

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

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#### 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

#### 28 Introduction

29 The hippocampus is critical for memory formation and spatial navigation (Buzsáki and Moser, 30 2013; Eichenbaum and Cohen, 2014), yet basic questions persist regarding the underlying 31 circuitry and cellular components. While area CA2 has been shown to play a significant role in 32 several hippocampal processes including social memory formation (Hitti and Siegelbaum, 33 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 34 35 mechanisms allowing these functions is lacking. There is mounting evidence that 36 generalizations cannot be made from the rich understanding of areas CA1 and CA3, as neurons 37 in area CA2 have been shown to have unique molecular expression profiles (Cembrowski et 38 al., 2016; Lein et al., 2004), morphology (Bartesaghi and Ravasi, 1999; No, 1934) and cellular 39 properties (Robert et al., 2020; Srinivas et al., 2017; Sun et al., 2014). Notably, and in contrast 40 to area CA1, CA2 pyramidal neurons do not undergo high frequency stimulation-induced 41 synaptic plasticity (Dasgupta et al., 2020; Zhao et al., 2007). Rather, the excitability of this 42 region is tightly controlled by a highly plastic network of inhibitory neurons (Leroy et al., 2017; 43 Nasrallah et al., 2015; Piskorowski and Chevaleyre, 2013). When active, CA2 pyramidal 44 neurons (PNs) can strongly drive area CA1 (Chevaleyre and Siegelbaum, 2010; Kohara et al., 45 2014; Nasrallah et al., 2019), thereby influencing hippocampal output. Furthermore, CA2 46 neurons also project to area CA3, where they recruit inhibition (Boehringer et al., 2017; Kohara 47 et al., 2014) and act to control hippocampal excitability. Thus, CA2 neurons are poised to have 48 long-reaching effects in the hippocampus, and a better understanding of the regulation of 49 neuronal activity in this region is needed.

50 The hypothalamic supramammillary (SuM) nucleus sends projections to both area CA2 and the 51 dentate gyrus (DG) (Haglund et al., 1984; Vertes, 1992). These long-range connections have 52 been shown in several species including rodents, primates and humans (Berger et al., 2001; 53 Haglund et al., 1984; Wyss et al., 1979) where they are present in early hippocampal 54 development. The SuM has been found to be active during a wide variety of conditions 55 including novel environment exposure (Ito et al., 2009), reinforcement learning (Ikemoto, 2005; 56 Ikemoto et al., 2004), food anticipation (May et al., 2019), and during REM sleep and arousal 57 (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 58 59 to the hippocampus or by modulation of the medial septum (Borhegyi et al., 1998; Vertes and 60 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 hippocampal94 excitability by controlling area CA2 output.

95

#### 96 **Results**

#### 97 SuM axons provide excitatory glutamatergic input to pyramidal neurons in area CA2 and CA3a

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 to area CA2, as defined by expression of the CA2-specific markers PCP4 (Supplemental Figure
1B) and RGS14 (Figure 1B), and did not spread to neighboring areas CA3 and CA1. In order
to maximize the precision of our experiments, we frequently only achieved partial infection of
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).

We performed whole-cell current and voltage clamp recordings of PNs across the hippocampal CA regions and activated projecting axons with pulses of 488 nm light in acute hippocampal slices. Following all recordings, we performed post-hoc anatomical reconstructions of recorded 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,  $\chi^2$  test for CA2 and CA3 PNs, p = 0.572) or between deep and superficial PNs (Table 2,  $\chi^2$  test for deep and superficial PNs, p = 0.946). Light-evoked 146 147 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  $-16 \pm 4.8$  pA in control and  $-1.8 \pm 0.3$  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 PNs in area CA2 receive mixed excitatory and inhibitory responses from the SuM input

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<sub>A</sub> and GABA<sub>B</sub> 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  $\pm$  0.05 mV in control and 0.24  $\pm$  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.

Given the combination of direct excitation and feed-forward inhibition from SuM inputs onto CA2 pyramidal cells, we asked how this input would summate with other synaptic inputs in the CA2 dendritic arbor. Hippocampal area CA2 receives synaptic input from CA3 in *stratum radiatum* (SR). Stimulation of CA3 inputs evokes a very strong feed-forward inhibition, such that it is exceptionally difficult to evoke action potential firing in CA2 pyramidal neurons when 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,  $p = 2.3 \times 10^{-4}$ ) and 215 SLM (n = 10; repeated-measures ANOVA,  $p = 8.5 \times 10^{-4}$ ). 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 Basket cells are strongly recruited by the SuM input

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

- specific aspects of PN excitability, we wished to establish which kind of IN was targeted by the
- 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  $-12 \pm 1.6$  pA 256 for PNs, n = 166; -29  $\pm$  7.8 pA for BCs, n = 18; -5.9  $\pm$  1.5 pA for non-BCs, n = 13; Kruskal-

Wallis test with Dunn-Holland-Wolfe *post hoc* test, p = 0.022). Consequently, EPSPs recorded 257 258 at -70 mV were of larger amplitude in BCs than in PNs and non-BCs (Figure 3D; amplitudes 259 were  $0.44 \pm 0.06$  mV for PNs, n = 20;  $1.71 \pm 0.57$  mV for BCs, n = 10;  $0.53 \pm 0.07$  mV for non-260 BCs, n = 4; Kruskal-Wallis test with Dunn-Holland-Wolfe *post hoc* test, p < 0.001). When 261 recording cell-attached or current-clamping BCs at their resting membrane potential (V<sub>M</sub>), 262 photostimulation of SuM axons was able to evoke AP firing (Figure 3E) in multiple instances 263 (n = 7 of 13). However, this was never observed in PNs (n = 0 of 78), non-BCs (n = 0 of 16), 264 SR INs (n = 0 of 9) or SO INs (n = 0 of 8). These results show that SuM projections to area 265 CA2 preferentially provide excitation to BCs that are likely responsible of the feedforward 266 inhibition observed in PNs. This is in accordance with an efficient control of area CA2 PNs 267 excitation by the SuM inhibitory drive as axons from BCs deliver the feedforward inhibition to 268 the peri-somatic region of PNs, effectively shunting incoming PSPs from both the SuM and 269 from dendritic-targeted inputs in SR and SLM.

