1197   for the third PSP). D-H. Summation of SuM synaptic potentials with SR and SLM electrical

1198   input stimulation. D. Diagram illustrating the recording configuration similar to panel A but

1199   with stimulating electrodes positioned in *stratum lacunosum moleculare* (SLM) and *stratum*

1200   *radiatum* (SR). E. Left, example traces of PSPs evoked by SuM fiber light stimulation alone

1201   (blue trace). Center, PSPs evoked by electrical stimulation of SR inputs alone (black) or paired

1202   with simultaneous SuM stimulation (orange). Right, PSPs evoked by electrical stimulation of

1203   SLM inputs alone (black) or paired with SuM stimulation (green). F, Plots of the difference

1204   between the mathematical summation of the amplitudes of the SuM PSP amplitude and

1205   electrical stimulation (linear summation) and the measured SuM + electrical PSP. Left, SR

1206   inputs are not significantly different from zero, indicating that SuM and SR inputs linearly

1207   summate. Right, for the first pulse, the measured SLM + SuM amplitude is significantly smaller

1208   (n = 10; T test, p = 0.014) than the expected linear summation. G. Left, example traces of 10

1209   Hz trains of PSPs of either electrical stimulation (black traces) or trains of paired electrical and

1210   light stimulation of SuM fibers (SR + SuM in orange or SLM + SuM in green). Right, traces

1211   with amplitudes normalized to the first PSP for both the electrical and simultaneous light and

1212   electrical PSPs. The amplitudes for all PSPs are measured from the potential immediately

1213   before each stimulus. H. Summary plots of the summation ratio of the 2nd, 3rd and 4th PSP for

1214   electrical stimulation (black symbols) or paired stimulation of SR + SuM (left, orange) or SLM

1215   + SuM (right, green).

1216

1217   **Figure 3. SuM input provides strong excitatory glutamatergic transmission to basket cells**

1218   **(BCs) in area CA2.** A-B. Left, diagrams illustrating whole-cell recordings in area CA2 and

1219   SuM fiber stimulation in acute slice preparation. Middle, example reconstruction of different

1220   cell types (soma and dendrites in thick lines, axon in thin lines, hippocampal strata in dotted

1221   grey lines). Right, sample traces of light-evoked EPSPs (top, individual traces in grey, average

1222   trace in black) and EPSCs (bottom, individual traces in grey, average trace in black). A. Basket

1223   cell in area CA2. B. Non-basket cell in area CA2. C. Summary graph of light-evoked EPSC

1224   potencies in PNs, BCs and non-BCs in area CA2 (individual cells shown as dots, population

1225   average shown as thick line, error bars represent SEM, PNs: n = 166; BC INs: n = 18; non-BCs:

1226   n = 13; Kruskal-Wallis test with Dunn-Holland-Wolfe *post hoc* test, p = 0.022). D. Summary

1227   graph of light-evoked PSP amplitudes in PNs, BCs and non-BCs (individual cells shown as

1228   dots, population average shown as thick line, error bars represent SEM, PNs: n = 20; BCs: n =

1229   10; non-BCs: n = 4; Kruskal-Wallis test with Dunn-Holland-Wolfe *post hoc* test, p < 0.001). E.

1230   Left, proportion of post-synaptic CA2 PNs, BCs and non-BCs firing action potentials time-

1231   locked to light stimulation of SuM input. Right, sample traces of light-evoked action potentials

1232   in a BC recorded in current-clamp at resting membrane potential (top) and in cell-attached

1233   (bottom) configurations.

1234

1235   **Figure 4. SuM input provides excitation to Parvalbumin-expressing BCs.** A. Three

1236   biocytin reconstructions of BC INs with dendrites in red and axons in light red. Inset, current

1237   clamp steps to -400 pA and +400 pA display high-frequency AP firing and repolarizing sag

1238   current. B. Corresponding light-evoked EPSCs and EPSPs for the three reconstructed neurons

1239   (individual traces in grey, average trace in black). C. Corresponding PV immunostaining of the

1240   three interneurons: parvalbumin staining, biocytin labeling of the recorded cell, and merge (PV

1241   in magenta and biocytin in green).

1242

1243   **Figure 5. Parvalbumin-expressing BCs mediate the feedforward inhibition recruited by**

1244   **photostimulation of SuM fibers.** A-C. Silencing of PV+ INs by inhibitory DREADDs reduces

1245   SuM feedforward inhibition onto area CA2 PNs. A. Diagram illustrating the method to infect

1246   SuM neurons and selectively inhibit PV+ INs in area CA2. An AAV allowing the Cre-

1247   dependent expression of inhibitory DREADD was injected bilaterally into area CA2 of the

1248   dorsal hippocampus and another AAV allowing the expression of ChR2 was injected into the

1249   SuM of PV-Cre mice, allowing optogenetic stimulation of SuM inputs and pharmacogenetic

1250   inhibition of PV+ INs by application of the DREADD agonist CNO at 10 μM. B. Example

1251   immunostaining against PV, DREADD and biocytin labelling in area CA2 from a slice used in

1252   these experiments. C. Left, diagram of the recording configuration in hippocampal slices.

1253   Center, sample traces (control in red, CNO in grey). Right, summary graph of light-evoked

1254   IPSC amplitudes recorded in CA2 PNs before and after application of 10 μM CNO (n = 13,

1255   error bars represent SEM). D. Application of the P/Q-Type voltage activated calcium channel

1256   blocker ω-agatoxin TK results in nearly complete loss of feed-forward inhibition recruited by

1257   light activation of SuM inputs in area CA2. D1, sample traces, (top, control in red, ω-agatoxin

1258   TK in grey) and summary graph of light-evoked IPSC amplitudes recorded in CA2 PNs before

1259   and after application of 200 nM ω-agatoxin TK (bottom, n = 5, error bars represent SEM). D2,

1260   sample traces (top, SR95531 & CGP55845A in black, ω-agatoxin TK in grey) and summary

1261   graph of light-evoked EPSC amplitudes before and after application of 200 nM ω-agatoxin TK

1262   (bottom, n = 6, error bars represent SEM). E. Application of the mu-opioid receptor agonist,

1263   DAMGO, results in the complete abolition of light-evoked SuM inhibitory transmission. E1,

1264   sample traces (top, control in red, DAMGO in grey) and summary graph of light-evoked IPSC