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

271 In the hippocampus, BCs express either cholecystokinin (CCK) or parvalbumin (PV) 272 (Klausberger and Somogyi, 2008). We found that in response to a 1 second depolarizing pulse, 273 most BCs that received strong SuM excitatory input displayed very fast AP firing with little 274 accommodation in the AP firing frequency (Figure 4A-B, Table 3). This firing behavior is 275 similar to what has been reported for fast spiking PV-expressing BCs in CA1 (Pawelzik et al., 276 2002). In contrast, CCK-expressing BCs show a lower firing frequency and more 277 accommodation during the train (Pawelzik et al., 2002). This result suggests that BCs connected 278 by the SuM may be expressing PV. To directly confirm this hypothesis, we performed *post hoc* 279 immunostaining of recorded interneurons that received strong excitation from SuM input. 280 Because of the dialysis inherent to the whole-cell recording conditions, we encountered 281 difficulty staining for multiple cells. However, PV-immunoreactivity could unequivocally be 282 detected in either the soma or dendrites of 7 connected BCs (Figure 4C). Therefore, this data 283 demonstrates that at least a fraction of the recorded BCs connected by the SuM is expressing 284 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 290 feedforward IPSCs from area CA2 PNs in response to SuM stimulation. To achieve that, we 291 injected AAVs expressing a Cre-dependent hM4D(Gi) inhibitory Gi-DREADD in area CA2 of 292 PV-Cre mice together with AAVs expressing ChR2 with a pan-neuronal promoter in the SuM 293 (Figure 5A). While we were able to obtain very specific expression of DREADD in PV+ INs, 294 only a fraction of PV+ INs had detectable DREADD expression as quantified by 295 immunohistochemistry (Figure 5B; fraction of PV+ INs expressing DREADDs in CA2 =  $75 \pm$ 296 3.5 %, n = 13). We observed a substantial reduction of SuM-evoked IPSC amplitude recorded 297 in area CA2 PNs upon application of 10  $\mu$ M of the Gi-DREADD ligand CNO (Figure 5C; 298 amplitudes were 847  $\pm$  122 pA in control and 498  $\pm$  87 pA in CNO hence a 42  $\pm$  6.0 % block, 299 n = 13; paired-T test, p < 0.001). Although we never measured a complete block of inhibitory 300 responses, this result unequivocally places PV+ INs as mediators of the SuM feedforward 301 inhibition of area CA2 PNs. The incomplete block of IPSCs observed in these experiments 302 indicates that either additional non-PV+ INs are recruited by SuM input or that our silencing of 303 PV-mediated feedforward inhibition is incomplete. This could be a consequence of partial 304 infection of PV+ INs in area CA2 by AAVs carrying DREADDs and partial silencing of 305 DREADD-expressing PV+ INs by CNO. To address the latter, we performed whole-cell 306 recordings from Gi-DREADD-expressing CA2 PV+ INs labelled with mCherry and monitored 307 the variations in V<sub>M</sub> level and action potential firing to SuM input stimulation before and after 308 CNO application (Supplemental Figure 3A). We found that CNO application caused a 309 significant hyperpolarization of Gi-DREADD-expressing CA2 PV+ INs, albeit modest in 310 magnitude (Supplemental Figure 3B-D;  $V_M$  were -55.3 ± 2.3 mV in ACSF and -61.8 ± 2.7 mV 311 in CNO hence a  $-6.5 \pm 2.4$  mV hyperpolarization, n = 6; Wilcoxon signed-rank test, p = 0.031). 312 While this confirmed the relevance of our silencing strategy, it highlighted the possibility that 313 Gi-DREADD-expressing CA2 PV+ INs may not be fully silenced by CNO. Indeed, we 314 observed residual SuM-evoked AP firing in these cells after CNO application (Supplemental 315 Figure 3D-E). These data indicate that synaptically evoked somatic AP firing is not fully 316 blocked by CNO in Gi-DREADD-expressing CA2 PV+ INs. Because it is difficult to 317 distinguish between partial silencing of PV INs by Gi-DREADDs or recruitment of other types 318 of INs in the SuM-driven feedforwards inhibition, we adopted other complementary strategies 319 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 323 contrast, N-type calcium channels are primarily involved in GABA release from CCK+ INs 324 (Hefft and Jonas, 2005). Thus, we recorded SuM input-evoked EPSCs and IPSCs in CA2 PNs 325 before and after application of the P/Q-type voltage-gated calcium channels specific blocker ω-326 agatoxin TK (200 nM) (Figure 5D). We observed a near-complete block of IPSCs upon  $\omega$ -327 agatoxin TK application (Figure 5D1, IPSC amplitudes were  $245.5 \pm 92.6$  pA in control and 328  $35.0 \pm 15.4$  pA in  $\omega$ -agatoxin TK hence a  $81.8 \pm 3.9$  % block, n = 5; paired-T test, p < 0.001), 329 suggesting a major contribution from PV+ INs to SuM-driven feedforward inhibition consistent 330 with our previous results. However, we observed that excitatory transmission from SuM axons 331 was also partially blocked by ω-agatoxin TK application, as SuM input-evoked EPSCs were 332 significantly reduced although not abolished (Figure 5D2, EPSC amplitudes were  $-51.8 \pm 5.9$ 333 pA in SR95531 & CGP55845A and -26.5  $\pm$  5.4 pA after  $\omega$ -agatoxin TK hence a 49.6  $\pm$  5.6 % 334 block, n = 6; paired-T test, p < 0.001). This observation indicates that glutamate release from 335 SuM axons relies on P/Q-type voltage-gated calcium channels to some degree, thereby 336 complicating the interpretation of the reduction of IPSC amplitude in CA2 PNs.