1265   amplitudes recorded in CA2 PNs before and after application of 1 μM DAMGO (bottom, n =

1266   6, error bars represent SEM). E2, sample traces (top, SR95531 & CGP55845A in black,

1267   DAMGO in grey) and summary graph of light-evoked EPSC amplitudes before and after

1268   application of 1μM DAMGO (bottom, n = 17, error bars represent SEM). F. Application of the

1269   delta-opioid receptor agonist, DPDPE, results in the long-term depression of light-evoked SuM

1270   inhibitory transmission. F1, sample traces (top, control in red, DPDPE in grey) and summary

1271   graph of light-evoked IPSC amplitudes before and after application of 0.5 μM DPDPE (bottom,

1272   n = 7, error bars represent SEM). F2, sample traces (top, SR95531 & CGP55845A in black,

1273   DAMGO in grey) and summary graph of light-evoked EPSC amplitudes before and after

1274   application of 0.5 μM DPDPE (bottom, n = 7, error bars represent SEM).

1275

1276   **Figure 6. Area CA2 PNs receive a net inhibitory drive from SuM that controls AP firing**

1277   **properties.** A. Diagram illustrating whole-cell recordings of area CA2 PNs and SuM fiber light

1278   stimulation in acute slice preparation. B. Example traces of a CA2 PN action potential firing in

1279   response to current injection in the absence (black traces) or presence of 10 Hz photostimulation

1280   of SuM inputs (red traces). C. Action potential onset latency is increased with 10 Hz SuM input

1281   photostimulation. Left, sample traces of the first AP in control and with inhibition blocked by

1282   1 μM SR95531 & 2 μM CGP55845A application (light-off in black, light-on in red, light-off

1283   in SR95531 & CGP55845A in grey, light-on in SR95531 & CGP55845A in purple). Right,

1284   summary graph of photostimulation-induced delay of AP firing in area CA2 PNs before and

1285   after application of SR95531 & CGP55845A (control shown in red, n = 12, paired-T test, p =

1286   0.016; SR95531 & CGP55845A shown in purple, n = 6; Wilcoxon signed-rank test, p = 0.44;

1287   individual cells shown with dots, boxplot represents median, quartiles, 10th and 90th percentiles).

1288   D. Sample traces of AP firing in repeated trials (light-off in black, light-on in red, light-on in

1289   SR95531 & CGP55845A in purple; during experiment photostimulation was interleaved with

1290   control but traces are grouped here for demonstration purposes). E. AP jitter in CA2 PNs is

1291   reduced by activation of SuM inputs. Left, summary graph of the standard deviation of AP

1292   firing with or without 10 Hz photostimulation (n = 12; Wilcoxon signed-rank test, p < 0.001

1293   for the first AP, p = 0.008 for the second AP, p = 0.004 for the third AP; individual cells shown

1294   with thin lines, population average shown as thick line, error bars represent SEM). Right,

1295   photostimulation-induced reduction of AP firing standard deviation in control and in SR95531

1296   & CGP55845A (control, n = 12; Wilcoxon signed-rank tests, p < 0.001 for the first AP, p =

1297   0.008 for the second AP, p = 0.004 for the third AP; SR95531 & CGP55845A, n = 6; Wilcoxon

1298   signed-rank tests, p = 0.22 for the first AP, p = 0.16 for the second AP, p = 0.09 for the third

1299   AP; individual cells shown with dots, boxplot represents median, quartiles, 10th and 90th

1300   percentiles).

1301

1302   **Figure 7. SuM input shapes CA2 PN AP bursts in conditions of elevated cholinergic tone.**

1303   A. Diagram illustrating whole-cell recordings of area CA2 PNs with light stimulation of SuM

1304   fibers in an acute slice preparation. B. Sample trace of spontaneous AP bursting activity

1305   recorded from a CA2 PN during bath application of 10 μM CCh. For every even-numbered

burst, a 10 Hz photostimulation (blue bars) was delivered to excite SuM inputs in area CA2 allowing a comparison of burst AP firing in the same cell. C. Sample traces of AP firing during bursts for light-off (left, black) and light-on (right, red) epochs. D. Comparison of AP number per burst for light-off (black) and light-on (red) events (n = 7; individual cells shown as thin lines, population average shown as thick line, error bars represent SEM; paired-T test, p = 0.031). E. Average firing rate during spontaneous burst events with SuM photostimulation (red, light-on) and controlled interleaved burst events (black, light-off). Shaded area represents SEM for 7 cells each with between 3 and 13 bursts analyzed in light-on and light-off conditions (2-way ANOVA, light factor: $p < 0.001$, time factor: $p < 0.001$, light x time factor: $p = 0.052$). F. Example burst events with (red) and without (black) SuM photostimulation overlayed and on a scale that shows the rapidly hyperpolarizing membrane potential that occurs with SuM input stimulation. G. Comparison of bursts duration for events with (red) and without (black) photostimulation (n = 7; individual cells shown as thin lines, population average shown as thick line, error bars represent SEM; paired-T test, p = 0.037). H. Comparison of time elapsed to next burst onset following bursts with (red) or without (black) photostimulation (n = 7; individual cells shown as thin lines, population average shown as thick line, error bars represent SEM; paired-T test, p = 0.001).

Figure 8. Consequences of SuM input on area CA2 output to CA1. A. Diagram illustrating *in vivo* recording in CA1 with tetrodes and SuM axon terminals stimulation over CA2 with an implanted optical fiber. B. Representative data from 4 multi-unit recordings. Raster plot (top) showing CA1 AP firing activity before and during photostimulation of SuM fibers in area CA2. The corresponding firing rate histogram (middle) of four tetrodes placed in the CA1 pyramidal cell layers, as well as plots of standard deviation (SD; bottom). Red lines indicate +/- 3SD. C. Individual (grey) and average (red) normalized firing rates from 34 multiunit recordings, 3 consecutive light stimulation epochs are displayed to help visualizing the consistency of the effect of SuM input light stimulation over area CA2 on CA1 multi-unit firing; the shaded area represents the SEM. D. Diagram illustrating whole-cell recordings of area CA1 PNs and SuM fiber light stimulation over area CA2 in acute slice preparation. E-H. Example waterfall plots (E, G) and corresponding peri-stimulus time histogram (F, H, population average shown as thick line, shaded area represents SEM) of EPSCs (black) and IPSCs (red) recorded from a CA1 PN *ex vivo* during photostimulation of SuM input over area CA2 with bath application of 10 μM CCh.

Supplemental figure legends

Supplemental Figure 1.

1340   A. Diagram illustrating the intersectional strategy used to label CA2-projecting SuM neurons.

1341   B-E. Labelling of CA2-projecting SuM neurons with the retrograde CAV-2 carrying Cre-

1342   recombinase injected in CA2 and the anterograde AAV carrying DIO-EGFP injected in SuM

1343   of wild type mice. B. Labelling of SuM fibers in the hippocampus from CA2-projecting SuM

1344   neurons. Left, nissl staining (blue) and EGFP expression (green) in the hippocampus. Right,

1345   PCP4 staining (magenta) and EGFP expression (green) in area CA2. C. Retrograde-labeled

1346   SuM neurons that project to hippocampal area CA2. Left, nissl staining (blue) and EGFP

1347   expression (green) in SuM (mtg = mammillotegmental tract, rmx = retromammillary

1348   decussation, SuMl = lateral SuM, SuMm = medial SuM, pm = principal mammillary tract, MM

1349   = medial mammillary nucleus). Right, calretinin staining (magenta) and EGFP expression

1350   (green) in SuM. D. Higher magnification image of CA2-projecting SuM neurons. Left, nissl

1351   staining (blue) and EGFP expression (green) in SuM. Center, nissl (blue) and calretinin staining

1352   (magenta) in SuM. Right, calretinin staining (magenta) and EGFP expression (green) in SuM.

1353   E. VGluT2 expression of CA2-projecting SuM neurons. Left, nissl staining (blue) and EGFP

1354   expression (green) in SuM. Right, VGluT2 staining (red) and EGFP expression (green) in SuM.

1355   F. Top, diagram illustrating the injection of AAVs into the SuM. Bottom, sagittal image of the

1356   injection site in SuM to express hCHR2(H134R)-EYFP (green) in the VGluT2-Cre line. G-H.

1357   Anterograde labelling of SuM projections to the hippocampus from AAV carrying DIO-ChR2-

1358   EYFP injected in SuM of VGluT2-Cre mice. G. Left, VGluT2 (red) and nissl staining (blue) in

1359   the hippocampus. Right, hCHR2(H134R)-EYFP -expressing SuM fibers (green) and nissl

1360   (blue) staining in the hippocampus. H. Left, higher magnification image of area CA2 with

1361   VGluT2 (red) and nissl (blue) staining. Center, hCHR2(H134R)-EYFP -expressing SuM fibers

1362   (green) and nissl staining (blue). Right, hCHR2(H134R)-EYFP -expressing SuM fibers (green)

1363   and VGluT2 staining (red).

Supplemental Figure 2.

1365   A. Diagram illustrating the whole-cell recording configuration of PNs in area CA2 and SuM

1366   fiber stimulation in acute hippocampal slices. B. Light-evoked EPCSs from SuM inputs are

1367   completely blocked following application of tetrodotoxin (TTX). Sample traces (top, control

1368   shown in black, +TTX shown in grey) and power-response curves (bottom) of light-evoked

1369   EPSC amplitudes recorded in PN before (black) and after application of $0.2\,\mu$M TTX (grey) at

1370   different light intensities (n = 5, error bars represent SEM). C. Light-evoked EPCSs from SuM

1371   inputs are completely blocked following application of NMDA and AMPA receptor blockers

1372   (NBQX & APV). Sample traces (top, control shown in black, NBQX & APV shown in grey)

1373   and time course (bottom) of light-evoked EPSC amplitudes upon application of 10 μM NBQX

1374   & 50 μM APV (n = 6, error bars represent SEM).

Supplemental Figure 3.

1376   A. Diagram illustrating the whole-cell recording configuration of hM4D(Gi)-mCherry

1377   DREADD-expressing PV INs in area CA2 and SuM fiber stimulation in acute hippocampal

1378   slices. B. Time course of the change of membrane potential (V_M) level of Gi-DREADD-

1379   expressing CA2 PV INs with application of 10 μM CNO (n = 6, error bars represent SEM). C.

1380 V_M level before and after application of CNO (n = 6; Wilcoxon signed-rank test, p = 0.031;

1381   individual cells shown as thin lines, population averages shown as thick lines, error bars

1382   represent SEM). D. Sample traces of PSPs and spikes recorded from a Gi-DREADD-expressing

1383   CA2 PV IN before (left, PSPs in black, spikes in red) and after CNO application (right, PSPs

1384   in grey, spikes in light red). E. Same as D with traces displayed as waterfall.

Supplemental Figure 4.

1386   A. Diagram illustrating the whole-cell recording configuration of PNs in area CA2 and SuM

1387   fiber stimulation in acute hippocampal slices. B-C. Effect of 10 μM CCh on SuM light-evoked

1388   PSCs recorded in CA2 PNs under different conditions: voltage clamp at -70 mV with inhibitory

1389   transmission blocked (B, SR95531 & CGP55845A in grey, SR95531 & CGP55845A + CCh in

1390   orange), and voltage clamp at +10 mV (C, control in red, CCh in orange). Left, sample traces.

1391   Middle, power-response curves (B, n = 7; two-way ANOVA with repeated measures, p < 0.001;

1392   C, n = 17; two-way ANOVA with repeated measures, p < 0.001; error bars represent SEM).

1393   Right, comparison of PPRs (B, n = 7; paired-T test, p < 0.001; C, n = 17; paired-T test, p =

1394   0.001; individual cells shown as grey lines, population average shown as horizontal line, error

1395   bars represent SEM). D-G. Short term dynamics of PSCs evoked by repeated SuM input

1396   stimulation at 10 Hz within the same CA2 PNs in voltage clamp at -70 mV or +10 mV. Both

1397   SuM-evoked EPSCs and IPSCs were recorded in the same cells before and after application of

1398   10 μM CCh (EPSCs before CCh in black, EPSCs after CCh in grey, IPSCs before CCh in red,

1399   IPSCs after CCh in orange; n = 13; error bars represent SEM). D. Sample traces. E. PSC

1400   amplitude. F. Pulse #n over pulse #1 ratio. G. E/I ratio. See Supplemental Table 1 for statistics.