337 It has previously been demonstrated that PV+ BC transmission can be strongly attenuated by 338 mu opioid receptor activation (MOR) while CCK+ BC transmission is insensitive to MOR 339 activation (Glickfeld et al., 2008). Thus, we recorded from PNs in area CA2 and examined the 340 sensitivity of light-evoked IPSCs to the application of the MOR agonist DAMGO (Figure 5E). 341 We found that there was a near complete block of the light-evoked IPSC amplitude following 342 1  $\mu$ M DAMGO application (Figure 5E1; IPSC amplitudes were 343  $\pm$  123 pA in control and 343  $31 \pm 12.4$  pA in DAMGO hence a  $88 \pm 5.0$  % block, n = 6 PNs; Wilcoxon signed-rank test, p 344 = 0.031), while direct excitatory transmission remained unaffected (Figure 5E2; EPSC) 345 amplitudes were  $-6.7 \pm 1.1$  pA in SR95531 & CGP55845A and  $-5.6 \pm 0.9$  pA after DAMGO, n 346 = 17 PNs; Wilcoxon signed-rank test, p = 0.19). While this result is in agreement with our 347 DREADD and  $\omega$ -agatoxin TK results showing a major contribution of PV+ INs to the SuM-348 driven feedforward inhibition, it should be noted that the dichotomy between PV+ versus CCK+ 349 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  $\mu$ M of the DOR 356 agonist DPDPE led to a long-term reduction of light-evoked IPSCs recorded in area CA2 PNs, 357 similar to the iLTD seen by CA3 input stimulation (Figure 5F1; IPSC amplitudes were  $168 \pm$ 358 28 pA in control and 64  $\pm$  22 pA in DPDPE hence a 61  $\pm$  14 % block by DPDPE, n = 7; paired-359 T test, p = 0.015), while leaving direct EPSCs unaffected (Figure 5F2; EPSC amplitudes were 360  $-4.0 \pm 1.6$  pA in SR95531 & CGP55845A and  $-3.1 \pm 1.1$  pA after DPDPE, n = 7; Wilcoxon 361 signed-rank test, p = 0.22). Further confirming the PV+ nature of INs responsible for the SuM 362 feedforward inhibition, this result reveals that both the local CA3 and long-range SuM inputs 363 converge onto an overlapping population of INs to inhibit area CA2 PNs, thus enabling cross-364 talk between these routes through synaptic plasticity of PV+ INs. However, since DORs are 365 only expressed in a fraction of PV+ INs and therefore only reduces but does not fully block 366 PV+ IN-mediated GABA release (Nasrallah et al., 2019; Piskorowski and Chevaleyre, 2013), 367 it is difficult to know if the remaining SuM-evoked IPSCs are from PV+ INs not expressing 368 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 SuMdriven 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.

374 The feedforward inhibitory drive from SuM controls pyramidal neuron excitability

375 Given SuM axonal stimulation triggers an excitatory-inhibitory sequence in post-synaptic PNs, 376 we asked which effect would prevail on PN excitability. In order to assess this, we mimicked 377 an active state in PNs by injecting constant depolarizing current steps sufficient to sustain AP 378 firing during 1 second while photostimulating SuM axons at 10 Hz (Figure 6A-B). We observed 379 that recruitment of SuM inputs significantly delayed the onset of the first AP (Figure 6C; 380 latency to the first AP were  $221 \pm 19.9$  ms in control and  $233 \pm 19.1$  ms with photostimulation, 381 hence a  $12.1 \pm 4.3$  ms increase upon photostimulation, n = 12; paired-T test, p = 0.016). In 382 addition, given SuM neurons display theta-locked firing in vivo, we asked if rhythmic inhibition 383 driven by SuM inputs in area CA2 could pace AP firing in PNs by defining windows of 384 excitability. Indeed, photostimulation of SuM axons at 10 Hz led to a significant decrease of 385 variability in the timing of AP firing by PNs (Figure 6D-E; standard deviations of the first AP 386 timing were  $36.9 \pm 11$  ms in control and  $24.7 \pm 7.4$  ms with photostimulation, hence a  $12.3 \pm 12.3 \pm 12$ 387 5.3 ms decrease upon photostimulation, n = 12; Wilcoxon signed-rank tests, p < 0.001 for the 388 first AP, p = 0.008 for the second AP, p = 0.004 for the third AP). Both the delay of AP onset