1401

Table 1. Electrophysiological properties of pyramidal neurons in SuM-innervated area

	V_M (mV)	R_M (MOhm)	C_M (pF)
CA2 PN (n = 81)	-69.8 ± 0.70	59.2 ± 2.65	209 ± 11.4
CA3 PN (n = 31)	-70.3 ± 1.06	72.4 ± 4.82	211 ± 15.7
Statistics	Mann-Whitney U test p = 0.997	Student T test p = 0.020*	Mann-Whitney U test p = 0.625
PN deep (n = 57)	-71.1 ± 0.76	64.0 ± 3.94	200 ± 12.3
PN superficial (n = 76)	-69.3 ± 0.67	64.9 ± 3.19	196 ± 11.8
Statistics	Student T test p = 0.077	Mann-Whitney U test p = 0.777	Mann-Whitney U test p = 0.588

Table 2. Characteristics of SuM light-evoked transmission onto pyramidal neurons

	EPSC					
cell type	connectivity (%)	amplitude (pA)	rise time (ms)	decay time (ms)	latency (ms)	success rate
CA2 PN	56 (n = 58 of 103)	-16 ± 1.9	2.9 ± 0.1	14 ± 0.8	2.4 ± 0.2	0.44 ± 0.03
CA3 PN	49 (n = 22 of 45)	-23 ± 5.9	3.0 ± 0.2	14 ± 0.9	2.7 ± 0.3	0.56 ± 0.06
Statistics	χ^2 test p = 0.572	Mann-Whitney U test p = 0.409	Mann-Whitney U test p = 0.391	Mann-Whitney U test p = 0.797	Mann-Whitney U test p = 0.156	Student T test p = 0.074
PN deep	56 (n = 35 of 63)	-15 ± 2.0	3.5 ± 0.2	16 ± 1.0	3.5 ± 0.4	0.39 ± 0.03
PN superficial	56 (n = 53 of 94)	-20 ± 3.0	3.1 ± 0.2	15 ± 0.9	2.7 ± 0.3	0.51 ± 0.04
Statistics	χ^2 test p = 0.946	Mann-Whitney U test p = 0.306	Mann-Whitney U test p = 0.051	Mann-Whitney U test p = 0.314	Mann-Whitney U test p = 0.083	Mann-Whitney U test p = 0.072
	IPSC					
cell type	connectivity (%)	amplitude (pA)	rise time (ms)	decay time (ms)	latency (ms)	success rate
CA2 PN	35 (n = 19 of 55)	197 ± 41.3	3.8 ± 0.4	25 ± 1.2	6.3 ± 0.7	0.55 ± 0.06
CA3 PN	57 (n = 16 of 28)	145 ± 23.4	4.5 ± 0.4	25 ± 1.2	7.5 ± 0.9	0.54 ± 0.05
Statistics	χ^2 test p = 0.134	Mann-Whitney U test p = 0.870	Student T test p = 0.203	Mann-Whitney U test p = 0.896	Mann-Whitney U test p = 0.303	Student T test p = 0.893
PN deep	47 (n = 16 of 34)	199 ± 40.6	3.8 ± 0.4	25 ± 1.4	7.2 ± 0.8	0.52 ± 0.07
PN superficial	47 (n = 26 of 55)	167 ± 27.5	4.9 ± 0.4	26 ± 1.2	6.8 ± 0.7	0.50 ± 0.05
Statistics	χ^2 test p = 0.987	Mann-Whitney U test p = 0.258	Student T test p = 0.047*	Student T test p = 0.564	Student T test p = 0.706	Student T test p = 0.796

Table 3. Electrophysiological properties of interneurons in SuM-innervated area

	V_M (mV)	R_M (MOhm)	C_M (pF)	firing adaptation index	sag (mV)
Basket cell (n = 16)	-57.3 ± 1.38	144 ± 28.1	64.0 ± 8.70	0.74 ± 0.05	9.4 ± 1.0
non-Basket Cell (n = 12)	-55.6 ± 1.84	224 ± 46.8	52.0 ± 5.90	0.57 ± 0.06	5.9 ± 1.4
interneuron SO (n = 6)	-57.0 ± 3.16	201 ± 21.0	44.7 ± 5.31	0.61 ± 0.11	7.6 ± 1.9
interneuron SR (n = 8)	-60.1 ± 2.89	282 ± 49.8	39.6 ± 3.18	0.65 ± 0.09	8.1 ± 2.1
Statistics	1-way ANOVA test p = 0.527	1-way ANOVA test p = 0.100	Kruskal-Wallis test p = 0.354	1-way ANOVA test p = 0.238	1-way ANOVA test p = 0.292

Table 4. Characteristics of excitatory SuM light-evoked transmission onto interneurons & pyramidal cells

cell type	connectivity (%)	amplitude (pA)	rise time (ms)	decay time (ms)	latency (ms)	success rate
Pyramidal Cell	63 (n = 166 of 263)	-19 ± 1.6*	3.4 ± 0.1*	15 ± 0.5*	2.9 ± 0.1	0.46 ± 0.02
Basket Cell	82 (n = 18 of 22)	-43 ± 8.7*	1.7 ± 0.3*	8.4 ± 1.3*	3.1 ± 0.4	0.59 ± 0.07
non-Basket Cell interneuron SO interneuron SR	39 (n = 10 of 26) 12 (n = 2 of 17) 11 (n = 1 of 9)	-16 ± 2.8	2.6 ± 0.5	12 ± 1.4	3.4 ± 0.7	0.36 ± 0.06
Statistics	χ^2 test p = 0.006*	Kruskal-Wallis test p = 0.016 Dunn-Holland-Wolfe *post hoc* p < 0.05*	1-way ANOVA test p < 0.001 Tukey *post hoc* p < 0.001*	1-way ANOVA test p < 0.001 Tukey *post hoc* p < 0.001*	1-way ANOVA test p = 0.580	1-way ANOVA test p = 0.066

Supplemental Table 1. Statistical comparisons related to Supplemental Figure 4.

Measurement	Conditions	Factors	2-way ANOVA p-values
amplitude	EPSC amplitude +/- CCh (n = 13)	treatment	0.00171693
		pulse #	0.00286193
		treatment x pulse #	0.0521822
	IPSC amplitude +/- CCh (n = 13)	treatment	0.413564
		pulse #	0.0247487
		treatment x pulse #	0.316489
	PSC amplitude in ACSF (n = 13)	holding level	0.0121691
		pulse #	0.0115431
		holding level x pulse #	0.391097
	PSC amplitude in CCh (n = 13)	holding level	2.85112E-11
		pulse #	0.189593
		holding level x pulse #	0.55014
Pn/P1 ratio	EPSC Pn/P1 ratio +/- CCh (n = 13)	treatment	1.05342E-10
		pulse #	9.99201E-16
		treatment x pulse #	0.0110396
	IPSC Pn/P1 ratio +/- CCh (n = 13)	treatment	0.000184435
		pulse #	0.00209369
		treatment x pulse #	0.297716
	PSC Pn/P1 ratio in ACSF (n = 13)	holding level	0.325751
		pulse #	2.08101E-08
		holding level x pulse #	0.941122
	PSC Pn/P1 ratio in CCh (n = 13)	holding level	0.0948351
		pulse #	3.07005E-05
		holding level x pulse #	0.889375
E/I ratio	PSC E/I ratio +/- CCh (n = 13)	treatment	7.61696E-06
		pulse #	0.99245
		treatment x pulse #	0.982047

A

AAV9.EF1a.DIO.hCHR2(H134R).EYFP

Csf2rb2-Cre

ChR2-EYFP

500 µm

csf2rb2-Cre

SuM

500 µm

B

nissl / ChR2-EYFP

CA1

CA2

CA3

DG

500 µm

nissl / ChR2-EGFP

ChR2-EGFP / RGS14

50 µm

C CA2 pyramidal neuron

C1

so

sp

sr

mf

C2

C3

20mV

200ms

C4

light

current-clamp @ -70mV

1mV

50ms

light

voltage-clamp @ -70mV

10pA

50ms

D CA3a pyramidal neuron

D1

so

sp

sr

mf

D2

D3

20mV

200ms

D4

light

current-clamp @ -70mV

1mV

50ms

light

voltage-clamp @ -70mV

10pA

50ms

Figure 1.

Figure 2.

A   Basket cells

light-evoked EPSC potency (pA)

light-evoked PSP amplitude (mV)

Figure 3.

Figure 4.

Figure 5.
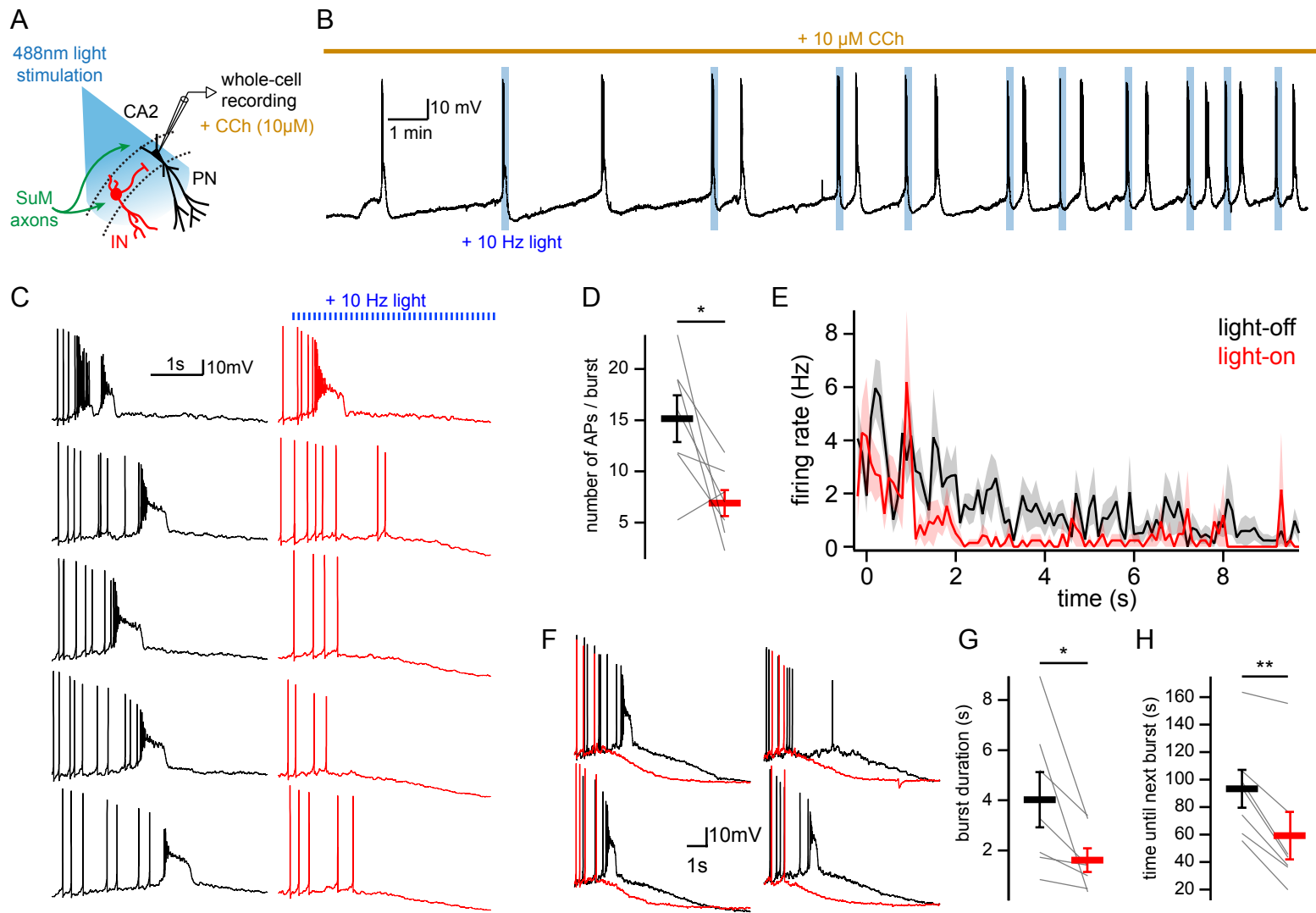
Figure 6.
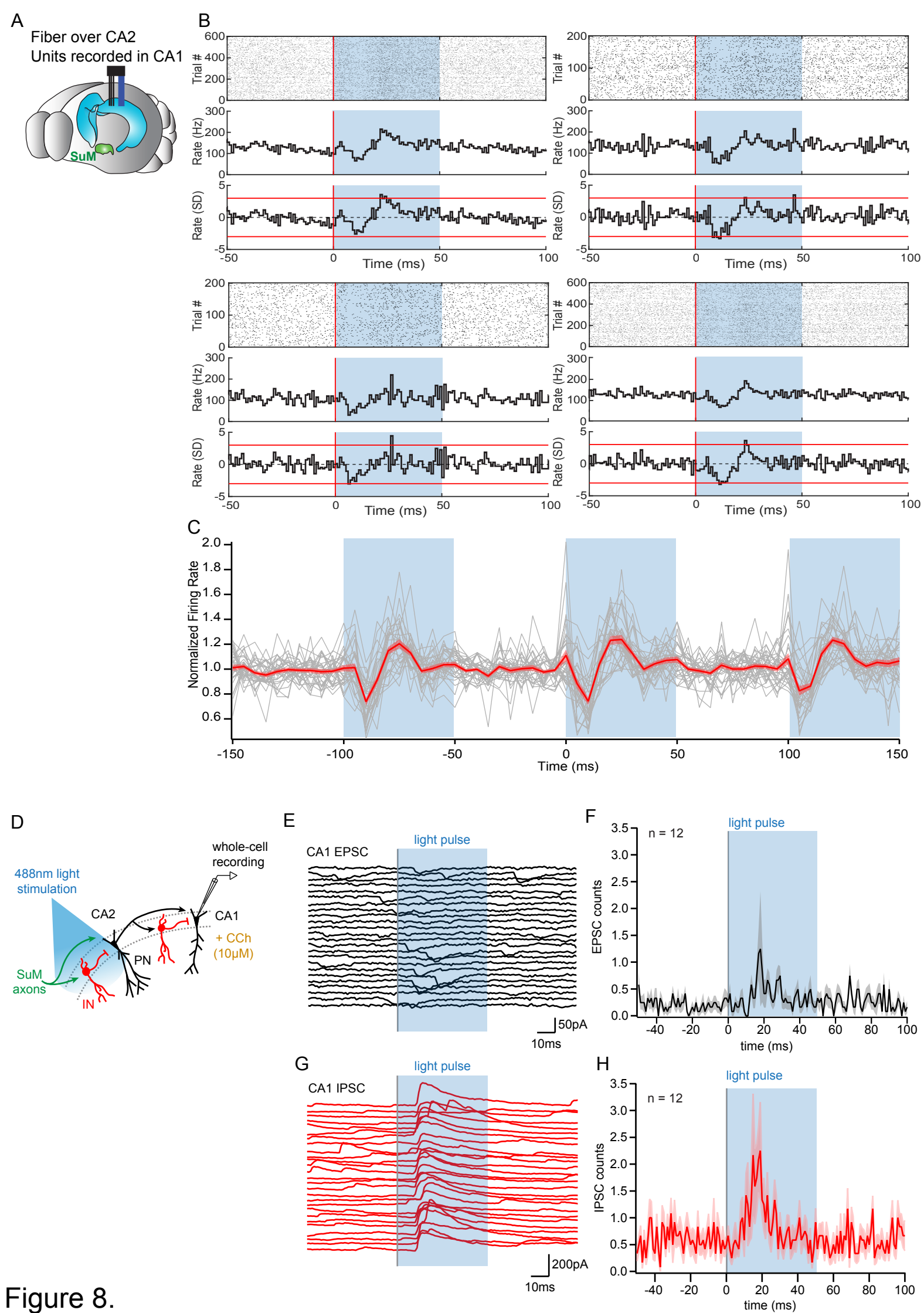
Figure 7.