389 and the reduction of AP jitter stemmed from the feedforward inhibition recruited by SuM inputs 390 as application of GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonists abolished these effects of SuM 391 stimulation (Figure 6C-E; latency to the first AP were 232 ± 19.8 ms in SR95531 & 392 CGP55845A and  $235 \pm 18.0$  ms with photostimulation, n = 6; Wilcoxon signed-rank test, p = 393 0.44; standard deviations of the first AP timing were  $11.9 \pm 2.0$  ms in SR95531 & CGP55845A 394 and  $7.1 \pm 1.5$  ms with photostimulation, n = 6; Wilcoxon signed-rank tests, p = 0.22 for the first 395 AP, p = 0.16 for the second AP, p = 0.09 for the third AP). These results reveal that the purely 396 glutamatergic SuM input, by recruiting feedforward inhibition, has an overall inhibitory effect 397 on PN excitability and can influence the timing and jitter of area CA2 PN action potential firing. 398 One drawback of these results is that the injection of current steps to evoke action potential

399 firing is not entirely representative of CA2 PN activity, as there is no synaptic input leading to 400 AP firing. It has been reported that the AP discharge of SuM neurons in vivo is phase-locked to 401 the hippocampal theta rhythm (Bernat Kocsis and Vertes, 1994). Because theta rhythm is a 402 brain state characterized by elevated levels of acetylcholine, we approximately mimicked these 403 conditions in the hippocampal slice preparation by bath application of  $10 \,\mu$ M of the cholinergic 404 agonist carbachol (CCh). Under these conditions, CA2 PNs depolarize and spontaneously fire 405 rhythmic bursts of APs, and the properties of these AP bursts are tightly controlled by excitatory 406 and inhibitory synaptic transmission (Robert et al., 2020). Thus, we decided to examine how 407 SuM input stimulation influenced CA2 PN firing under these conditions.

408 Because SuM neurons fire in bursts at theta frequency in vivo (Kirk et al., 1996), and because 409 the elevated cholinergic tone accompanying theta can activate muscarinic receptors that alter 410 the synaptic release properties of many synapses in the brain, we examined how synaptic 411 transmission from the SuM input to area CA2 was affected by the application of 10  $\mu$ M 412 carbachol (CCh) (Supplemental Figure 4A) (Kirk et al., 1996; B Kocsis and Vertes, 1994). With 413 GABA receptors blocked to first assess the SuM excitatory transmission only, we observed that 414 CCh decreased the amplitude and increased the PPR of SuM-evoked EPSCs in CA2 PNs 415 (Supplemental Figure 4B). This suggests a decrease of glutamate release by SuM axons induced 416 by CCh. We found similar results for SuM-evoked feedforward inhibitory transmission to CA2 417 PNs as IPSC amplitude was decreased and PPR increased with CCh application (Supplemental 418 Figure 4C). Next, we examined the relative short-term dynamics of SuM-evoked excitatory and 419 inhibitory transmission to CA2 PNs. For this, both EPSCs and IPSCs were recorded from the 420 same individual CA2 PNs upon repeated SuM input stimulation with 5 pulses delivered at 10 421 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  $\mu$ M 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  $15.2 \pm 2.3$  in control and  $6.9 \pm 1.3$  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 <</li>
- 457 0.001; time factor, p < 0.001; light x time factor, p = 0.052).
- 458 In the presence of CCh, CA2 PNs undergo a depolarization of the membrane potential that is 459 followed by a period of AP firing as the membrane potential remains depolarized for several 460 seconds, and then slowly hyperpolarizes until the next bursting event (Robert et al., 2020). We 461 observed that photostimulation of SuM inputs resulted in a striking reduction in the amount of 462 time the membrane potential remained depolarized, and this is likely why the burst duration 463 was significantly shorter in bursts with SuM photo-stimulation (Figure 7F-G; burst duration 464 was  $4.0 \pm 1.1$  s in control and  $1.6 \pm 0.5$  s with photostimulation, n = 7; paired-T test, p = 0.037). 465 The rate and level of V<sub>M</sub> repolarization following bursts were not significantly changed by SuM 466 input photostimulation (V<sub>M</sub> repolarization rate was  $-3.3 \pm 0.6$  mV/s in control and  $-3.6 \pm 0.7$ 467 mV/s with photostimulation, n = 7; paired-T test, p = 0.601; post-burst V<sub>M</sub> was -62.8  $\pm$  1.7 mV 468 in control and  $-62.0 \pm 2.0$  mV with photostimulation, n = 7; paired-T test, p = 0.173), however 469 the inter-burst time interval was reduced. Indeed, AP bursts with SuM input activation were 470 followed more rapidly by another burst of APs than the ones without SuM input activation 471 (Figure 7B and 7H; time until next burst was  $93 \pm 14$  s in control and  $59 \pm 17$  s with 472 photostimulation, n = 7; paired-T test, p = 0.001), which could be due to both short-term 473 depression of inhibitory transmission after repeated activation during the SuM input 474 photostimulation train and reduced activation of hyperpolarizing and shunting conductances 475 during bursts shortened by SuM input photostimulation. Thus, in our preparation, SuM input 476 activation is able to modify the spontaneous bursting activity of CA2 PNs under conditions of 477 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).