Figure 8.

Supplemental Figure 1.

A

488nm light
stimulation

whole-cell
recording

CA2

SuM
axons

PN

IN

B

light | + TTX |

control

10pA

25ms

● control
● + TTX

EPSC amplitude (pA)

LED power (mW/mm²)

C

light | + NBQX & APV |

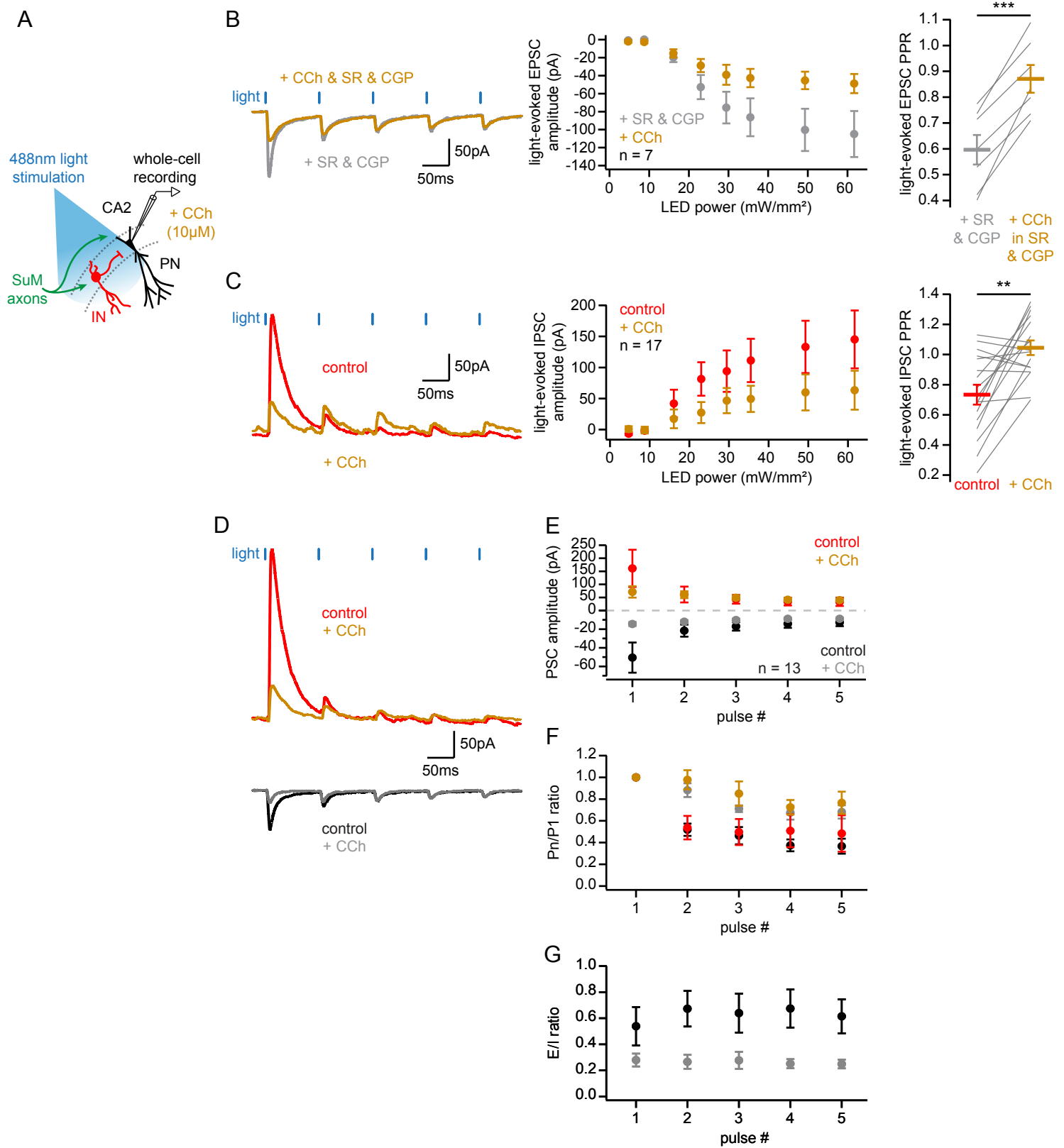control

10pA

25ms

+ NBQX & APV

EPSC amplitude (pA)

time (min)
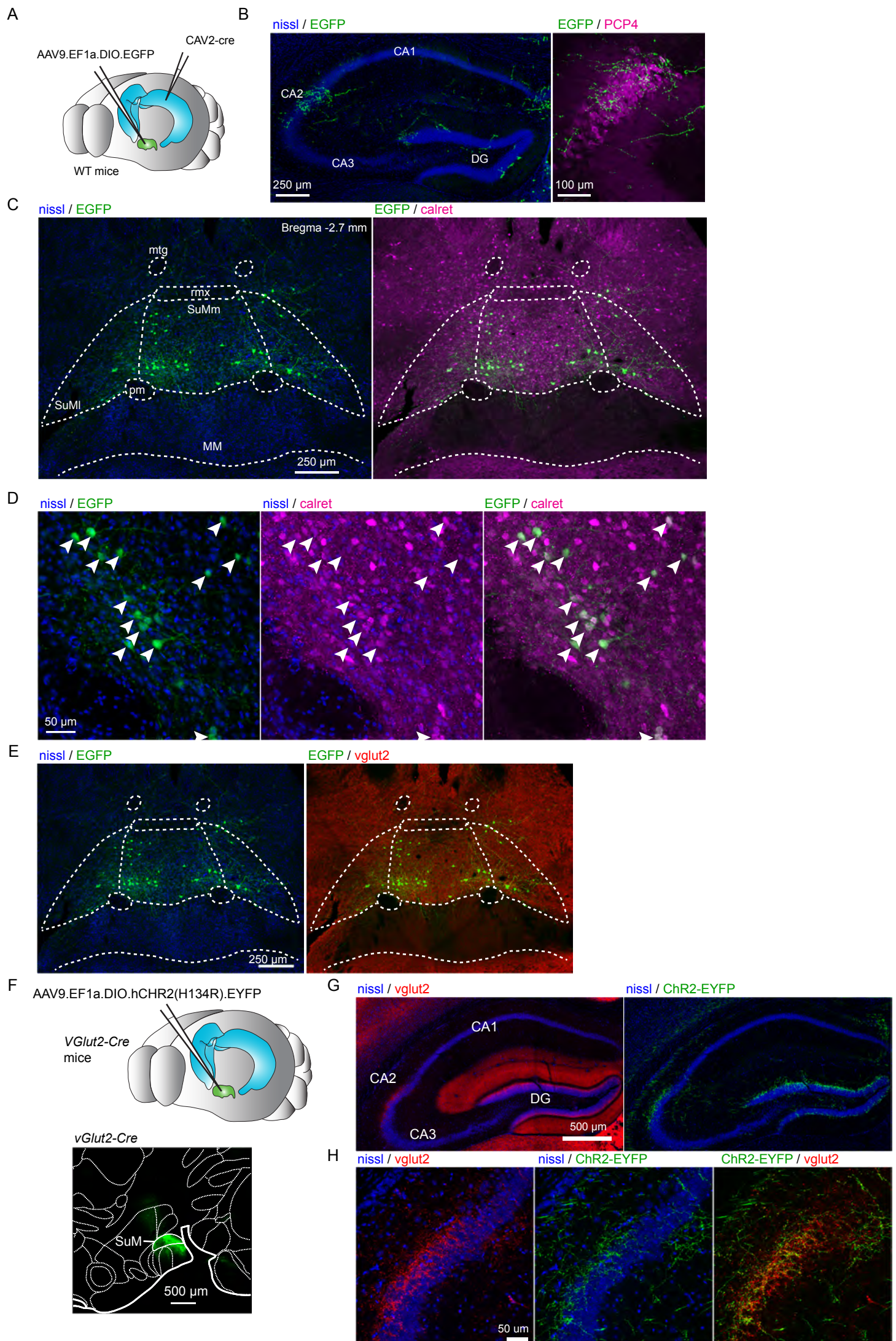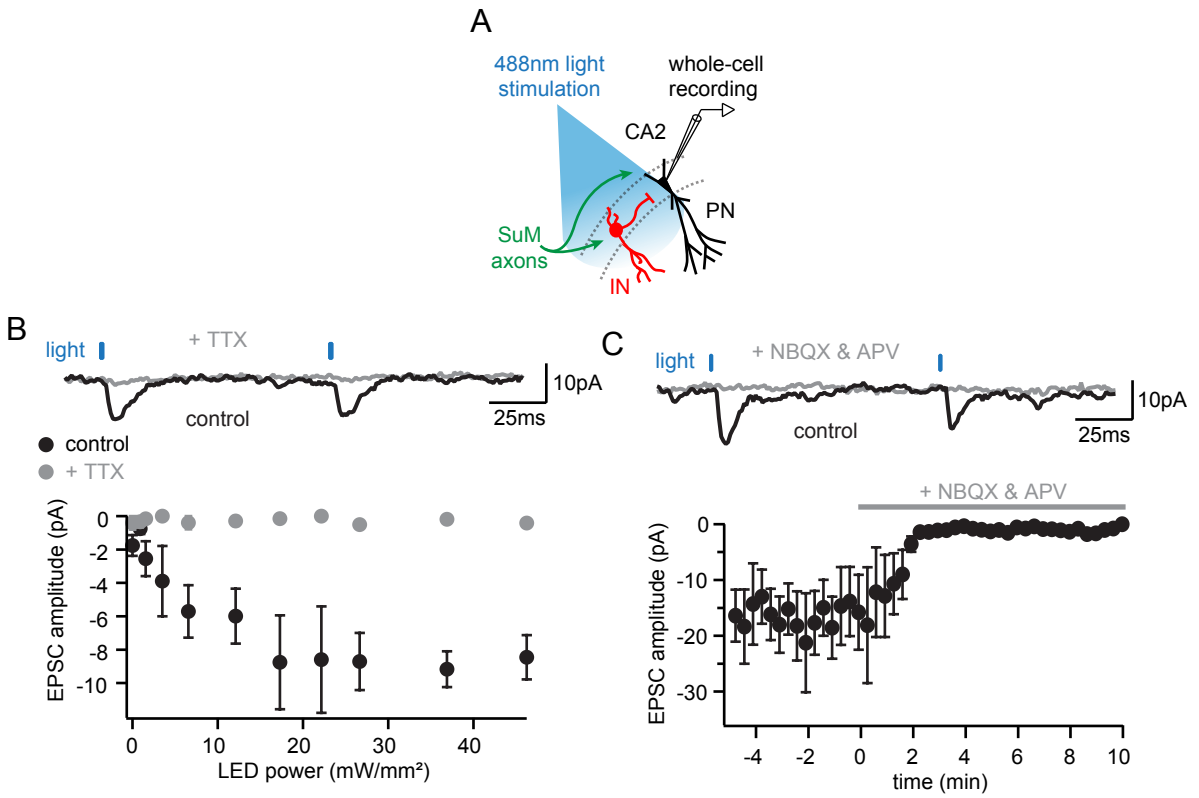