484 ChR2-EYFP was expressed in the SuM of Csf2rb2-Cre mice in a Cre-dependent manner and 485 the mice were implanted with a microdrive targeting tetrodes to region CA1 and an optical fiber 486 to the SuM terminals in CA2 (Figure 8A). Mice were placed in a small box (familiar context) 487 and left free to explore as blue (473 nm) laser light pulses (50 ms pulse width) were applied to the SuM terminals at 10 Hz. Across 23 recording sessions in five mice we found that the activation of SuM terminals in CA2 resulted in a significant and reproducible change in the multiunit spiking activity recorded in the pyramidal cell layer of CA1 on 34 of 55 tetrodes. The firing rate change was similar across individual tetrodes (Figure 8B-C), with a decrease in the normalized firing rate starting shortly after laser onset and continuing for about 10 ms, followed immediately by a rebound-like increase to about 20 % greater than baseline firing rate (Figure 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 objective centered on area CA2. Whole-cell patch-clamp recordings of CA1 PNs were obtained 500 501 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 - 20508 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 lightstimulation 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

522 PV+. The resulting feedforward inhibition recruited by SuM input stimulation enhanced the 523 precision of AP timing of CA2 PNs in conditions of low and high cholinergic tone relevant to

524 different brain states. The modified CA2 output evoked poly-synaptic inhibition in area CA1,

525 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.

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

528 <u>neurons</u>

529 Glutamatergic innervation of area CA2 by the SuM has been previously described by tracing 530 studies (Kiss et al., 2000; Soussi et al., 2010) and presumed to form synapses exclusively onto 531 PNs (Maglóczky et al., 1994). Our experimental strategy allowed for the direct examination of 532 the post-synaptic targets of SuM glutamatergic axons. Our results confirm that PNs in area CA2 533 indeed receive excitatory synapses from SuM axons. However, in contrast to what had been 534 proposed in previous studies, we observed that SuM inputs target not only PNs but also INs in 535 area CA2. Importantly, we identified a specific subpopulation of INs as BCs which were the 536 cell type most potently excited by SuM. These BCs could fire action potentials upon SuM input 537 photostimulation leading to a substantial feedforward inhibition of neighboring PNs. We found 538 that at resting membrane potentials, the mixed excitatory/inhibitory SuM input resulted in a net 539 depolarization of the membrane potential in CA2 PNs. However, when the SuM input was 540 paired with either inputs in SR or SLM, we observed a decrease in the summation ratios of 541 trains of synaptic inputs consistent with a perisomatic shunting inhibition driven by SuM in 542 area CA2. Furthermore, we found that with elevated cholinergic tone, recruitment of BCs by 543 SuM controlled PNs excitability and shaped spontaneous burst firing. This finding 544 demonstrates that SuM activity can pace action potential firing in PNs through recruitment of 545 feedforward inhibition.

546 The population of INs potently excited by SuM transmission display many features that 547 motivate us to classify them as PV+ BCs. They have somas located in the somatic layer, have 548 densely packed perisomatic-targeted axons, are fast spiking and show PV immuno-reactivity. 549 The selective expression of GiDREADD in PV+ cells allows for selective silencing that reduces 550 SuM-driven feedforward inhibition of area CA2 PNs. With these techniques, however, we were 551 unable to sufficiently silent PV+ cells in area CA2, leaving open the possibility that another 552 population of basket cell is targeted by SuM input. The feedforward inhibitory transmission 553 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  $\omega$ -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)

Recent studies have indicated that the SuM input to CA2 plays a key role in social novelty discrimination (Chen et al., 2020). Our findings are very consistent with the finding that DOR-mediated inhibitory synaptic plasticity of PV+ INs in area CA2 is required for social recognition memory (Domínguez et al., 2019). Furthermore, exposure to a novel conspecific induces a DOR-mediated plasticity in this same inhibitory network in area CA2 (Leroy et al., 2017). Thus, our finding that SuM input acts via PV+ interneurons fits with previous studies and provides a 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 Consequences of SuM input on area CA2 output

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 influencing CA1 activity (Tirko et al., 2018). Optogenetic experiments have recently shown 611 612 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 (Ito et al., 2018). Altogether, these studies point to the SuM as a crucial component in the

- regulation of hippocampal oscillations and our findings shed light on an aspect of this circuit.
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#### 655 Gating of area CA2 activity by PV+ INs and significance for pathologies

The density of PV+ INs in area CA2 is strikingly higher than in neighboring areas CA3 and CA1 (Botcher et al., 2014; Piskorowski and Chevaleyre, 2013). This population of INs has been shown to play a powerful role in controlling the activation of CA2 PNs by CA3 inputs (Nasrallah et al., 2015). We show in this study that long-range inputs from the SuM can strongly recruit PV+ BCs, which in turn inhibit PNs in this area. Hence, both intra-hippocampal inputs from CA3 and long-range inputs from the SuM converge onto PV+ INs to control CA2 PN excitability and output.

663 Postmortem studies have reported losses of PV+ INs in area CA2 in pathological contexts including bipolar disorder (Benes et al., 1998), Alzheimer's disease (Brady and Mufson, 1997), 664 665 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.

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#### 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(cr e)Arbr/J (PV-Cre)	Jackson	Stock No. 017320				
genetic reagent (adeno-associated virus)	AAV9.EF1a.D IO.hChR2(H13 4R).EYFP	Addgene	Addgene20298				
genetic reagent (adeno-associated virus)	AAV9.hSynap sin.EGFP.WPR E.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 µ M
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	Carbamoylch oline chloride (CCh)	Tocris	2810	10 µM
chemical compound, drug	ω-agatoxin TK	Alomone labs	STA-530	200 nM
software, algorithm	Matlab	Mathworks	www.mathwo rks.com	
software, algorithm	Igor Pro	Wavemetrics	www.waveme trics.com	
software, algorithm	OriginPro	OriginLab Corporation	www.originla b.com	

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

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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.

689 Use of the Tg(Slc17ab-icre)10Ki mouse line: we used the Tg(Slc17ab-icre)10Ki mouse line

690 that was previously generated (Borgius et al., 2010) and expresses the Cre recombinase under

691 control of the slc17a6 gene coding the vesicular glutamate transporter isoform 2 (VGluT2).

<u>Use of the csf2rb2-Cre mouse line</u>: 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.

695 <u>Use of the Pvalbtm1(cre)Arbr/J mouse line</u>: we used the Pvalbtm1(cre)Arbr/J mouse line that 696 was previously generated (Hippenmeyer et al., 2005) and expresses the Cre recombinase under 697 control of the Pvalbm gene coding parvalbumin (PV).

698 Stereotaxic viral injection: Animals were anaesthetized with ketamine (100 mg/kg) and 699 xylazine (7 mg/kg). The adeno-associated viruses AAV9.EF1a.DIO.hChR2(H134R).EYFP and 700 AAV9.hSynapsin.EGFP.WPRE.bGH  $3x10^{8}$ were used at vg, the 701 AAV.Synapsin.DIO.hM4D(Gi).mCherry was used at  $3.6 \times 10^9$ vg and the 702 AAV2/9.hSyn.hChR2(H134R).EYFP.WPRE.hGH was used at 3.7x10<sup>13</sup> vg. The retrograde tracer CAV2-cre virus was used at 2.5x10<sup>12</sup> vg. 500 nL of virus was unilaterally injected into 703 704 the brain of 4 week-old male wild type C57BL/6, Tg(Slc17ab-icre)10Ki (VGluT2-Cre), 705 csf2rb2-cre (SuM-Cre) or Pvalbtm1(cre)Arbr/J (PV-Cre) mice at 100 nL/min and the injection 706 cannula was left at the injection site for 10 min following infusion. In the case of 707 AAV.Synapsin.DIO.hM4D(Gi)-mCherry injection in PV-Cre mice, bilateral injections were 708 performed in dorsal CA2. The loci of the injection sites were as follows: anterior-posterior 709 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 Electrophysiological recordings: 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<sub>2</sub>PO<sub>4</sub> 1.25, NaHCO<sub>3</sub> 30, HEPES 719 20, glucose 25, thiourea 2, Na-ascorbate 5, Na-pyruvate 3, CaCl<sub>2</sub> 0.5, MgCl<sub>2</sub> 10. Brains were 720 then rapidly removed, hippocampi were dissected out and placed upright into an agar mold and 721 cut into 400  $\mu$ m 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<sub>2</sub>PO<sub>4</sub> 1.25, NaHCO<sub>3</sub> 724 26, glucose 10, Na-pyruvate 2, CaCl<sub>2</sub> 2, MgCl<sub>2</sub> 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<sub>2</sub>GTP 0.4, Na<sub>2</sub>-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/mm<sup>2</sup> 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-

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

corrected for. Pharmacological agents were added to ACSF at the following concentrations (in  $\mu$ M): 10 NBQX and 50 D-APV to block AMPA and NMDA receptors, 1 SR95531 and 2 CGP55845A to block GABA<sub>A</sub> and GABA<sub>B</sub> receptors, 1 DAMGO to activate  $\mu$ -opioid receptors (MOR), 0.5 DPDPE to activate  $\delta$ -opioid receptors (DOR), 10 clozapine N-oxide (CNO) to activate hM4D(Gi) DREADDs, 10 CCh to activate cholinergic receptors, 0.2 tetrodotoxin (TTX) to prevent sodic action potential generation, 200 nM  $\omega$ -agatoxin TK to block P/Q-type 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  $\mu$ m diameter), gold-plated to an impedance of 200 to 250 k $\Omega$  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<sup>2</sup> 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 Immunochemistry and cell identification:

786 Midbrains containing the injection site were examined post-hoc to ensure that infection was787 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  $\mu$ m 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.

Reconstructed neurons were classified as either PNs or INs based on the extension and localization of their dendrites and axons. PNs were classified as deep (closest to *stratum oriens*) or superficial (closest to *stratum radiatum*) based on the radial position of their soma in the pyramidal layer. CA1, CA2 and CA3 PNs were identified based on their somatic localization, dendritic arborization and presence of thorny excrescences (TE). Among INs with somas located in the pyramidal layer (*stratum pyramidale*, SP), discrimination between BCs and non-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<sub>M</sub>) and capacitance (C<sub>M</sub>) 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<sub>M</sub> and C<sub>M</sub>; the only statistically 814 difference being a larger R<sub>M</sub> 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<sub>M</sub> and lower C<sub>M</sub> 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 Data analysis and statistics: 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  $\pm$  SEM. Statistical significance was assessed using 829  $\chi^2$  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.

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#### 832 Author Contributions

RAP, VR & TM designed experiments. RAP, VR, VC, LT, EL, RB, AJYH performed
experiments. JC and CV provided technical support. VR, RAP, VC and DP completed analysis.
VR and RAP wrote the manuscript with input from all authors.

836

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- 845
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- 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 view of injection site in the Csf2rbr-Cre mouse line. B. Left, hCHR2(H134R)-EYFP -1166 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  $\mu$ m. (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  $\mu$ m. (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).

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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 < 0.0011197 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 before each stimulus. H. Summary plots of the summation ratio of the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> PSP for 1213 1214 electrical stimulation (black symbols) or paired stimulation of SR + SuM (left, orange) or SLM 1215 + SuM (right, green).

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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.