Supplemental Figure 2.
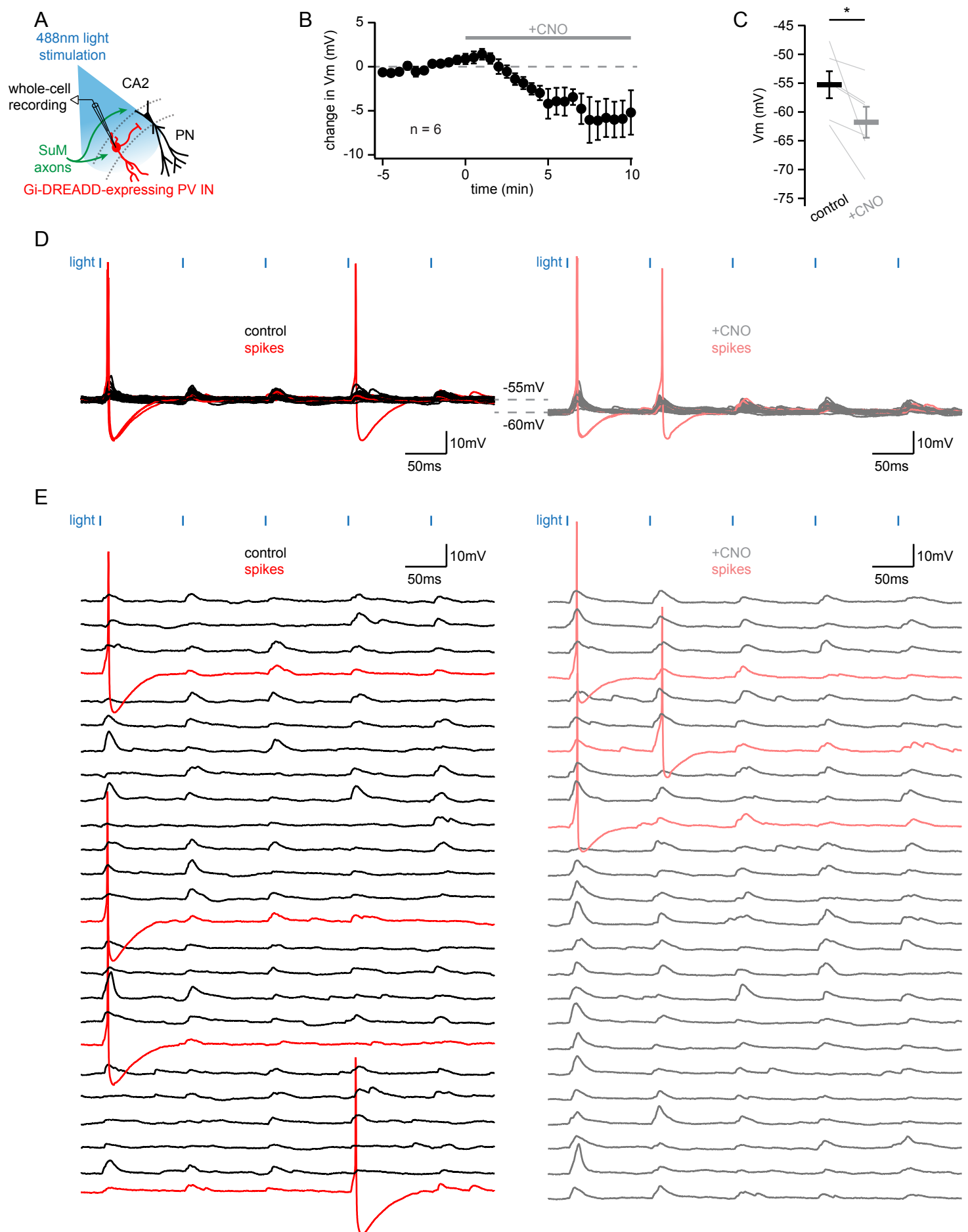
Supplemental Figure 3.

Supplemental Figure 4.