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Figure 4. SuM input provides excitation to Parvalbumin-expressing BCs. A. Three biocytin reconstructions of BC INs with dendrites in red and axons in light red. Inset, current clamp steps to -400 pA and +400 pA display high-frequency AP firing and repolarizing sag current. B. Corresponding light-evoked EPSCs and EPSPs for the three reconstructed neurons (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  $\mu$ M. 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  $\mu$ M 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  $\omega$ -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  $\mu$ M 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  $1\mu$ M 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  $0.5 \,\mu M$  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  $\mu$ M 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 in SR95531 & CGP55845A in grey, light-on in SR95531 & CGP55845A in purple). Right, 1283 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, 10<sup>th</sup> and 90<sup>th</sup> 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 < 0.0011293 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

Figure 7. SuM input shapes CA2 PN AP bursts in conditions of elevated cholinergic tone. A. Diagram illustrating whole-cell recordings of area CA2 PNs with light stimulation of SuM fibers in an acute slice preparation. B. Sample trace of spontaneous AP bursting activity recorded from a CA2 PN during bath application of 10  $\mu$ M CCh. For every even-numbered 1306 burst, a 10 Hz photostimulation (blue bars) was delivered to excite SuM inputs in area CA2 1307 allowing a comparison of burst AP firing in the same cell. C. Sample traces of AP firing during 1308 bursts for light-off (left, black) and light-on (right, red) epochs. D. Comparison of AP number 1309 per burst for light-off (black) and light-on (red) events (n = 7; individual cells shown as thin 1310 lines, population average shown as thick line, error bars represent SEM; paired-T test, p =1311 0.031). E. Average firing rate during spontaneous burst events with SuM photostimulation (red, 1312 light-on) and controlled interleaved burst events (black, light-off). Shaded area represents SEM 1313 for 7 cells each with between 3 and 13 bursts analyzed in light-on and light-off conditions (2-1314 way ANOVA, light factor: p < 0.001, time factor: p < 0.001, light x time factor: p = 0.052). F. 1315 Example burst events with (red) and without (black) SuM photostimulation overlayed and on a 1316 scale that shows the rapidly hyperpolarizing membrane potential that occurs with SuM input 1317 stimulation. G. Comparison of bursts duration for events with (red) and without (black) 1318 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 1319 1320 next burst onset following bursts with (red) or without (black) photostimulation (n = 7; 1321 individual cells shown as thin lines, population average shown as thick line, error bars represent 1322 SEM; paired-T test, p = 0.001).

1323 Figure 8. Consequences of SuM input on area CA2 output to CA1. A. Diagram illustrating 1324 in vivo recording in CA1 with tetrodes and SuM axon terminals stimulation over CA2 with an 1325 implanted optical fiber. B. Representative data from 4 multi-unit recordings. Raster plot (top) 1326 showing CA1 AP firing activity before and during photostimulation of SuM fibers in area CA2. 1327 The corresponding firing rate histogram (middle) of four tetrodes placed in the CA1 pyramidal 1328 cell layers, as well as plots of standard deviation (SD; bottom). Red lines indicate +/- 3SD. C. 1329 Individual (grey) and average (red) normalized firing rates from 34 multiunit recordings, 3 1330 consecutive light stimulation epochs are displayed to help visualizing the consistency of the 1331 effect of SuM input light stimulation over area CA2 on CA1 multi-unit firing; the shaded area 1332 represents the SEM. D. Diagram illustrating whole-cell recordings of area CA1 PNs and SuM 1333 fiber light stimulation over area CA2 in acute slice preparation. E-H. Example waterfall plots 1334 (E, G) and corresponding peri-stimulus time histogram (F, H, population average shown as 1335 thick line, shaded area represents SEM) of EPSCs (black) and IPSCs (red) recorded from a CA1 1336 PN ex vivo during photostimulation of SuM input over area CA2 with bath application of 10 1337 μM CCh.

#### 1338 Supplemental figure legends

#### 1339 Supplemental Figure 1.

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

#### 1364 Supplemental Figure 2.

A. Diagram illustrating the whole-cell recording configuration of PNs in area CA2 and SuM fiber stimulation in acute hippocampal slices. B. Light-evoked EPCSs from SuM inputs are completely blocked following application of tetrodotoxin (TTX). Sample traces (top, control shown in black, +TTX shown in grey) and power-response curves (bottom) of light-evoked EPSC amplitudes recorded in PN before (black) and after application of 0.2  $\mu$ M TTX (grey) at 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  $10 \,\mu M$  NBQX
- 1374 & 50  $\mu$ M APV (n = 6, error bars represent SEM).

#### 1375 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<sub>M</sub>) level of Gi-DREADD-1379 expressing CA2 PV INs with application of 10  $\mu$ M CNO (n = 6, error bars represent SEM). C.  $V_M$  level before and after application of CNO (n = 6; Wilcoxon signed-rank test, p = 0.031; 1380 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 in grey, spikes in light red). E. Same as D with traces displayed as waterfall. 1384

#### 1385 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 transmission blocked (B, SR95531 & CGP55845A in grey, SR95531 & CGP55845A + CCh in 1389 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  $\mu$ M 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

	$V_{M}(mV)$	R <sub>M</sub> (MOhm)	C <sub>M</sub> (pF)
CA2 PN $(n = 81)$	$-69.8 \pm 0.70$	$59.2 \pm 2.65$	$209 \pm 11.4$
CA3 PN (n = 31)	$-70.3 \pm 1.06$	$72.4 \pm 4.82$	211 ± 15.7
Statistics	Mann-Whitney U test	Student T test	Mann-Whitney U test
	p = 0.997	p = 0.020*	p = 0.625
PN deep $(n = 57)$	-71.1 ± 0.76	$64.0 \pm 3.94$	$200 \pm 12.3$
PN superficial $(n = 76)$	$-69.3 \pm 0.67$	$64.9 \pm 3.19$	$196 \pm 11.8$
Statistics	Student T test	Mann-Whitney U test	Mann-Whitney U test
	p = 0.077	p = 0.777	p = 0.588

Table 1. Electrophysiological p	properties of pyramidal neurons	in SuM-innervated area
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	EPSC					
cell type	connectivity (%)	amplitude	rise time	decay	latency	success
	_	(pA)	(ms)	time (ms)	(ms)	rate
CA2 PN	56 (n = 58 of 103)	$-16 \pm 1.9$	$2.9 \pm 0.1$	$14 \pm 0.8$	$2.4 \pm 0.2$	$0.44 \pm 0.03$
CA3 PN	49 (n = 22 of 45)	$-23 \pm 5.9$	$3.0 \pm 0.2$	$14 \pm 0.9$	$2.7 \pm 0.3$	$0.56 \pm 0.06$
Statistics	$\chi^2$ test	Mann-	Mann-	Mann-	Mann-	Student T
		Whitney U	Whitney U	Whitney	Whitney U	test
		test	test	U test	test	
	p = 0.572	p = 0.409	p = 0.391	p = 0.797	p = 0.156	p = 0.074
PN deep	56 (n = 35 of 63)	$-15 \pm 2.0$	$3.5 \pm 0.2$	$16 \pm 1.0$	$3.5 \pm 0.4$	$0.39 \pm 0.03$
PN superficial	56 (n = 53 of 94)	$-20 \pm 3.0$	$3.1 \pm 0.2$	$15 \pm 0.9$	$2.7 \pm 0.3$	$0.51 \pm 0.04$
Statistics	$\chi^2$ test	Mann-	Mann-	Mann-	Mann-	Mann-
		Whitney U	Whitney U	Whitney	Whitney U	Whitney U
		test	test	U test	test	test
	p = 0.946	p = 0.306	p = 0.051	p = 0.314	p = 0.083	p = 0.072
			IPSC			
cell type	connectivity (%)	amplitude	rise time	decay	latency	success
		(pA)	(ms)	time (ms)	(ms)	rate
CA2 PN	35 (n = 19 of 55)	$197 \pm 41.3$	$3.8 \pm 0.4$	$25 \pm 1.2$	$6.3 \pm 0.7$	$0.55 \pm 0.06$
CA3 PN	57 (n = 16 of 28)	$145 \pm 23.4$	$4.5 \pm 0.4$	$25 \pm 1.2$	$7.5 \pm 0.9$	$0.54 \pm 0.05$
Statistics	$\chi^2$ test	Mann-	Student T	Mann-	Mann-	Student T
		Whitney U	test	Whitney	Whitney U	test
		test		U test	test	
	p = 0.134	p = 0.870	p = 0.203	p = 0.896	p = 0.303	p = 0.893
PN deep	47 (n = 16 of 34)	$199 \pm 40.6$	$3.8 \pm 0.4$	$25 \pm 1.4$	$7.2 \pm 0.8$	$0.52 \pm 0.07$
PN superficial	47 (n = 26 of 55)	$167 \pm 27.5$	$4.9 \pm 0.4$	$26 \pm 1.2$	$6.8 \pm 0.7$	$0.50 \pm 0.05$
Statistics	$\chi^2$ test	Mann-	Student T	Student T	Student T	Student T
		Whitney U	test	test	test	test
		test				
	p = 0.987	p = 0.258	p = 0.047*	p = 0.564	p = 0.706	p = 0.796

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

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

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

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

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

Measurement	Conditions	Factors	2-way ANOVA p-values
	EDSC amplitude // CCh	treatment	0.00171693
	(n - 13)	pulse #	0.00286193
	(II – 15)	treatment x pulse #	0.0521822
	IPSC amplitude 1/ CCh	treatment	0.413564
	(n - 13)	pulse #	0.0247487
omplitude	(II – 15)	treatment x pulse #	0.316489
ampiltude	DSC amplitude in ACSE	holding level	0.0121691
	PSC amplitude in ACSF $(n - 13)$	pulse #	0.0115431
	(II – 15)	holding level x pulse #	0.391097
	DSC amplitude in CCh	holding level	2.85112E-11
	PSC amplitude in CCI (n = 13)	pulse #	0.189593
	(1-15)	holding level x pulse #	0.55014
	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
$D_{\rm P}/D1$ ratio		treatment x pulse #	0.297716
r II/r 1 Tatio	DSC Dr/D1 ratio in ACSE	holding level	0.325751
	PSC PII/PT ratio in ACSF (n = 13)	pulse #	2.08101E-08
	(1-15)	holding level x pulse #	0.941122
	DSC Dr/D1 ratio in CCh	holding level	0.0948351
	(n - 13)	pulse #	3.07005E-05
	(1-15)	holding level x pulse #	0.889375
	DSC E/Lengting 1/ CCh	treatment	7.61696E-06
E/I ratio	$r_{SU} = \frac{1}{1} r_{AUO} + - UUh$	pulse #	0.99245
	(11 - 13)	treatment x pulse #	0.982047

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

ChR2-EYFP AAV9.EF1a.DIO.hCHR2(H134R).EYFP 500 µm Csf2rb2-Cre



ChR2-EGFP / RGS14

В



- 50 µm
- CA2 pyramidal neuron С



Figure 1.

А



# Figure 2.



cell-attached 50ms



Figure 4.



Figure 5.



Figure 6.





time (ms)

Figure 8.



Supplemental Figure 1.



Supplemental Figure 2.



Supplemental Figure 3.



Supplemental Figure 4.